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Editors:
Basuki Supartono
Ariyani Noviantari

Discovering the Miracle of Stem Cells

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Menteng, Jakarta Pusat,
Daerah Khusus Ibukota Jakarta 10340
WhatsApp: +62 811-1064-6770
E-mail: penerbit@brin.go.id
Website: penerbit.brin.go.id
Penerbit BRIN
@penerbit_brin
@penerbit.brin

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Publisher's Note

Stem cell research in Indonesia has brought hope and good news for successful discoveries in treating various diseases. By paying particular attention to the unique opportunities and challenges related to stem cell research and application in Indonesia, researchers found that Indonesia has a specific context as a country that shapes this effort. This requires the presence of a role and full support from the government. Through the implementation of current regulations, Indonesian government policies have sufficiently explained various aspects of stem cell science, including production, application, and utilization, both from a medical and religious perspective. However, the application of stem cells in Indonesia still needs to be expanded in the context of research. Currently, funding for stem cell therapy is provided through research grants or community contributions without government support.

Discovering the Miracle of Stem Cells presents the results of an in-depth exploration of stem cell research, focusing on its transformative potential in various medical and scientific domains. This book comprehensively describes the basic characteristics of stem

cells, including their types, functions, and important roles in tissue regeneration and therapeutic interventions, with various application focuses and different research fields.

This book explains the culture technique for mesenchymal stem cells (MSCs), which is essential for advancing regenerative medicine. Then, applying stem cells in orthopaedics also shows effectiveness in treating bone and cartilage disorders. In addition, stem cells are also used in the treatment of diabetic wounds, skin rejuvenation after UV exposure, and the management of neurodegeneration and other neurological disorders through the therapeutic implications of stem cells.

Furthermore, this book examines the ethical dimensions of stem cell research that require the application of strict ethical standards to navigate the complex moral landscape encountered in using stem cells for therapeutic purposes. The government, as a policy maker, is expected to be able to establish regulations to ensure legal certainty, safety, and comfort for patients and stakeholders related to stem cell research.

This book is dedicated to all Indonesian people so that they can better understand stem cells, which are very useful in medicine. In particular, stem cell researchers also hope that the Indonesian government will fully support the integration of stem cell therapy services into the framework of Indonesian health services regarding regulation, budgeting, and infrastructure development in the stem cell domain in the future. This integration will ensure that all levels of Indonesian society who need such treatment can access stem cell therapy safely and affordably. Furthermore, Indonesia is expected to become a destination for global patients seeking stem cell therapy, thus making a significant contribution to the international medical community by improving the quality of life and health standards. Finally, we would like to thank all parties who contributed to and helped publish this book.

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Foreword

As a former Minister of Health for the Republic of Indonesia and a seasoned cardiologist, it is with great honor that I present the foreword for this remarkable book, *Discovering the Miracle of Stem Cells*. This book is edited by Prof. Dr. dr. Basuki Supartono, F.I.C.S, M.A.R.S. and Ariyani Noviantari, S.Si., M.Biomed.

Prof. Basuki Supartono is an esteemed orthopedic and trauma surgeon in Indonesia and a pioneering figure in stem cell therapy. His extensive expertise in treating musculoskeletal disorders, particularly in overcoming trauma-related complications in orthopedics, paired with his dedication to research and education, makes this book an invaluable resource. I have had the pleasure of knowing Prof. Basuki as a dedicated colleague who has relentlessly worked to improve healthcare in Indonesia. With a strong foundation in both research and education, he has mentored countless students and scholars across various academic levels. His commitment to advancing stem cell therapies has not only made a lasting impact in Indonesia but has also garnered international recognition. Meanwhile, Ariyani

Noviantari is the researcher at The Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency (BRIN).

This book was born out of the urgent need for innovative therapeutic approaches to address the rising incidence of degenerative diseases, including musculoskeletal conditions, skin disorders, diabetes, cardiovascular diseases, neurological issues, and other complex health problems. Stem cell technology has been pivotal in advancing regenerative medicine, and this publication showcases Indonesia's significant progress in both stem cell research and clinical applications, offering these developments to the international community. Within this volume, editors and their collaborators, provides a thorough and accessible explanation of how stem cells are transforming contemporary medical treatments, with the potential to repair and regenerate damaged tissues. The chapters are carefully structured, providing readers with an in-depth understanding of stem cells, from their discovery to their diverse clinical uses.

This book presents Indonesia's achievements in stem cell research and its clinical applications to the world, illustrating the country's contributions to pioneering medical science. I trust that healthcare professionals, researchers, and students will find this book invaluable. It not only deepens their knowledge of stem cells but also provides a strong ethical framework for those aiming to advance stem cell-based therapies. I am confident this book will inspire future generations of scientists and doctors to further explore this transformative and promising field.

Jakarta, October 12th, 2024

Dr. dr. Siti Fadillah Supari, Sp.J.P.(K)
Former Minister of Health of the Republic of Indonesia
(2004–2009)

Preface

The journey into stem cell research represents one of the most profound scientific endeavors of our time. From the early exploration of basic cell biology to the groundbreaking advances in regenerative medicine, stem cells have opened up new possibilities for healing and restoring the human body. *Discovering the Miracle of Stem Cells* seeks to highlight these extraordinary potentials, particularly focusing on the contributions made by Indonesian researchers to this rapidly evolving field.

This book covers a wide array of topics within stem cell science, encompassing both fundamental research and clinical applications. It underscores the pivotal role that stem cells have played in transforming modern medicine. The initial chapters, such as "Stem Cell Culture Techniques" by Jeanne Adiwinata Pawitan, provide key insights into culturing methods and the development of mesenchymal stem cells (MSCs), which are among the most widely applied stem cells in regenerative therapies. Clinical applications are thoroughly explored in chapters like "Stem Cell for Orthopaedics Application" by Ismail

Hadisoebroto Dilogo, as well as discussions on the potential use of stem cells in treating diabetes and skin aging, with contributions from experts such as Siufui Hendrawan and Winawati Eka Putri. These chapters are enriched with fascinating case studies in orthopaedics, cardiology, dermatology, and neurology, offering a comprehensive overview of how stem cells are being utilized in diverse medical fields.

One notable chapter, "The Potential of CD34+ Stem Cells in Increasing Fibroblast and Collagen Levels in Ultraviolet B Exposed Skin," co-authored by Basuki Supartono, explores the use of stem cells in anti-aging therapies, providing promising avenues for skin regeneration. In addition, the book delves into the ethical and legal dimensions of stem cell research, expertly analyzed by Dito Anurogo in the chapter "The Art of Socio-ethical and Legal Dimensions in Stem Cell Research." The articles in this book have been carefully selected, reviewed, edited, and proofread under the supervision of the National Research and Innovation Agency (BRIN), ensuring the highest level of academic integrity.

On a broader scale, this book not only consolidates existing knowledge in the field of stem cells, but also introduces new perspectives and clinical applications specific to the Indonesian healthcare context. While global research on stem cells is vast, this work offers a unique focus on Indonesia's advancements, illustrating how local research and clinical trials are contributing to global progress. By situating these developments alongside international breakthroughs, *Discovering the Miracle of Stem Cells* adds a regional dimension to the existing body of literature in regenerative medicine, offering insights into localized challenges and solutions often overlooked in mainstream research.

As a researcher and clinician, I have had the privilege of witnessing firsthand the remarkable potential of stem cell therapies to provide hope and relief to patients suffering from a wide range of conditions.

I am deeply thankful to my colleagues, research teams, and collaborators, whose unwavering dedication and tireless efforts have made this book possible. Their contributions have been instrumental in advancing our collective understanding of stem cells and propelling

this pioneering field to the forefront of medical innovation. I also extend my sincere gratitude to the National Research and Innovation Agency (BRIN) for their invaluable support.

I offer my heartfelt thanks to the readers of this book, particularly practitioners, researchers, and students in the biomedical sciences. My hope is that this book will serve both as a valuable reference and a source of inspiration, encouraging further research and innovation in stem cell science.

Lastly, I wish to acknowledge the steadfast support of my wife, Dr. Prita Kusumaningsih, SpOG, my family, my children, grandchildren, and friends, who have continuously encouraged me throughout the writing process. Their patience and understanding have been invaluable in bringing this work to completion.

As we continue to explore the vast potential of stem cells, I believe we are only beginning to realize the full scope of their capabilities. *Discovering the Miracle of Stem Cells* is not merely a record of scientific discoveries; it is a testament to the future of medicine, where healing and regeneration become achievable for all.

Jakarta, October 12th, 2024

Prof. Dr. dr. Basuki Supartono,
Sp.O.T., F.I.C.S., M.A.R.S

Chapter 1

Discovery of Stem Cells

Ariyani Noviantari

A. Introduction

Stem cells have been attracting a lot of attention in recent years, and the application of stem cells has been recognized as an extremely promising and advanced research area being studied. Stem cells are a special kind of cell that can self-renew and specialize into distinct cell lineages. They are found in any stage of life. Because of their unique capacity for self-replication and differentiation, they are becoming a common form for the investigation of fundamental biological issues, including transcription, cell fate decisions, replication, division, and replication. Fundamental queries at various developmental stages can be answered with the help of adult stem cells, which can give rise to the cells within a certain lineage, and embryonic stem cells (ESCs),

A. Noviantari

National Research and Innovantion Agency, e-mail: ariy007@brin.go.id

Noviantari, A. (2025). Discovery of stem cells. In B. Supartono & A. Noviantari (Eds.), *Discovering the miracle of stem cells* (1–21). BRIN Publishing. DOI: 10.55981/brin.1128.c1295, E-ISBN: 978-602-6303-50-9

which can generate any cell type in the mammalian body (Poliwoda et al., 2022; Zakrzewski et al., 2019).

Stem cell research is developing exponentially, altering the character of medical care as it will be performed in the future. Innovation in the treatment of degenerative diseases is made possible by cell-based therapy and its products in regenerative medicine by promoting regeneration (Cho et al., 2018). Due to the presence of trophic factors secreted by these cells, stem cell products such as secretomes provide another potential alternative to stem cells (Kim et al., 2013).

Stem cells are distinguished by their ability to self-renew and differentiate into various types of cells like osteoblasts, chondrocytes, adipocytes, neurons, glial cells, cardiomyocytes, muscle cells, hepatocytes, endothelial cells, and other types of cells (El Barky et al., 2017; Laverdet et al., 2014; Macrin et al., 2017). Based on their ability to differentiate, stem cells are categorized into two groups: (1) pluripotent stem cells (PSCs), such as ESCs and induced pluripotent stem cells [(iPSCs) and (2) multipotent stem cells or adult stem cells (ASCs), PSCs, including ESCs formed from embryos and iPSCs derived by gene transfer, can proliferate indefinitely and differentiate into several types of tissues depending on their treatment conditions. However, multipotent stem cells, like mesenchymal stem cells (MSCs), can only differentiate according to their lineage and can be formed from mature tissues such as bone marrow, adipose tissue, umbilical cord blood, placenta, or blood. Additionally, ESCs and ASCs offer a great tool for cell therapy, which increases the importance of stem cell research in regenerative medicine (Chang et al., 2019; Zakrzewski et al., 2019).

Stem cells have a high potential to become one of the most essential parts of medicine. Furthermore, they contribute significantly to the advancement of regenerative medicine (Zakrzewski et al., 2019). However, there are still many gaps in our understanding of stem cells and their applications in clinical settings. This book had the objective to provide information on stem cells and their applications, starting with the chapter about the MSCs culture technique and continuing

with applying stem cells and their secretome in diabetic, neurological, orthopaedic, and cardiovascular diseases, and stem cells for anti-aging. Therefore, the book concluded with an ethically responsible approach to stem cell research.

B. The Understanding of Stem Cells

Stem cells are special cells with the capacity to differentiate into different kinds of cells or other tissues as well as the ability to regenerate themselves over an extended time (El Barky et al., 2017; Laverdet et al., 2014). Stem cells can develop into different types of cells and eventually become specialized (Zakrzewski et al., 2019). Although stem cells are undifferentiated, they can differentiate into a variety of cells, including osteoblasts, chondrocytes, adipose tissue, neurons, glial cells, cardiomyocytes, muscle cells, hepatocytes, endothelial cells, and others (Catacchio et al., 2013).

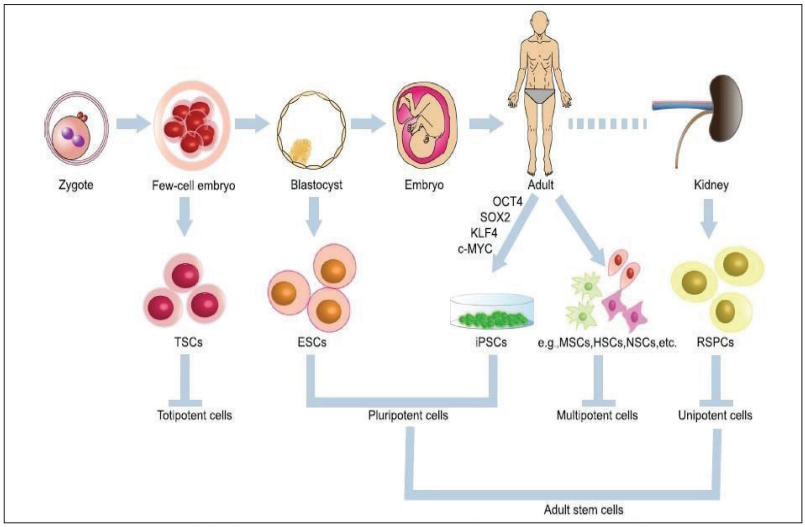
The following requirements must be met by stem cells before they can be used in clinical settings: they must be widely available (billions to billions of cells), non-invasive to obtain, able to differentiate into different cell types, and safe and effective when autologously transplanted (cell transplantation) to stem (stem cell transplantation for oneself) or allogeneic (stem cell expansion or production following Good Manufacturing Practices [GMPs] criteria) (Lindroos et al., 2011). Autologous stem cell therapy is the best type of stem cell therapy since it can lower the risk of rejection. Allogeneic stem cell therapy, on the other hand, has the potential to be therapeutic despite not coming from the patients themselves because it is simpler and more successful (Alwi, 2012; Hasanah & Nuban, 2021).

Stem cells can be classified into four categories based on their potential (Figure 1.1), as outlined below.

1) Totipotent stem cells can develop into embryonic and extra-embryonic (placental) cell types to generate an organism. The maximum capacity for cell differentiation is totipotent. This cell is created when sperm and egg cells combine to form a zygote.

These cells will grow into three germ layers (Zakrzewski et al., 2019).

2) Pluripotent stem cells can differentiate into embryonic cell types but not extra-embryonic tissue forms like the placenta. Comprising totipotent stem cells as their origin, these cells exhibit nearly limitless cell lineage potential, examples are iPSCs and ESCs (Amin et al., 2019; Liu et al., 2020; Zakrzewski et al., 2019).
3) Multipotent stem cells, such as hematopoietic stem cells, which can differentiate into many blood cell types, are cells that can differentiate into multiple cell types while remaining belonging to the same group. MSCs, neural stem cells (NSCs), and intestinal stem cells (ISCs) are further examples (Liu et al., 2020; Sobhani et al., 2017; Zakrzewski et al., 2019).
4) Unipotent stem cells possess the capacity to self-renew while only being able to develop into a single type of cell. Examples include renal stem cells, dermatocytes, and others (Balogh & Engelmann, 2011; Zakrzewski et al., 2019).



Source: Liu et al. (2020)

Figure 1.1 Potency of Differential of Stem Cells

Stem cells can be divided into three types: embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs).

1. Embryonic Stem Cells (ESCs)

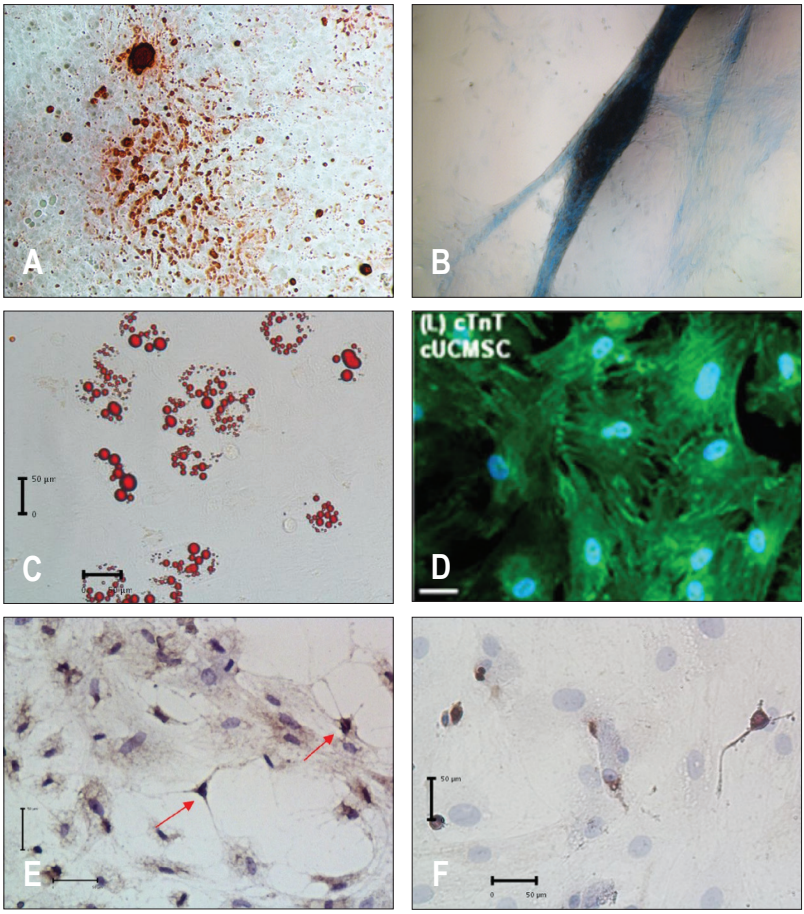
ESCs are derived from the blastocyst inner cell mass (ICM) stage, which is the early developmental stage of the embryo following 4–5 days of fertilization. These cells in the three germ layers—the ectoderm, mesoderm, and endoderm—are pluripotent, self-renewing, and able to develop into any kind of differentiated cell in the body (El Barky et al., 2017; Zhao et al., 2012).

2. Adult Stem Cells (ASCs)

Adult stem cells, also known as somatic stem cells, are undifferentiated cells present throughout the body. Adult stem cells can be obtained from bone marrow, fat tissue, peripheral blood, umbilical cord blood, Wharton's jelly, placenta, amniotic fluid, muscles, intestines, liver, kidneys, hair follicles, skin, blood vessels, urine, and menstrual blood. Only a small percentage of adult stem cells—such as those from umbilical cord blood—are pluripotent, while the majority are multipotent. Some are unipotent. MSCs and hematopoietic stem cells are two types of adult stem cells (Bacakova et al., 2018; S. Liu et al., 2016; Nguyen et al., 2016).

MSCs are a type of adult stem cells that can be obtained from bone marrow, adipose tissue, lung tissue, umbilical cord blood, peripheral blood, and other sources. The following criteria can be used to identify these stem cells, possess positive expression of CD73, CD90, and CD105, can adhere to plastic culture surfaces (plastic adherence) under standard culture conditions, able to differentiate into adipocytes, chondrocytes, and osteoblasts in vitro, does not express CD11b or CD14, CD19 or CD79α, CD34, CD45, and HLA-DR (Dominici et al., 2006).

Because of their multipotent characteristics, MSCs can develop into many types of cells. MSCs can differentiate in vitro into chondrocytes, osteoblasts, adipocytes, neurons, cardiomyocytes, and other cell types (Figure 1.2). In vitro, culture medium with growth factors is one common method for differentiating MSC utilizing



Notes: (A) Osteoblast; (B) Chondrocyte; (C) Adipocyte; (D) Cardiomyocyte (immunofluorecence – marker cTNT); (E) Neuron (imunocytochemistry - marker MAP-2); (F) Neuron (imunocytochemistry- marker Nestin).

Source: (A) & (B) Noviantari, Antarianto, et al. (2020), (C) Noviantari et al. (2023), (D) Hollweck et al. (2011), (E) Noviantari, Rinendyaputri, and Ariyanto (2020), (F) Noviantari, Antarianto, et al. (2020)

Figure 1.2 Differentiation Potential of MSCs

inducing factors (Huang et al., 2015; Macrin et al., 2017; Noviantari, Rinendyaputri, Yunindasari, et al., 2020; Taran et al., 2014).

The following tissues can be utilized for isolating MSCs.

1) The bone marrow

Bone marrow stem cells are also referred to as stromal cells. Bone marrow contains both hematopoietic and non-hematopoietic stem cells. Friedenstein first revealed in 1970 that there is a population of hematopoietic stem cells in femur bone marrow that attaches to plastic surfaces, whereas the majority of the cells are non-adherent. MSCs are these attached cells. The adhering cells multiply within a few days and, if grown in vitro, can develop into osteoblasts, adipocytes, and chondrocytes (Friedenstein et al., 1970; Morrison & Scadden, 2014; Pontikoglou et al., 2011).

MSCs from bone marrow compose a very small fraction of bone marrow, approximately 1 in 10,000 mononuclear cells (MNCs). Further research demonstrates the multipotency of these cells. These cells can develop into bone, cartilage, muscle, ligaments, tendons, dermis, and supporting tissue in addition to differentiating into osteoblasts, chondrocytes, and adipocytes (Caplan, 1991; Sandhaanam et al., 2013). The morphology of primary MSCs derived from rat bone marrow in Figure 1.3.

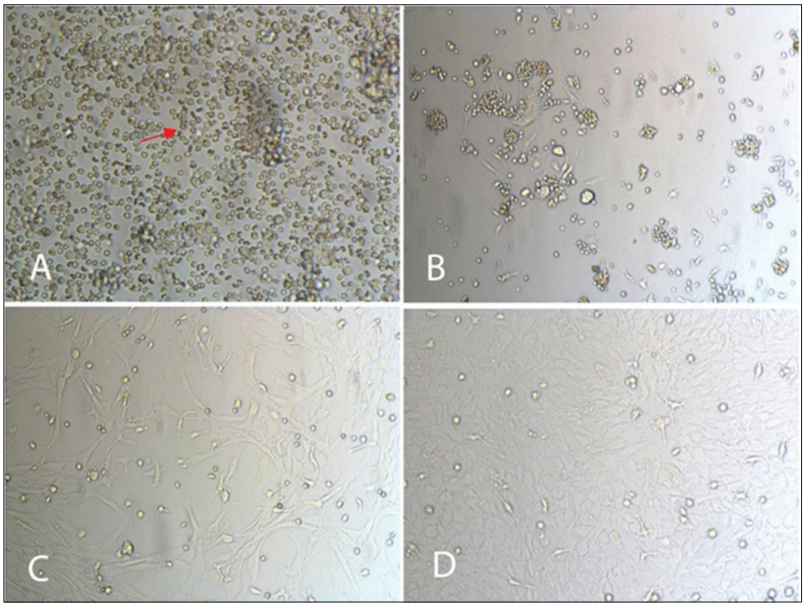
A variety of kinds of cells compose bone marrow stem cells. The expression of antigens on the cell surface is one of the characteristics noted. These bone marrow-derived MSCs express the hyaluronan receptor CD44, the transforming growth factor-b receptor III CD105, the thy-1 CD73, the melanoma cell adhesion molecule, or Mel-CAM, CD146, and do not express the hematopoietic cell markers CD11b, CD14, CD45, and CD34 (Pontikoglou et al., 2011).

2) Umbilical cord blood

The umbilical cord connects the fetus to the placenta. Furthermore, umbilical cord blood is a type of blood found in the placenta and

umbilical cord blood vessels. Three components make up the umbilical cord: Wharton's jelly, two arteries, one vein, and the cord lining (Figure 1.4) (Ali & Al-Mulla, 2012; Cassar & Blundell, 2016)

When compared to other stem cell sources, umbilical cord blood has the following advantages: it is simpler to obtain, does not threaten the donor, has low immunogenicity, reduces the risk of infection by cytomegalovirus or Epstein-Barr virus due to low placental transmission, and gives fewer ethical issues, minimal compared to stem cells from embryos. Umbilical cord blood stem cells do not require invasive treatments because they



Notes: (A) Rat bone marrow MSCs culture taken on day 0 (magnification 400x). Erythrocytes are shown by the arrow. Rat bone marrow MSCs culture on (B) day 1, (C) day 5, and (D) day 8 (B, C, D magnification 100x).

Source: Noviantari, Antarianto, et al. (2020)

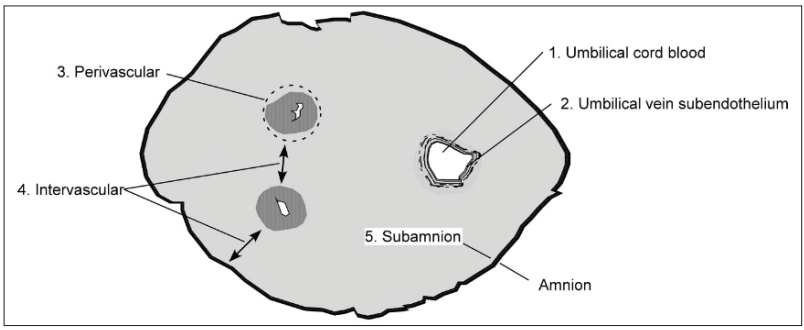
Figure 1.3 The Morphology of Primary MSCs Derived from Rat Bone Marrow

are derived from extraembryonic tissue, which is often removed during delivery. Despite their small quantity, umbilical cord blood stem cells possess the capacity to regenerate. This can be solved through cell expansion for clinical transplantation (Ilic et al., 2012; Yuliana et al., 2012).

3) Adipose tissue

One of the major organs in the body is adipose tissue. Thin adult women and men have between 3 and 4.5 kilograms of fat tissue in their bodies. Meanwhile, extremely obese people have about 45 kg of fat tissue (Halim et al., 2010). Adipocytes, fibroblasts, endothelial cells, vascular smooth muscle cells, and progenitor cells are some of the intricate constituents of adipose tissue (Lindroos et al., 2011).

After liposuction, adipose tissue is used to produce stem cells. Lipoaspirate may be processed right away or left unprocessed for up to three days before being processed again (Harris, 2014). It has been observed that bone marrow-derived stem cells and adipose tissue stem cells contain similar properties. Stem cells from adipose tissue have several advantages over those from bone marrow, including being easier to collect in large quantities, a low-risk and less painful technique, and easier cell replication.



Source: Troyer and Weiss (2008)

Figure 1.4 Anatomy of Umbilical Cord

In addition, these cells are immunosuppressive, simpler to get samples of adipose tissue, and can develop into other cells, including blood vessels. Thus, fat tissue stem cells hold great promise for regenerative therapy (Pawitan, 2009).

4) Wharton's jelly

Another source of stem cells is the gelatinous tissue called Wharton's jelly, known to be a component of the baby's umbilical cord. Stem cells from Wharton's jelly have several advantages, namely that they can be expanded or produced in large quantities, are easy to obtain, are not dangerous for donors both mother and baby, stemness properties can be maintained until passage 9–10, lower ethical problems compared to ESCs, and do not have the potential to develop to a tumor (Bagher et al., 2015).

5) Teeth

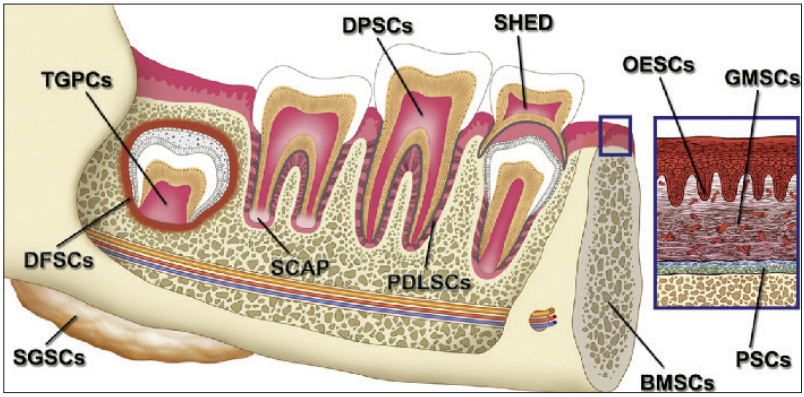
Stem cells can be obtained from teeth. Dental pulp stem cells (DPSCs) and human exfoliated deciduous stem cells (SHED) are two types of stem cells derived from teeth. Miura initially isolated SHED from a byproduct of extracting milk teeth. Because of this, there are an infinite number of stem cell sources available and the collection procedure is non-invasive. Other areas of the tooth (Figure 1.5) have sources of stem cells in addition to SHED and DPSCs (Egusa et al., 2012; Miura et al., 2003). SHED has a more effective proliferation potential than DPSCs and bone marrow-derived MSCs stem cells from teeth are also potential to differentiate into chondrogenic and osteogenic (Huang et al., 2009; Zakrzewski et al., 2019)

3. Induced Pluripotent Stem Cells (iPSCs)

Stem cells known as induced pluripotent stem cells (iPSCs) are produced by causing somatic cells to express characteristics similar to those of ESCs. To reprogram the somatic cell nucleus, exogenous

genes like OCT4, SOX2, NANOG, and LIN28 or OCT3/4, KLF4, and cMYC will be inserted. Using retroviral vectors to transduce the four transcription factors, OCT3/4, SOX2, KLF4, and cMYC are involved in the process of converting somatic cells into pluripotent iPSCs. Adult human somatic cells can be reprogrammed to become pluripotent, just as ESCs. In the future, iPSCs with this pluripotent quality may differentiate into any kind of adult cell, offering an alternative treatment for degenerative diseases (Takahashi & Yamanaka, 2013).

Since it was discovered that the transplanted cells mostly exerted their therapeutic impact through the secretion of paracrine substances, cell-free therapy has become a unique method in regenerative medicine over the past ten years. More and more data



Notes: BMSCs: bone marrow-derived MSCs;
DPSCs: dental pulp stem cells;
SHED: stem cells from human exfoliated deciduous teeth;
PDLSCs: periodontal ligament stem cells;
DFSCs: dental follicle stem cells;
TGPCs: tooth germ progenitor cells;
SCAP: stem cells from the apical papilla;
OESCs: oral epithelial progenitor/stem cells;
GMSCs: gingiva-derived MSCs,
PSCs: periosteum-derived stem cells;
SGSCs: salivary gland-derived stem cells.

Source: Egusa et al. (2012)

Figure 1.5 Sources of Stem Cells from Teeth

suggest that stem cell-derived secretomes, including growth factors, chemokines, cytokines, extracellular matrix (ECM), and extracellular vesicles (EVs), can repair wounded tissues as effectively as cells. This discovery has given rise to a novel concept for the use of secretome in regenerative medicine (Foo et al., 2021).

The secretome, referred to as the MSCs-conditioned medium (MSCs-CM), is a mixture of bioactive substances that have anti-inflammatory, anti-apoptotic, neuroprotective, and proliferative functions. Increasing data indicates that MSCs-CM plays a significant role in a variety of disorders, including bone, skin, muscle, and dental diseases. The conditioned medium includes growth and soluble factors like nerve growth factor (NGF), brain-derived nerve factor (BDNF), insulin growth factor (IGF); interleukin 10 (IL-10), tumor necrosis factor (TNF); and basic fibroblast growth factor (bFGF), that involved in neurogenesis and neuroprotection. The secretome can influence the differentiation of cells that retain their pluripotent or multipotent characteristics. Growth and neurotrophic factors from CM-rat bone marrow MSCs, such as bFGF and NGF, enhanced the ability of neural stem/progenitor cells (NPCs) to differentiate into astrocytes (GFAP) and neurons (NeuN) (Budiariati et al., 2021; Rinendyaputri et al., 2018).

C. Important Aspects of the Book

The previous section provided a brief overview of stem cells. The chapter is presented in an organized structure that facilitates the understanding of stem cells and their use in regenerative therapy. To this end, we would bring out that this book, *Discovering the Miracles of Stem Cell*, will discuss an in-depth comprehensive overview of the current state of stem cell research, including recent advances, challenges, and future directions, and contribute to filling such gaps by seeking contributions from researchers and practitioners interested in investigating the latest advances and challenges in stem cell research and its clinical applications. In summary, this book explores a wide spectrum of stem cell-related subjects that proceed from bench to bedside.

In Chapter 2, Jeanne Adiwinata Pawitan discusses "Stem Cells Culture Techniques: Mesenchymal Stem Cells". In this chapter, Pawitan has developed easy methods for establishing a culture of MSCs from the umbilical cord, bone marrow, and adipose tissue. These techniques involve a simple washing step with a filter for coffee, repeated harvest explant method, and simultaneous mononuclear cells (MNCs) separation followed by centrifugation. Pawitan also explains how to upscale culture, cryopreservation of MSCs, and aseptic technic to prevent MSCs culture contamination.

Ismail Hadisoebroto Dilogo in Chapter 3 discusses "Stem Cells for Orthopaedics Application". Dilogo describes that stem cells have shown a great deal of promise for promoting the regeneration of tendons, muscles, bones, and cartilage as well as for starting healing processes and making up for impairments in orthopaedics and trauma surgery. Among the many benefits of stem cell therapy include decreased pain, improved mobility and function, and tissue regeneration. Stem cell therapy for orthopaedic conditions has a promising success rate. Before its use can be broadly applied, its drawbacks—such as high cost, a lack of standardization, and restricted availability—must be resolved. Thus, we recommend more investigation, the creation of standardized procedures, the integration of tissue engineering and its methodology, and an in-depth study of the metabolites derived from stem cells, such as exosomes and secretomes.

Next, Siufui Hendrawan, Jennifer Lheman, David Victorious Lukas, and Sukmawati Tansil Tan discuss "Mesenchymal Stem Cells Secretome for Diabetic Wound". This chapter will focus on the potential use of stem cells in treating diabetes, including the differentiation of stem cells into insulin-producing cells and the transplantation of these cells into patients. As a diabetes alternative medicine, this one has a lot of promise and potential. New approaches, such as the use of encapsulated stem cells and nanotechnology, are required to overcome the limits of stem cells and their secretome to be

employed in a wide range of illnesses. Furthermore, expedited clinical applications necessitate collaboration between all parties involved.

After that, Winawati Eka Putri, Cita Rosita Sigit Prakoeswa discuss "Mesenchymal Stem Cells and Its Conditioned Medium: for Skin Aging". Putri et al. describe the application of conditioned medium and MSCs as a treatment for skin aging, for example from bone marrow, amniotic fluid or amniotic membrane, chorion, umbilical cord, umbilical cord blood, and adipose tissue. Recent research has shown that MSCs and CM of MSCs are safe to use. The high cost of manufacture and lack of standard operating procedures for creating stem cell conditioned medium are the limitations. More research is required to determine the best way to produce and give MSCs to optimize their anti-aging effects on the skin.

Teguh Santoso, Idrus Alwi, Cynthia Retna Sartika, Cosphiadi Irawan, Dewi Wulandari, Ika Prasetya Wijaya, Eka Ginanjar, Elizabeth Merry Wintery, Mohamad Syahrir Azizi, Aw Tar Choon, Bayu Winata Putera, Yanni Dirgantara, Angliana, Ditta Kalyani Devi, Nadya Karina, Rima Haifa, Nabilla Farah Naura, and Billy Yosua Costantin Pongajow discuss "Stem Cells for Acute Myocardial Infarction: Safety and Efficacy". This chapter explores the role of stem cells in development and regeneration, their use in therapy, and challenges. Santoso et al describe several factors that influence the success of stem cell therapy. Several potential influencing factors for the outcomes of allogeneic umbilical cord MSCs therapy (UC-MSCs) for ST-segment elevation myocardial infarction (STEMI) have been effectively found by the case series. To reach deeper conclusions, more investigation is necessary. Despite the noted notable improvements, the study's weaknesses include a small sample size of only four patients, which makes it difficult to generalize the findings.

Somia Gul, Saba Majeed, and Aisha Aziz discuss about "Stem Cells Based Therapies for Neurological Disorders". This chapter covers the potential use of stem cells in treating neurological disorders. Neurodegenerative diseases have a concerning side effect profile

when treated with traditional pharmaceutical therapy. For individuals with neurodegenerative diseases, stem cell therapy is currently most likely the most effective and desirable method of treatment. Stem cells have now been thoroughly tested in clinical settings for the treatment of a variety of neurological diseases, including Parkinson's disease, Alzheimer's disease, and others, having been studied in vitro and animal models.

Mochamad Syaifudin, Wimpie Pangkahila, Ida Sri Iswari, I Gusti Kamasan Nyoman Arijana, Basuki Supartono, and Mochamad Wildan in the next chapter discuss "The Potential of Cd34+ Hematopoietic Stem Cells to Increase Fibroblast and Collagen Skin in Ultraviolet B Exposed Skin". Syaifudin et al. describe that UV radiation can cause cellular component damage and photoaging caused by UV radiation can result in severe skin damage. Male Wistar rats that exposed to ultraviolet B (UVB) radiation showed an increase in fibroblasts and collagen in their skin following subcutaneous injection of human peripheral blood CD34+ stem cells.

Ahmad Faried and Yulius Hermanto discuss "Induced Pluripotent Stem Cells (iPSCs) and Neurological Diseases". The author explains that iPSCs were created using reprogramming technology, which allows researchers to examine cell fate decision mechanisms and model diseases in humans. It has provided novel possibilities for stem cell research and unique candidates in the pharmaceutical and clinical areas. iPSCs have potential use in toxicology, drug development, pathology, regenerative medicine, and the evaluation of pharmacological side effects. New insights into the biology of diseases and the possibility of developing novel therapeutics will be provided by the modeling of neurodevelopmental and neurodegenerative diseases.

Dito Anurogo discusses "The Art of Ethical Dimensions in Stem Cell Research". This chapter explores the ethical and regulatory considerations associated with stem cell applications. Anorogo describes that the study of stem cells has tremendous medicinal potential, but it also presents difficult ethical challenges, including

ESCs use. The complex rules and patents that impact research and affordability are part of the legal environment. Collaboration between researchers, ethicists, patients, and the general public is necessary to ensure a responsible approach and it is backed by international collaborations and ethical boards.

Finally, in Chapter 11, Basuki Supartono discusses "Stem Cells Are a New Hope, a New Horizon for Humanity and the Future of Human Beings: Representing Indonesia to the World". Supartono explains an overview of the development of stem cells in Indonesia, the role of the government in stem cell applications in the country, the limitations on stem cell research in Indonesia, and recommendations and suggestions for improving stem cell therapy in Indonesia.

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Chapter 2

Stem Cell Culture Techniques: Mesenchymal Stem Cells

Jeanne Adiwinata Pawitan

A. Introduction

Cell therapy, especially stem cell therapy, has gained lots of attention worldwide, including in Indonesia. However, most cell therapies are not standardized, except the use of hematopoietic stem cells (HSCs) to repopulate the bone marrow after radiation or chemotherapy in hematological malignancies. For every condition, most centers have their own methods of cell production, preservation, and cell therapy application methods with regard of the source of cells, dose, route of administration, and how many repeats that are needed, as well as the intervals between the repeats (Yoneda et al., 2022).

As a developing country, Indonesia needs to be independent in health services, including in cell therapy services. As Indonesia

J. A. Pawitan

National Research and Innovation Agency, e-mail: ariy007@brin.go.id

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regulation prohibits the use of nonhuman cell sources, as well as embryonic stem cells, only adult stem cells can be used. Adult stem cells can be derived from various adult tissues, such as bone marrow, adipose tissue, dental pulp, hair bulb, and mobilized peripheral blood, as well as partition waste, such as umbilical cord tissue, umbilical cord blood, amnion or placenta (Sipp et al., 2018). Moreover, some studies have succeeded to isolate stem cells from menstrual discharge (Sipp et al., 2018), urine (Fu et al., 2014), or milk (Kersin & Ozek, 2021).

As for cell therapy, usually lots of stem cells are needed. While cell extraction from tissues is usually limited in number, multiple donors are needed when allogeneic cell is intended to be used, or the cells should be expanded in vitro by culturing, which also apply when autologous cells will be used. The type of adult stem cell that is easy to be expanded in vitro is mesenchymal stem cell (MSCs). In order to be independent in cell therapy for various conditions, Pawitan et al. (2013, 2014, 2014-2015) began to isolate MSCs from various tissues, and succeeded to develop methods for easy, and economical MSCs production using an in house developed domestic derived supplement. Pawitan et al. (2013, 2014, 2014-2015) methods to produce MSCs from bone marrow, adipose, and umbilical cord tissue have been applied in a current Good Manufacturing Practices (cGMP) laboratory, and the cells have been used in various animal studies, and clinical trials. In this occasion, Pawitan et al. (2013, 2014, 2014-2015) would like to share their methods of MSCs isolation, propagation, and cryopreservation, which were derived from three kinds of tissues, bone marrow, adipose, and umbilical cord tissue. Therefore, the author will address the MSCs, sample collection and transportation, isolation and culture of MSCs from bone marrow, adipose tissue and umbilical cord tissue, upscale culture of MSCs, preservation of the cells by cryopreservation, and the importance of aseptic technic to prevent culture contamination.

B. Mesenchymal Stem Cell (MSC) from Bone Marrow, Adipose, and Umbilical Cord Tissue

The term mesenchymal stem cell (MSC) was first used in 1991 by Arnold Caplan, who is a US biologist, to describe a cell type, which was derived from bone marrow that could differentiate into various types of tissues of mesenchymal origin. After that, MSC can be isolated from various adult tissues. They are similar but have subtle differences in their potential to differentiate and their other potencies. Moreover, different laboratories might use different surface markers for MSC characterization, and there were ambiguities and inconsistencies in MSC potencies. Therefore, in 2006, International Society for Cell Therapy (ISCT) proposed the term multi-potent 'mesenchymal stromal cell'. According to ISCT, MSCs should fulfill a certain criteria, i.e., they are fibroblastic in morphology, adherent to plastic vessel where they are cultured, have specific surface markers (CD 90, CD73, and CD105 should be ≥95%, and lineage negative CD should be ≤2%), and should be able to differentiate into three lineages namely osteogenic, chondrogenic, and adipogenic lineages. MSCs from bone marrow, adipose, and umbilical cord tissue, all can differentiate into the three lineages. However, the lipid droplets of MSCs from umbilical cord tissue, when they are differentiated into adipocytes, are much smaller compared to those from bone marrow or adipose tissue. Further, in 2017, Arnold Caplan, who at that time no longer believed that MSCs were stem cells, proposed 'medicinal signaling cell' as abbreviation of MSC. However, many studies still refer MSC as 'mesenchymal stem cells' (Sipp et al., 2018).

C. Sample Collection and Transportation

Samples of various tissues that will be used for MSC production should be collected by aseptic technic according to current Good Tissue Practice (cGTP), from individuals who are devoid of infectious

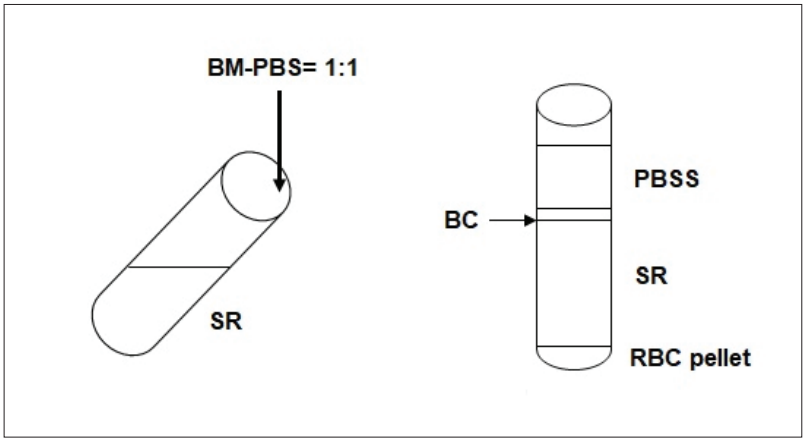
and hereditary diseases and have signed the informed consent form to donate their tissue. Therefore, the donor should be checked for the presence of immunoglobulin G (IgG) and M (IgM) of human immunodeficiency virus 1 and 2 (HIV1-2), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and toxoplasma, rubella, cytomegalo, herpes (TORCH) viruses. In addition, anamnesis of donor pedigree should be carried out to exclude hereditary diseases (Yoneda et al., 2022).

After collection, sample should be placed in a transport medium and kept in a refrigerator at 4°C before and after transportation, and should be process before 24 hours after collection. Transport medium is basal medium such as alpha minimum essential medium (alpha MEM) or Dulbecco's modified Eagle medium (DMEM) that contain antibiotics and antimycotic three times of the usual dose (Pawitan et al., 2014).

D. Isolation and Culture of MSCs from Bone Marrow

The majority of stem cells in bone marrow are HSCs, which should be matching between donor and recipient. Otherwise, the transplanted HSCs will be rejected by the recipient, or when given to repopulate the host' bone marrow, the transplanted cells can regarded recipient's tissue/organ as foreign that can be dangerous for the recipient. Besides HSCs, bone marrow also contains a few MSCs that are found in the mononuclear cell fraction, together with the HSCs (Lucas, 2021).

Isolation of MSCs from bone marrow can be done by either isolating mononuclear cells (MNCs) using a separation medium (Gudleviciene et al., 2014) or directly separating the pellet after centrifugation, which is followed by culture to expand the MSCs (Pawitan et al., 2014-2015). When separation reagent such as Ficoll Hypaque or its equivalent such as Lymphoprep™ is used to isolate the MNCs, bone marrow is mixed with an equal volume of phosphate buffered saline (PBS), and the mixture is dropped carefully onto



Notes: BM: bone marrow;
PBS: phosphate buffered saline;
SR: separation reagent;
PBSS: PBS – serum;
BC: buffy coat;
RBC: red blood cells.

Figure 2.1 Separation of MNCs Using a Separation Reagent

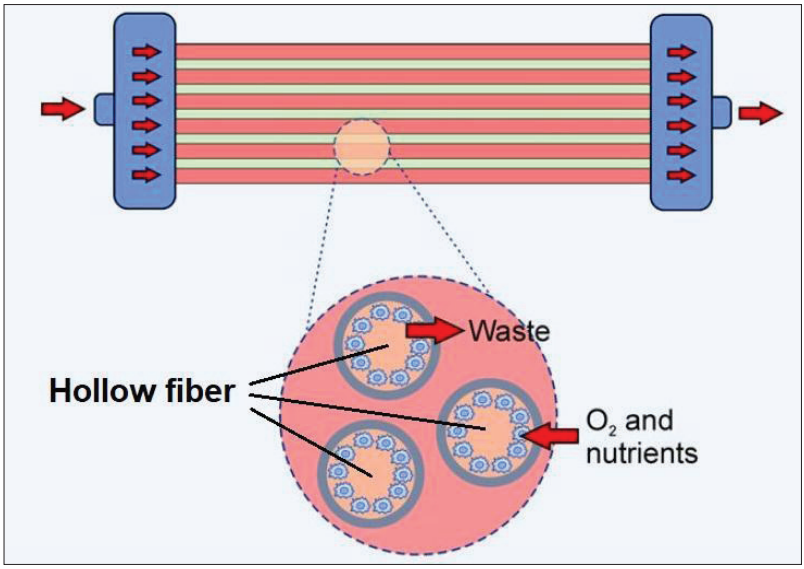
an equal volume of separation reagent, so that the bone marrow mixture does not mix with the separation reagent, but floating. After centrifugation, red blood cells will be pelleted at the bottom, at the upper part of red blood cells is the separation reagent, then the MNCs that form a buffy coat that can be visualized as a white ring, and the uppermost is the PBS and serum (Gudleviciene et al., 2014) (Figure 2.1).

The white ring is carefully taken, and then is washed with PBS, and centrifuged to be pelleted. The pellet is reconstituted with special complete medium for MSCs and then cultured (Gudleviciene et al., 2014).

For direct separation of MNCs, Pawitan et al. (2014-2015) have developed a simple method to culture bone marrow-derived MSCs

(BM-MSCs) without the use of Ficoll Hypaque. In brief, 5–6 ml of bone marrow is mixed with an equal amount of complete MSC medium. The complete medium is alpha MEM that is supplemented with in-house prepared platelet lysate and glutamax. The mixture is centrifuges and pellet that contains the buffy coat together with red blood cells is separated from the supernatant, and transferred into a T25 flask, then a same amount of MSC complete medium is added and cultured. An alternative to culturing whole pellet is to culture only the buffy coat and upper part of erythrocyte pellet. The supernatant is homogenized, and 1 ml is taken and placed in a 12 well plate and cultured. The supernatant is observed beginning day 3 to see cell attachment. Observation of supernatant culture is used to determine the time of washing the pellet culture, as the pellet culture is full of erythrocytes that makes impossible to see MSC attachment, while the supernatant only contains a few MNCs and erythrocytes, so it is readily visible when the MSCs are attached. After the MSCs in the supernatant culture are attached, the pellet culture is washed gently to remove erythrocytes and unattached non-MSCs, then 5–10 ml fresh complete MSC medium is added, and culture is resumed. Further, medium change is done every 3 to 4 days, until enough colonies appear, and the MSCs can be harvested (Pawitan et al., 2014-2015).

Harvesting is done by detaching the cells using an enzyme, which is TrypLE Select, that is milder compared to Trypsin. Before adding TrypLE Select, the cells should be washed gently and thoroughly by PBS, as platelet lysate in the complete medium has anti-enzyme activity. After washing, 1 ml of TrypLE select is added and put in the incubator for five minutes. After five minutes, the flask is observed to see whether detachment occurs, which is visible as rounded shining cells that are floating in the enzyme solution. When the cells are rounded, but still attached to the bottom, the bottom of the flask is tapped gently, or the flask is banged gently to a hard surface. When not all cells are detached, the flask is put in the incubator for another 5 minutes, until all cells are detached. After all cells are detached,



Source: Jankovic et al. (2023)

Figure 2.2 Quantum Bioreactor

cell suspension in TrypLE Select is transferred to a tube, and an equal amount of complete medium is added to stop the enzyme. The mixture can be directly cultured to a new flask or washed by PBS, reconstituted in complete medium and cultured to be passage-1. Before culturing, the amount of cells needs to be counted, and culturing is done by seeding 5000 viable cells/cm^2 of vessel surface area (Pawitan et al., 2014-2015).

Another method for direct separation and culture of MSCs may use a Quantum bioreactor that contains hollow fibers (Figure 2.2). Before culture, the hollow fibers' outer surface is coated overnight at 37°C with human fibronectin (huFN) or other reagents, such as gelatin, collagen, human serum albumin, poly-L-lysine, pooled human cryoprecipitate (PHCP), or human vitronectin (huVN) as a cell attachment factor. After coating, 12.5–25 ml of whole bone marrow that is mixed with heparin and complete medium until the volume reaches 100 ml is loaded in the Quantum bioreactor and

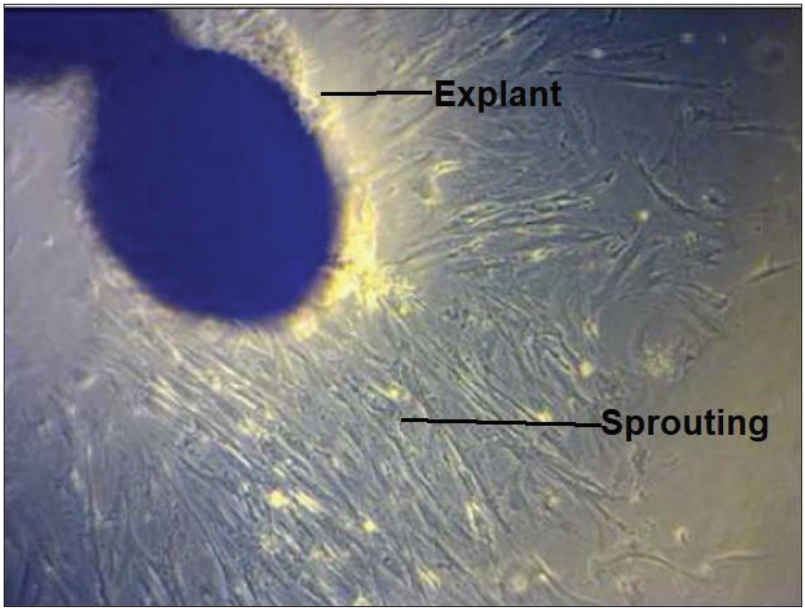
fresh complete MSC medium continuous flow is maintained at the outer and inner space of the hollow fibers, MSCs attached to coated hollow fibers, while other cells are flushed by the continuous medium flow. Comparison between coating reagents showed that coating with huFN, PHPC, or huVN, yielded similar results that were much better than other coating reagents (Frank et al., 2019).

E. Isolation and Culture of MSCs from Adipose Tissue

In adipose tissue, MSCs are found in the vicinity of blood vessels. Isolation of MSCs from adipose tissue can be done by explants' method (Li et al., 2018) or after extraction of cells by either enzymatic (Pawitan et al., 2013) or mechanical method (Gentile et al., 2019).

1. Isolation and Culture of MSCs from Adipose Tissue by Explant Method

In explant method, adipose tissue is minced into small fragments, and put on plastic vessels, such as petri disks, multi-wells, or flasks. As adipose tissue tends to float, explants' method needs a special care to prevent the tissue from floating, either by placing a cover slip on the explants or by putting the explants at the upside of a flask, while giving medium at the bottom to prevent drying until the explants attached. After the explants are attached, the flask is reversed, so that the upside becomes the bottom. The act of reversion should be done very carefully to prevent detachment of the tissues. When using cover slips, care should be taken to avoid air bubble formation. Another method is by putting the explant at the bottom and giving a just enough medium to wet the explants and put the vessel in an incubator until the explants are attached, before carefully giving the usual amount of medium to prevent detachment of the explants. Care should be taken to prevent floating of explants, because cells will only sprout out of the explants if the explants are attached to plastics (Figure 2.3) (Li et al., 2018).



Source: Pawitan et al. (2014)

Figure 2.3 Plastic vessel attached explant culture facilitates "sprouting".

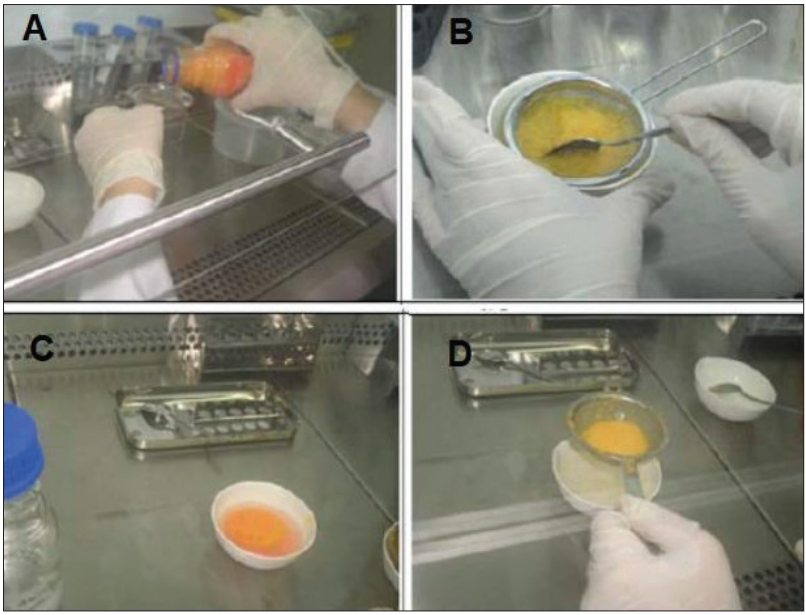
2. Isolation and Culture of MSCs from Adipose Tissue After Extraction of Cells

As isolation by explant method needs special expertise to prevent floating and make the adipose tissue attach to the plastic vessel, cultures after extraction of cells are preferable. Extraction of cells to separate the cells from extracellular matrix can be done by mechanical or enzymatic methods. In mechanical method, the tissue is minced into small pieces, put into erythrocyte lysis solution, followed by agitation by vortex, or other mechanical methods, such as using ultrasonic cavitation device, ultrasound, combination of centrifugation and filtration, or using various mechanical devices. Studies showed that this method yielded enough cells that was comparable to enzymatic method (Gentile et al., 2019). However, another study

showed that enzymatic method gave a lot more cells compared to mechanical method (Aronowitz et al., 2015), which was proven by Pawitan et al. (2013) own experience. Therefore, extraction of cells by enzymatic method is preferable to mechanical method though it is more expensive, as an appropriate enzyme to detach the cells from the tissue matrix is needed. When compared to explant or mixed (enzymatic-explant) method, enzymatic method is preferable, as fat tissue tends to float that leads to explant or mixed culture failure.

In enzymatic method, when the adipose tissue is derived from surgical incision procedure, the tissue should be minced to small pieces, before subjected to enzyme treatment. However, when the tissue is derived from liposuction and already in the form of small pieces, it needs to be washed thoroughly to remove liposuction solution, which is toxic to cells. Moreover, washing step is important to remove red blood cells and free lipids from damaged fat cells that may interfere with cell attachment. Pawitan et al. (2013) found a faster, simpler, and washing solution saver method to wash lipoaspirate by using a coffee filter (Figure 2.4), compared by a previous method, which used extensive washing by repeated centrifugation steps (Zuk et al., 2001), to wash the lipoaspirate until it is really cleaned from contaminants.

To extract cells from adipose tissue, various enzymes and enzyme combinations can be used, such as trypsin, TrypLE Select or collagenase type 1, which can digest adipose tissue matrix. In Pawitan et al. (2013)'s laboratory, they followed a previous method with modification (Zuk et al., 2001), by digesting the lipoaspirate using 0.75% collagenase type 1 in PBS with a lipoaspirate: collagenase solution ratio is 1:2, for one hour at 37°C with manual mixing every five minutes until the tissue become oily and bright yellow in color, which show that the digestion is complete. If the color is still pale yellow, then digestion time needs to be prolonged until the color become bright yellow and oily (Pawitan et al., 2013). After digestion, adipose tissue remnants are discarded, and the infranatant is filtered

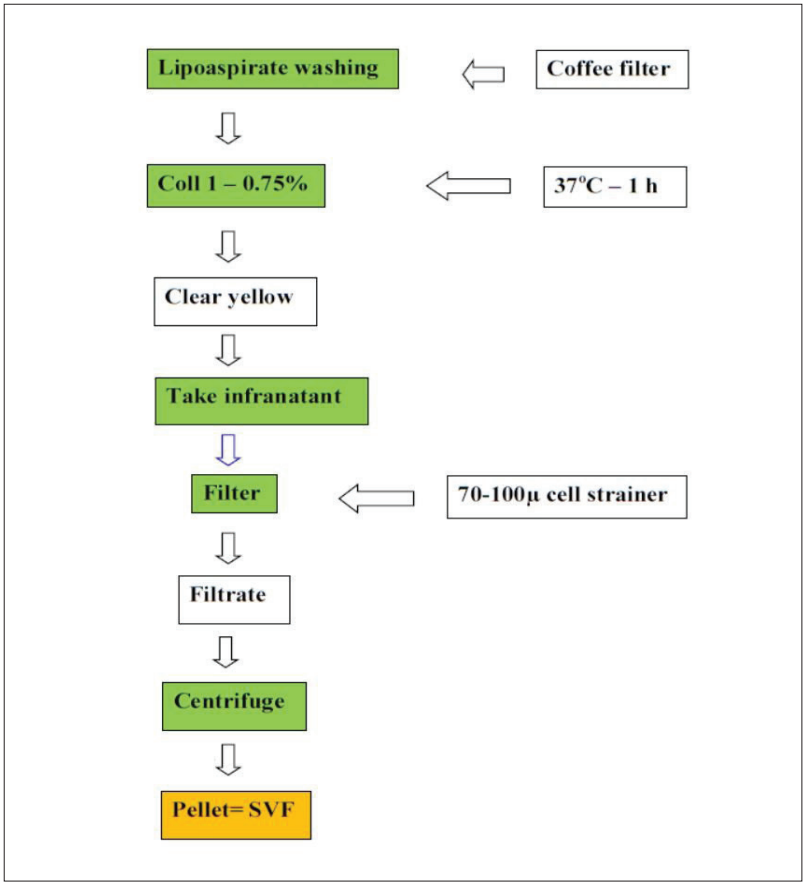


Notes: (A) Lipoaspirate is poured in a sterile stainless steel coffee filter and the fluid was collected in a glass beaker;
(B) Lipoaspirate washing: the lipoaspirate containing coffee filter is soaked in a phosphate buffered saline containing porcelain bowl;
(C) Contaminants and free lipids are left in the bowl;
(D) Clean lipoaspirate.

Source: Pawitan et al. (2013)

Figure 2.4 Simple Lipoaspirate Washing Using a Coffee Filter

using a 70 or 100µ cell strainer, centrifuged, and the pellet is washed using PBS. If the pellet appears red, then it should be subjected to erythrocyte lysis buffer for 10 minutes at room temperature and washed by PBS, followed by centrifugation to get the pellet. The pellet, which contains myriads of cells including MSCs, is called processed lipoaspirate (PLA) or stromal vascular fraction (SVF), as MSCs are found near blood vessels, and some researchers regarded these cells as derivative of pericytes. The SVF pellet is re-suspended in complete medium (Figure 2.5).



Notes: Coll 1: collagenase 1;
1 h: one hour;
µ: micrometer;
SVF: stromal vascular fraction.

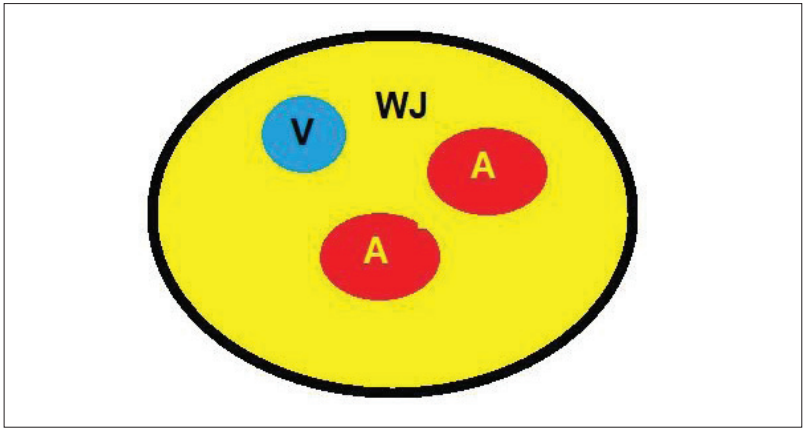
Figure 2.5 Flowchart of the Isolation of Adipose Tissue Derived Stromal Vascular Fraction

Complete medium that is used for culture should be xenofree if the cells are intended for the patient. There are various xenofree commercial media, each sold with their respective supplement, such as MesenCult ™ Basal Medium (human), and Stem Pro culture medium.

However, in Pawitan et al. (2013)'s laboratory, they use Alpha MEM that is supplemented by 10% in house prepared platelet lysate and glutamax. Initial seeding is 170,000 viable nucleated cells or more in a twelve well plate or 42,500 viable nucleated cells/cm^2. Less seeding density may delay the culture to become confluent, while more seeding density may lead to faster confluency. Harvest can be done after 80%–90% confluent. Care should be taken to prevent 100% or more, as harvest after 100% or more may cause senescence of the MSCs. After harvest, the cells can be passaged with a seeding density of 5,000 viable cells/cm^2.

F. Isolation of MSCs from Umbilical Cord Tissue

In umbilical cord tissue MSCs are mainly found in Wharton's jelly (Figure 2.6). Isolation of MSCs from umbilical cord tissue can be done by either explants or enzymatic method. In isolation of MSCs from Wharton's jelly umbilical cord tissue by explant method, care should be taken so that the Wharton's jelly is facing downward and not vice versa. On the other hand, isolation by enzymatic method should



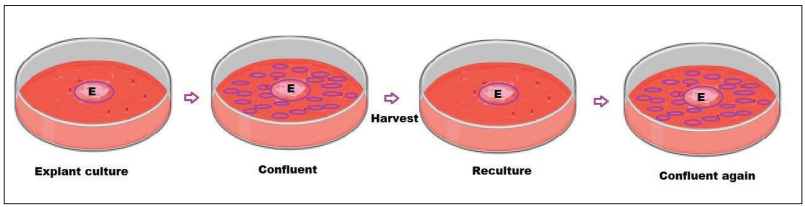
Notes: A: Artery;
V:Vein;
WJ: Wharton's jelly.

Figure 2.6 Schematic Picture of Umbilical Cord

pay attention on the extracellular matrix (ECM) that surrounds the MSCs. Knowledge of the ECM is crucial for selecting the enzymes that are needed to digest the ECM to free the MSCs from the ECM (Varaa et al., 2019).

1. Isolation of MSCs from Umbilical Cord Tissue Using Explant Method

For explant method, Pawitan et al. (2014) have developed multiple harvest explant method (Figure 2.7), as the explant can be re-cultured for several times after the first harvest.



Notes: E: Explant

Figure 2.7 Schematic Picture of Multiple-Harvest Explants Method

Before processing the umbilical cord, the cord tissue should be decontaminated, especially when it is derived from a normal delivery, as the umbilical cord is contaminated with vaginal flora. Around 5–10 cm umbilical cord tissue is washed briefly in 0.5% povidone iodine (betadine) containing PBS pH 7.4, followed by washed in PBS twice. Both ends are discarded and the umbilical cord is cut into several pieces, about 1 cm long. The umbilical veins and artery are pulled out and the umbilical cord is dissected. The tissue can be either directly minced into small pieces around 2–5 mm in with and length, or thinly sliced to remove the outer glistening part (amnion) and then minced into small pieces. Mincing of the umbilical cord tissue is done while wetting the tissue with complete medium to prevent the tissue from drying. Explant culture can be done in 24 or 12 well plates or T25 flasks. For 24 or 12 well plates, one, two, to three pieces are placed in the well, respectively. For T25 flask, many pieces are placed in the flask

and arranged at a distance from each other. The plates and flasks are put in an incubator at 37°C for 1 hour. After the explants are attached, a few complete MSC medium is given cautiously, to prevent floating of the pieces, and the plates and flasks are put back in the incubator. When using direct mincing method, care should be taken that the Wharton's jelly is facing downward, otherwise sprouting of cells will not occur. However, when the amnion is removed before mincing, both surfaces may face downward. The culture needs to be observed daily to detect contamination, the need to add complete MSC medium due to drying or sprouting. After sprouting, usual amount of medium is added and the medium is changed every 3 to 4 days, until 80%–90% confluent. After the culture is confluent, the cells can be harvested and passaged. After harvesting, the explant can be re-cultured followed by several times harvests to yield more cells (Pawitan et al., 2014).

As alternative to Alpha MEM, Budiyanti et al. (2015)'s result in culturing umbilical cord derived MSCs showed that DMEM low glucose or high glucose (DMEM-LG or HG) and a commercial medium MesenCult showed lower performance compared to Alpha MEM.

2. Isolation of MSCs from Umbilical Cord Tissue Using Enzymatic Method

Enzymatic method aims to extract the MSCs from the tissue, which differs from explant method that directly culture tissue pieces without cell extraction. For enzymatic method, after decontamination and mincing of umbilical cord tissue, the tissue fragments are subjected to an enzyme, i.e., 0.1% collagenase for 20 hours at 37°C, followed by filtration using a 100 µm cell strainer to remove tissue remnants. The cell suspension that passes through the strainer is centrifuged; the pellet is washed by PBS twice and the pellet is resuspended in complete MSC medium and cultured (Chen et al., 2016). However, when the author followed Chen et al.'s method, after collagenase digestion, digestion result was a very sticky mucous that could not pass through the strainer (Pawitan et al., 2011). This failure is due to the fact that

Wharton's jelly ECM is composed of collagen and hyaluronic acid, and the hyaluronic acid cannot be digested by collagenase alone. Another method that was more reliable used an enzyme cocktail, which consists of collagenase, hyaluronidase, and trypsin for 45 minutes to two hours at 37°C. After digestion with the enzyme cocktail, the resulting digest was a liquid that could be filtered using a cell strainer. Further, the cells that passed the cell strainer were cultured to yield MSCs (Varaa et al., 2019). When enzymatic is compared to explant method, it is much more expensive as it needs various enzymes to be successful, and initial processing is more laborious so that it takes longer time, but the MSCs grow faster to become confluent, so the MSCs can be harvested faster in primary culture (Varaa et al., 2019). However, explant method is preferable as it is much cheaper, simpler in initial processing, and after primary culture, re-culture takes similar time with enzymatic method (Pawitan et al., 2014).

a. Upscale Culture of MSCs

Initial MSC culture is usually done using T25 flasks. To expand the MSCs, larger vessels can be used, such as T75, T150, T175, and T225 flasks. Since patient may need a lot of cells, to get enough cells for patients, many large flasks can be used that may occupy the whole incubator and be time consuming and labor intensive. To address this issue, hollow fiber system (Frank et al., 2019), multiple flasks (Jankovic et al., 2023), hyperflask (Kearney et al., 2017) and cell factory (Rout-Pitt et al., 2018), both of which have ten layers and micro-carrier-bioreactor system, are developed (Nurhayati et al., 2021).

Two replications of whole bone marrow expansion using Quantum bioreactor, which contained huFN coated hollow fibers, with fresh circulating complete MSC medium in inner and outer space of the hollow fibers showed that the MSC yield were 1.7×10^8 and 5.5×10^7 cells respectively (Frank et al., 2019).

A Beckton-Dickinson (BD) multiple flask with T175 dimension can be found as a Triple flask that has three layers, or a Multi flask that has five layers, with a growth surface area of 525 and 875 cm^2,

respectively or more. These Triple- and Multi flasks are designed to allow easy pipetting to add reagents or to recover detached MSCs, as well as minimal residual liquid retention when pouring technique is preferred over pipetting (Jankovic et al., 2023).

Corning Hyperflask® is a multi-layer flask with a T175 flask dimension, which walls in between layers are in the form of gas permeable membranes. These membranes provide gas and culture media exchange between layers, which harbor the cells. The total area is 1720 cm^2 or around ten times that of a T175 flask area. Each Hyperflask may contain a total of 560–565 ml media without air space. The total media volume is equivalent to eleven T175 flasks. The Hyperflask can be stacked to save place (Kearney et al., 2017).

NUNC™ EasyFill™ Cell Factory™, which is developed by Thermo Fisher Scientific, consists of ten layers. The whole surface area of a Cell Factory is equivalent to 36 pieces of T175 flasks, while the whole dimension is much less than the area that is occupied by 36 pieces of T175 flasks (Rout-Pitt et al., 2018).

For attached cells such as MSCs, culture in a spinner bioreactor needs Collagen-coated micro-carrier beads for cell attachment. Nurhayati et al. (2021) compared flask with spinner bioreactor culture in two experiments using different umbilical cord, and platelet lysate donors. In spinner bioreactor culture, the cells were seeded by mixing 14×10^3 cells/cm^2 with two grams of micro-carrier beads in 50 ml culture media, initially without stirring to facilitate cell attachment. After 16 hours, the medium was added to become 100 ml, and stirring was set at 50 rpm. The spinner flask was put in a fully humidified CO_2 incubator at 37°C. Harvesting of cells from a spinner bioreactor was done using TrypLE select to detach the cells, followed by filtration using cell strainers to separate the cells from the micro-carrier beads. Their result showed that the population doubling time (PDT) of experiment-1 were 12.3 (flask) and 14.8 hours (spinner bioreactor), whereas in experiment-2, they were 17.7 (flask) and 16.9 hours (spinner bioreactor), which were comparable (Nurhayati et al., 2021).

Up-scaling using larger spinner bioreactor may need cell strainer with larger diameter to speed up separation of cells from the micro-carrier.

b. Cryopreservation of Mesenchymal Stem Cells

When MSCs are used for therapy in patient, they should be used within 24 hours, as Krishnanda et al. (2017) and Nofianti et al. (2018) showed that the quality of MSCs was reduced when they were kept for longer time. When harvested cells are not used, they should be stored in a cryopreservation medium, frozen, and kept in a cryo-tank at a temperature of -196^{o}C. In freezing the cells, a decrease in temperature should occur slowly, i.e., 1^{o}C per minute, which can be done in either a controlled rate freezer, or in a device that is called Mr. Frosty, to avoid the formation of ice crystal that is harmful to cells. However, when the cells are to be used, thawing should be done fast, by putting the cryo-vial in a water bath of 37^{o}C. As for cryopreservation medium, there are various cryopreservation media that might differ in type of reagents and their concentrations. Most cryopreservation media use 10% dimethyl sulfoxide (DMSO), but other reagents might differ, such as the use of fetal bovine serum (FBS) that is a xenomaterial, which might contain nonhuman sialic acid Neu5Gc that can be internalized into cryopreserved MSCs (Nasonkin & Koliatsos, 2006), and thus elicit immune response (Villacrés et al., 2021). Therefore, Goei et al. (2015) studied the use of platelet lysate to replace the FBS and found that platelet lysate usage in cryopreservation medium showed better performance after cryopreservation in proliferation rate and cell size, which is a surrogate for senescence. Goei et al. (2015)'s in house cryopreservation medium, which was composed of 10% DMSO and 10% platelet lysate in alpha MEM, was used to cryopreserved Passage-1 umbilical cord derived MSCs, and the results showed that passaging of the cryopreserved cells until Passage-8 with cumulative population doublings (CPD) of more than 34.34 showed less than 5% senescence (Pawitan et al.,2017). Further, the use of Goei et al. (2015)'s cryopreservation medium in adipose derived (AD)- MSCs and BM-MSCs showed that AD-MSCs showed better performance

after cryopreservation, in term of senescence and population doubling time, which is a surrogate of proliferation rate (Ismail et al., 2018).

c. Aseptic Technic to Prevent Culture Contamination

MSCs are easy to be isolated and propagated by culturing from bone marrow, adipose tissue, or umbilical cord, as long as the laboratory officer can keep them in sterile condition. To keep the culture in sterile condition, care should be taken to avoid contamination by bacteria, yeast, fungi, or other microorganism that are difficult to detect, such as mycoplasma, protozoa, viruses, and other cell lines (Biocompare, 2022). Avoiding contamination can be done by using sterile tools, equipments, and all needed culture materials. In addition, every step from isolation, culture, and harvest should be done by aseptic technique (Biocompare, 2022). When operators are not sure of their aseptic technique, antibiotic and antimycotic can be used, but they are only for contamination prevention measures, and cannot replace sterilization and aseptic technique.

1) Sterilization

Before every step, preparation of sterile items should be done one day before. In preparation, tools, vessels, reagents and media need to be prepared and sterilized, or purchased, and large equipment, such as biosafety cabinet and incubator, should be checked whether they work well or not (Pawitan et al., 2012).

Sterilization can be done by heat for heat resistant things, by irradiation using gamma or UV rays for plastics that cannot be autoclaved, or by filtration for non-heat resistant liquids. Sterilization by heat can be done either by dry heat in an oven at 150 to 170°C, for very heat resistant things, such as metal tools, or Pyrex glass wares, or by autoclave at 115 to 121°C and 8 kg or one atmosphere pressure for low heat resistant things, such as blue capped Schott bottles, or heat resistant solutions, such as PBS (Biocompare, 2022).

For things that are purchased in sterile condition, such as large volume of media, regents, and supplements, attention should be paid,

when the volume be only a little left, as they might be contaminated from the frequent taking. Therefore, aliquots need to be done for large volumes, and the aliquot is used for taking. When aliquot has not been done, filtration might be needed for the little left over. In addition, sterile plastic wares, such as flasks, petri dishes, 5 or 15 or 50 ml tubes, are usually not individually packed; flask and petri dishes may contain 10 to 20 per pack, and tubes may contain 25 to 50 per pack. These packs should be opened in the BSC, and directly closed after the needed amount is taken. When a little number is left in the pack, they might not be sterile anymore. Therefore, the left-over need to be sterilized by UV irradiation for a minimal of 15 minutes, together with other things that need to be UV irradiated before the work begin (Pawitan et al., 2012).

Therefore, for every step, knowledge of type and number/amount of tools, vessels, reagents and media that are needed is very important. For a beginner, the number should be doubled or tripled, as everything that has been in contact with non-sterile things should not be used. Unsterile things can be the wall or the floor of incubator, outer surface of reagent or waste bottles, beaker glass, operator hands, etc (Pawitan et al., 2011).

Small metal tools, such as tweezers, scalpels, or scissors, should be placed in a metal box with clips to prevent accidental opening. Before placing them in the box, the end of the tool that will come into contact with specimen or cells to be cultures should be wrapped in aluminum foil and placed regularly in the box. The sterile end of the tool is placed at one side, while the other end that will be held by the hand is placed at the opposite side of the box. Small things, such as Eppendorf tube or cryovials should be placed in a heat resistant pouch and sealed by a special tape. As an alternative of heat resistant pouch, a laboratory officer can use autoclave resistant bottles with metal cap, such as used bottles of jam, spaghetti, or barbeque sauce. The metal box, small thing containing pouch or bottles may be opened only inside a BSC (Pawitan et al., 2012).

2) Aseptic Technique

Every isolation and culture related step should be done by aseptic technique, i.e., all works should be done in a BSC and before usage the UV lamp should be on for a minimum of 15 minutes, with all things that need to be UV irradiated inside. Operators should use culture attributes, which are laboratory coat, hair cap, mask, and gloves (Biocompare, 2022). Before working, the BSC table should be wiped with disinfectant, disinfectant spray should be sprayed on all things that will be put inside the BSC, and during work disinfectant spray should often be used to disinfect the gloves (Pawitan et al., 2012).

When working, the BSC should be on, so that sterile air wall from the BSC can protect the culture, as air from outside BSC cannot come into the BSC, and air from inside BSC cannot go outside, which protect the operator, in case the sample that is handled is infectious. Things should be placed in the BSC according to operator convenience; there is no strict rule on how to place things in the BSC, as every person has their own convenience that might be different between persons. To make the culture work easy, usually things that are not readily used are put far from the operator, at the back of the BSC, and things that are in use are put in front in the BSC, near the operator. However, one thing that should be kept in mind is that the row of holes at the front and back of BSC should be free of things, otherwise the air wall will be disturbed and there will be holes in the air wall, so that air from outside can move inside and vice versa, which may cause contamination of the culture (Pawitan et al., 2012).

The most important things that should be kept in mind are that operators must always be aware that they should never touch unsterile things to specimen, medium, or whatever that is related to the culture, and never pass unsterile things on specimen, medium, or whatever that is related to the culture that is in open condition. Unsterile things include tools that have been fallen to BSC table, have touched BSC wall, or the outside of a medium or reagent bottle or tube

or waste vessel or gloves or whatever that is unsterile, such as pipettor, micropipette, racks, outside of boxes, etc (Pawitan et al., 2012).

3) Antibiotic and Antimycotic

Antibiotic and antimycotic that are used should be those that are special for tissue culture, the final concentration in culture media can be seen in Table 2.1. Concentration of antibiotic and antimycotic for tissue/cell culture is different from for drugs and can differ from brand to brand. Therefore, care should be taken when counting the amount that is needed to make a final concentration. For instance, penicillin is usually sold as combination penicillin 10,000U/ml, and streptomycin 10,000µg/ml, so dilution to get a final concentration is 1:100 (Pawitan, et al., 2012).

Table 2.1 Final Concentration of Various Antibiotics and Antimycotics

Reagent	Function	Final Concentration
Penicillin	Antibiotic	100U/ml
Streptomycin	Antibiotic	100µg/ml
Kanamycin	Antibiotic	100µg/ml
Gentamycin	Antibiotic	50µg/ml
Polymyxin B sulphate	Antibiotic	100U/ml
Nystatin (mycostatin)	Antimycotic	50µg/ml
Amphotericin B (fungizon)	Antimycotic	0.25 – 2.5µg/ml

Notes: U: unit;
µg: microgram;
ml: milliliter.

G. Concluding Remark

Pawitan et al. (2013, 2014, 2014-2015) have developed simple methods for isolation and culture of BM-MSCs, AD-MSCs, and umbilical cord derived MSCs using direct MNC separation after centrifugation, simple washing step using a coffee filter, and multiple harvest explant method, respectively. In addition, a xeno-free cryopreservation medium used in house processed platelet lysate was developed. For

the success of isolation and culture of MSCs from various tissues, mastering an aseptic technique is very important.

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Chapter 3

Stem Cells for Orthopaedics Application

Ismail Hadisoebroto Dilogo

A. Introduction

Over the past decade, fundamental science and experimental studies concerning stem cells have expanded. The use of stem cells in orthopaedics is expanding in line with the rise of basic scientific research. Stem cells in orthopaedic cases are transforming orthopaedic practices towards regenerative medicine, beginning with the treatment of bone abnormalities and the regeneration of nerves, tendons, ligaments, and cartilage (Maniar et al., 2015). Stem cells are cells with the ability to divide, self-renew, and differentiate into several types of body cells. On the basis of their differentiation potential, stem cells are classified as totipotent, pluripotent, or multipotent. Totipotent stem cells are capable of differentiating into any type of human cell, including placental and extraembryonic cells. Only embryonic stem

I. H. Dilogo

Universitas Indonesia, e-mail: ismailortho@gmail.com

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cells can produce totipotent stem cells. However, the use of totipotent stem cells in clinical practice has not been established due to medical ethics problems. Endoderm, mesoderm, and ectoderm are the three embryonic layers that pluripotent cells can transform into. Precisely, pluripotent stem cells originate from embryonic cells after the blastocyst phase. Multipotent stem cells, unlike their predecessors, can differentiate into more than one germ layer, but not all three. Adult stem cells and blood stem cells are examples of mesenchymal stem cells (MSCs). There are bones, cartilage, muscles, and ligaments involved in orthopaedics. MSCs have the ability to differentiate into mesoderm-derived tissues. Therefore, the use of mesenchymal stem cells is one of the most common in orthopaedic disorders (Im, 2017).

According to study done by Kabat et al. in 2018, the bulk of clinical studies employing MSCs were undertaken by the Neurology Department. In clinical studies involving mesenchymal stem cells, orthopaedics comes in second place. The concept of tissue engineering, especially in orthopaedics, adheres to the 5 R principles: repair, replace, restore, regenerate, and rejuvenate. MSCs can be either autologous or allogeneic, with its own advantages and disadvantages. Allogenic MSCs sources have advantages, including being more ready to use and not causing donor-site morbidity. Allogenic MSCs have also been proven safe to use in clinical trials since it has low HLA-II expression and triggers minimal immune reactions. In addition, allogeneic MSCs also do not trigger lymphocyte proliferation and modulate the immune system. The bulk of clinical studies employing MSCs were undertaken by the Neurology Department, according to study published in 2018 by Kabat et al. In terms of clinical studies utilizing MSCs, orthopaedics is ranked second (Gerth et al., 2019).

B. Stem Cells for Critical-Sized Bone Defects

Fracture is one of the most common cases encountered in orthopaedics and traumatology. Unlike other organs, bones can heal perfectly as before a fracture occurs (Santolini et al., 2015). The fracture healing process is depended on the diamond concept, which Giannoudis first

introduced. The diamond concept states that for a fracture to achieve optimal healing, four pillars must be addressed: osteogenic cells, osteoconductive, osteoinductive, and mechanical stability (Giannoudis et al., 2007). The osteogenic column is comprised of osteoprogenitor cells from the periosteum and endogenous MSCs from the bone marrow. Shortly after the fracture, the creation of a hematoma in the region around the fracture activates these cells. The production of cytokines initiates an inflammatory phase marked by increased vascularity and permeability of the blood vessels. Osteoclasts and fibroblasts transform the hematoma into granulation tissue and lay down a fibrin meshwork, which is eventually penetrated by vascular capillaries after the migration of MSCs. Endothelial cells, MSCs, chondrocytes, osteocytes, and osteoblasts then produce cytokines. This procedure is followed by the proliferation and differentiation of MSCs to create hard and soft calluses (Schubert et al., 2013).

Osteoconductive pillars are scaffolds, specifically the extracellular matrix, which works as a scaffold and supports the migration and adhesion of osteoinductive and osteogenic cells at the fracture site, which is necessary for fracture healing. In fractures when there is insufficient scaffolding, an autograft or allograft is necessary. Previous research has revealed that cells can perceive the mechanical environment surrounding them through electrochemical signals created by fluid changes in the canaliculi. Different cell membranes also serve as mechanoreceptors in a variety of other cell types (Utomo et al., 2019; Shang et al., 2021).

Osteoconductive pillars are scaffolding, namely the extracellular matrix, which stimulates the migration and adhesion of osteoinductive and osteogenic cells at the fracture site, which is crucial for fracture healing. In situations of fractures with insufficient scaffolding, an autograft or allograft is necessary. Previous research suggests that cells can perceive their surrounding mechanical environment through electrochemical signals created by fluid changes in the canaliculi. Other cells also possess cell membranes that serve as mechanoreceptors (Stamnitz & Klimczak, 2021; Venkataiah et al., 2021).

A critical-sized bone defect is defined as one that is greater than 2.5 times the bone's diameter. The primary issue with significant bone abnormalities is the restricted capacity of the fracture and its surrounding environment to mend optimally. In the initial stages of the healing process, the hematoma serves as a source of signaling chemicals that stimulate cellular cascades. MSCs are drawn to the fracture site and develop into osteoblasts in response to growth factor stimulation to enhance fracture repair. In MSCs-based treatment, the migration of MSCs to the wounded site is considered the initial stage in bone production and defect healing. BMPs and platelet-derived growth factors (PDGFs) are crucial for bone production and fracture repair, while MSCs stimulate angiogenesis to promote bone regeneration (Dilogo et al., 2017; 2019).

Ten to fifteen percent of the time, the healing and regeneration of bones, particularly those with substantial abnormalities, are disrupted, resulting in delayed union and non-union. Non-union has several reasons, including bleeding problems, periosteum injury, bone loss, and poor fracture stabilization. The gold standard for stability is the administration of an autologous bone transplant. However, this approach is limited by its limited graft resources. Despite the effectiveness of autologous bone grafting surgeries, problems such as prolonged discomfort at the donor site (49%), deformity, and impaired function still occur. The utilization of autologous or allogenic MSCs is crucial for the development of cell-based therapies for nonunion patients (Giannoudis et al., 2016).

After the inflammatory phase, the wounded region recruits endogenous MSCs, which then develop into chondrocytes or osteoblasts. Chondrocytes undergo bone mineralization by endochondral ossification, whereas osteoblasts deposit bone via intramembranous ossification. Periosteum, endosteum, and bone marrow are sources of endogenous MSCs that can promote bone repair. Endogenous stem cells release a variety of bioactive molecules that influence tissue function and possess anti-inflammatory, immunomodulatory, and regenerative capabilities. In addition to endogenous stem cells, foreign

stem cells can also be injected. Exogenous MSCs acts as an osteogenic, osteoinductive, and osteoconductive function in promoting fracture repair (Knight & Hankenson, 2013). MSCs factor can be administered systemically via the circulation, or it can be administered locally at the fracture site. Our previous studies have shown that MSCs are still found in areas with atrophic non-union fractures and can differentiate into osteogenic cells (Ismail et al., 2013).

The concept of tissue engineering in orthopaedic cases saves time, reduces complications and lengthy surgical procedures, reduces donor site morbidity, and provides effective results. After the inflammatory phase, endogenous mesenchymal stem cells are recruited and differentiated into chondrocytes or osteoblasts in the damaged region. Osteoblasts deposit bone by intramembranous ossification, whereas chondrocytes mineralize bone via endochondral ossification. The periosteum, endosteum, and bone marrow are sources of endogenous MSCs that can promote bone repair. Endogenous stem cells release many bioactive substances that influence tissue function and possess anti-inflammatory, immunomodulatory, and regenerative capabilities. Exogenous stem cells can be supplied in addition to endogenous stem cells. In clinical use, BMP (Bone Morphogenic Protein) also has some side effects. These side effects include leakage and triggering ectopic bone formation. In addition, BMP-2 increases osteoclastic activity, thereby increasing osteolysis. BMP also induces local inflammation and forms a seroma or soft tissue swelling (James et al., 2016). BMP-2 is the only FDA-approved osteoinductive growth factor for clinical usage at a 1.5 mg/ml dosage (Kamal et al., 2019; Pearson et al., 2019).

Six patients with critical-sized bone lesions were treated with a combination of Bone Marrow MSCs (BM-MSCs), hydroxyapatite (HA) granules, BMP-2, and internal fixation in a translational trial. Within six months of follow-up, we discovered that the patient's pain rating decreased dramatically. Within one year of follow-up, clinical and radiological results were also dramatically improved, and no adverse effects, such as tumorigenesis, were noted (Dilogo et al., 2019). In addition, we conducted a trial employing umbilical

cord mesenchymal stem cells (UC-MSCs) to treat seven patients with critical-sized bone lesions. In addition, we discovered that all patients reported considerable functional improvement during a follow-up period spanning from 12 to 36 months (Dilogo et al., 2021).

C. Stem Cells for Articular Cartilage Defect

Articular cartilage consists of an extracellular matrix and chondrocytes that absorb and even out the mechanical loading received by the joint. However, articular cartilage has poor vascularization and does not have good healing potential. Damage to the cartilage that is sustainable will develop into end-stage arthritis. Various surgical modalities are known to treat articular cartilage defects, such as the microfracture method, mosaicplasty, and osteochondral grafting. However, the outcome of surgical modalities such as microfracture and osteochondral grafting will result in cartilage repair in the form of fibrous cartilage. Studies have also shown a tendency towards failure with surgical modalities. Therefore, recent research has moved towards stem cell-based therapy to slow or reverse cartilage damage (Dewan et al., 2014; Phull et al., 2016).

Mesenchymal stem cells can be grown culturally to form various tissues derived from the mesoderm, including cartilage. In the defect area, MSCs release bioactive factors, which are regenerative and immunomodulatory. Factors secreted by exogenous MSCs stimulate endogenous MSCs in the body and form new cartilage tissue. Endogenous MSCs are found in many synovial joint tissues, representing 1% of the total cell population. It modulates the inflammatory cascade, which is essential in cartilage repair. Unfortunately, endogenous MSCs decline functionally with age (Lam et al., 2020; Le et al., 2020; Lee et al., 2021).

Chondrogenesis begins with the condensation of progenitor cells, which is then followed by the migration of MSCs. This process leads to the creation of cartilage and bone via endochondral ossification. To repair cartilage injury, cartilage damage will activate the creation of chondrogenic factors that recruit endogenous MSCs

from the synovium, synovial fluid, and bone marrow. The MSCs and scaffold are then placed at the fault site. Scaffolds offer MSCs with a microenvironment in three dimensions for proliferation and differentiation. Endogenous MSCs that have been stimulated will release bioactive substances that alter tissue function and have anti-inflammatory, immunomodulatory, and regenerative effects (Xiang et al., 2022; Zha, Li, et al., 2021; Zha, Sun, et al., 2021).

Exogenous sources of MSCs can be administered by direct implantation through a surgical incision for cases of severe defects or by intra-articular injection for small defects. If not satisfied with MSCs administration, we can add chondrogenic factors and scaffolds to increase cartilage repair. Chondrogenic factors include bone morphogenic protein (BMP), transforming growth factor beta (TGF-B), insulin-like growth factor-1 (IGF), and platelet-rich plasma (PRP). BMPs, particularly BMP-2 and BMP-7, promote cartilage regeneration by promoting MSCs differentiation and increasing the recruitment of endogenous MSCs to injured cartilage regions. Chondrocytes are effectively stimulated to produce proteoglycans and type 2 collagen by transforming growth factor-beta. IGF-1 is a cartilage homeostasis mediator with the function of increasing chondrocyte proliferation and stimulating proteoglycans. PRP also stimulates the proliferation of MSCs and enhances the formation of extracellular matrix (Goldberg et al., 2017).

A scaffold is required and must be present for cartilage regeneration. To be regarded suitable for cartilage regeneration, scaffolds must satisfy three requirements. These criteria include being composed of biodegradable and biocompatible materials to support the chondrogenesis process, having a degradation rate that adapts to the rate of cartilage formation, and possessing mechanical properties that can withstand physical loading to provide adequate space for the tissue to undergo the regeneration process (Daneshmandi et al., 2020; Kadir et al., 2021; Muhammad et al., 2019).

MSCs activities in tissue regeneration include chondrocytes, extracellular matrix, the immune system, mitochondria, and paracrine

actions. Against chondrocytes, MSCs enhance chondrogenesis, decreases apoptosis, and preserves chondrocyte autophagy. MSCs balance the ratio of MMP-13 to TIMP-1 in cartilage and decreases the expression of hypertrophy indicators in cartilage, including collagen X, FGF receptor, PTH-related protein, and MMP-13. MSCs promote immunosuppressive factors and hinders the maturation of monocytes into dendritic cells. Through mitochondrial transfer, MSCs enhance the membrane potential in mitochondria. MSCs have paracrine actions that are mediated via extracellular vesicles produced from MSCs (Platas et al., 2013; Stoddart et al., 2015).

Supartono et al. found in a prior study that microfractures had no meaningful effect on profound osteochondral abnormalities. Therefore, the inflammatory process without microfractures is adequate to drive MSCs homing (Supartono et al., 2018). A systematic review by Dilogo et al. demonstrated that intra-articular umbilical cord MSCs injection without surgical treatment improved clinical outcomes in patients with knee osteoarthritis. This finding is supported by the ability of MSCs to reduce cartilage erosion due to synovitis in pre-clinical studies without any reports of serious adverse events (Dilogo et al., 2023).

During cartilage development, MSCs contribute to the production of an extracellular matrix that is crucial for cartilage repair. In addition, MSCs produce cytokines, growth factors, and chemokines that recruit endogenous MSCs to the site of the defect and maintain the optimal microenvironment. Despite all the benefits of MSCs, their use has a number of drawbacks, including the inability to be used immediately and high storage costs. Based on the limits of MSCs, research has also demonstrated that the therapeutic potential of MSCs derives from their paracrine components, which are also present in their derivative product, the secretome. The secretome, also known as conditioned medium (CM), is a substance that is released by MSCs and comprises extracellular vesicles and other biological components, such as growth factors and cytokines. Due to its cell-free nature, secretome offers a lesser risk of immunogenicity compared to MSCs, as well as a manipulable dosage and potency, large-scale production, ready-to-use

status, comparatively cheap cost, and storage simplicity. Secretome's therapeutic impact may be attributed to its pro-angiogenic, anti-fibrotic, anti-apoptotic, anti-inflammatory, and immunomodulatory properties. In contrast to MSCs, however, secretome has limitations such as a shorter half-life that necessitates repeated administration and a poorer anti-inflammatory capacity. The secretome increases collagen type 2 expression to preserve cartilage integrity and viability. It enhances matrix synthesis by inhibiting the generation of nitric oxide. Additionally, the secretome lowers chondrocyte inflammation by downregulating degradation enzymes and pro-inflammatory cytokines (such as Interleukin 6 and TNF alpha) and upregulating anti-inflammatory cytokines such as interleukin 10 (Chang et al., 2018; Contentin et al., 2022).

Due to its immunomodulatory, regenerative, anti-catabolic, and chondroprotective capabilities, secretome administration has been shown to be useful in instances of osteoarthritis by preclinical research. Previous research by Lubis, Luthfi et al. stated that secretome administration from UC-MSCs, along with microfracture procedures, is effective as an alternative treatment for cases of cartilage defects (Lubis, Luthfi, et al., 2022). Growth hormone (GH) is a biological factor that has the potential to regenerate cartilage by regulating the body's metabolism, namely by talking about the production of insulin-like growth factor (IGF-1). However, during the ageing process, there is a phenomenon known as somatopause, so giving external GH becomes rational. Giving GH and regulating IGF-1 also triggers neovascularization, which is crucial in morphoangiogenesis. In addition, GH also stimulates chondrocytes to synthesize matrix components such as type II collagen, which is essential in cartilage regeneration. Lubis et al. (2019) also shown that the treatment of intra-articular growth hormone injections resulted in improved macroscopic and microscopic results in osteoarthritis in New Zealand rabbits than the administration of hyaluronic acid and no injection (control group). In subsequent studies, Lubis, Wijaya, et al. demonstrated that repeated treatment of growth hormone had a

greater effect than a single dosage, specifically by administering five injections at 1-week intervals. Lubis, Wijaya, et al. also reported in their comprehensive analysis that growth hormone injections had encouraging outcomes for cartilage regeneration in knee osteoarthritis without systemic side effects (Lubis, Wijaya, et al., 2022).

The development of stem cells and their derivatives in cartilage regeneration is growing. Currently, exosome research, especially in pre-clinical studies, is increasingly supporting their use in cases of cartilage regeneration. Exosomes are extracellular vesicles measuring 50–130 nanometers which function for intercellular communication and signal transduction between cells. The advantages of using exosomes include having a potent paracrine function, less rejection from the immune system, no risk of triggering tumors, can be combined with existing carriers, and being easier to store (Contentin et al., 2022; Lee et al., 2021). Exosomes induce endogenous stem cells, increase endogenous chondrocytes, and decrease pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha to regenerate cartilage via many methods. Exosomes were shown to boost cell proliferation and minimize the frequency of apoptosis in an in vitro examination of osteoarthritis patients in the temporomandibular joint. Exosomes from Bone Marrow MSCs successfully enhance cartilage repair and extracellular matrix formation and relieve pain in rabbits with knee osteoarthritis, according to additional preclinical research (Lee et al., 2021; Liu et al., 2019).

D. Stem Cells for Avascular Necrosis of Femoral Head

Exosomes contain many pathways for cartilage regeneration, including activating endogenous stem cells, boosting endogenous chondrocytes, and suppressing pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha. In an in vitro examination of osteoarthritis patients in the temporomandibular joint, exosomes were shown to boost proliferation and decrease apoptosis incidence. Exosomes derived from bone marrow mesenchymal stem cells efficiently stimulate cartilage repair, extracellular matrix formation, and pain reduction

in rabbits with knee osteoarthritis, according to another preclinical research (Xu et al., 2020).

Early therapy results in a favourable clinical outcome. Unfortunately, many patients present with severe osteonecrosis and missed opportunities in the early stages of development, necessitating THR (Total Hip Replacement). THR has downsides, particularly in young adult patients, because of decreased mobility and the need for revision. Osteonecrosis of the femoral head is an orthopaedic disorder characterized by disruption of blood vessels and necrosis of the subchondral bone, leading to femoral head necrosis. In addition, this illness is defined by elevated intra-osseous pressure and disturbances in bone metabolism, which result in an imbalance between bone absorption and remodeling. Now available therapies include core decompression, vascularized bone graft, osteotomy, tissue engineering material transplantation, and complete hip replacement. No medicine, however, can halt the progression of avascular necrosis. Forty percent or more of patients who had core decompression required total hip arthroplasty due to disease progression (Houdek et al., 2014).

There are several approaches to utilizing stem cells in femoral head avascular necrosis cases, including combining stem cells with core decompression, autologous bone transplant, platelet-rich plasma, and other biomaterials. Other research suggests that using stem cells can improve short-term therapeutic outcomes. Mao et al.'s recent meta-analysis indicated that stem cell treatment could dramatically decrease development and increase the long-term survival of the hip joint. Furthermore, the study by Mao et al. explained that the administration of stem cells was most effective, particularly when given to individuals under the age of 40 (Mao et al., 2020).

The most prevalent treatment for femoral head osteonecrosis is MSCs therapy. Depending on the source, MSCs can develop from bone marrow, adipose tissue, peripheral blood, or the umbilical cord. Unknown is the precise manner of stem cell-based treatment for avascular necrosis of the femoral head. One rationale is the biological characteristics theory, which asserts that stem cells may self-replicate

and replenish. When stem cells are injected into a necrotic femoral head, they can develop into osteoblasts, chondrocytes, and other tissues, enabling the regeneration of dead bone. In addition, stem cells release numerous biological substances like cytokines, growth factors, and exosomes to promote angiogenesis, reduce femoral head hemorrhage, and reduce intra-osseous pressure, therefore decreasing the course of femoral head osteonecrosis (Li et al., 2021).

Houdek et al.'s paper discusses the indications and contraindications for using stem cells in femoral head avascular necrosis cases. According to the Steinberg classification, additional indications include individuals with symptomatic stage 1 or stage 2 avascular necrosis of the femoral head. On MRI, patients show bilateral symptomatic avascular necrosis on at least one side and >30% asymptomatic lesions. Stem cell administration is contraindicated for patients with avascular necrosis of the femoral head stage 3 or higher, fast-developing avascular necrosis, and active or persistent infections (Elgaz et al., 2020; Houdek et al., 2014).

E. Stem Cells for Brachial Plexus Injury

Brachial plexus injury (BPI) is a damage to the peripheral nerves that results in paralysis of the upper extremities. It occurs in 2.8% of trauma patients. These injuries are frequently the result of high-energy trauma, such as automobile collisions. Injury to the BPI can vary from nerve compression to total nerve transection. A nerve damage is characterized by muscular weakness, altered reflexes, and loss of sensory function. Improving nerve function is hampered by the sluggish regeneration of nerves, which is around 1 mm every day. Various attempts have been made to repair peripheral nerve damage, such as the administration of drugs and also microsurgery interventions in the last few decades. However, there are still limitations regarding functional outcomes in long-term follow-up. The denervation from the injury causes muscle contractility and atrophy loss. A state of denervation left for a long time causes irreversible atrophy. Resident muscle stem cells enable regeneration of skeletal muscle tissue. In muscles that replace injured

myofiber, the regeneration phase is marked by the activation of a population of stem cells known as satellite cells.

To treat motor neuron degeneration, cell replacement procedures have been established throughout the past decade. Due to their capacity to develop into neural progenitor cells, mesenchymal stem cells have garnered attention as a nerve regeneration technique (Sumarwoto et al., 2022). MSCs can also develop into neuronal cells, such as Schwann cells, which aid in nerve regeneration by creating myelin. Mesenchymal stem cells can also stimulate myogenesis and angiogenesis by secreting angiogenic, mitogenic, and anti-apoptotic substances such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and Bcl-2. In addition, MSCs generate paracrine proteins such as stem cell factor and heat-shock protein 20, which enhance organ function by promoting remodeling, regeneration, and neovascularization. MSCs can also move to sites of damage or hypoxia, where they enhance tissue healing. This is due to the release of anti-inflammatory, anti-apoptotic, and trophic substances, such as brain-derived neurotrophic factors and nerve growth factors.

MSCs can be administered locally or systemically. Local MSCs implantation has the advantage that MSCs can directly reach the target organ, also known as "non-systemic homing". Meanwhile, intravenous MSCs implantation has a weakness where MSCs can be trapped in the lungs, liver, or spleen, considering that these organs are more significant, and their adhesion molecules will reduce the number of cells that reach the target site (about 2%). Following the principles of tissue engineering, stem cell implantation can be optimized by creating a micro-environment that supports regeneration by combining MSCs administration with scaffold biomaterials and growth-promoting factors (Guo et al., 2020).

F. Stem Cells for Degenerative Disc Disease

Additionally, MSCs can develop into neuronal cells such as Schwann cells, which promote nerve regeneration by creating

myelin. Mesenchymal stem cells can also stimulate myogenesis and angiogenesis by secreting many angiogenic, mitogenic, and anti-apoptotic substances, including vascular endothelial growth factor (VEGF), IGF-1, HGF, and Bcl-2. In addition, MSCs generate paracrine proteins such as stem cell factor and heat-shock protein 20, which contribute to remodeling, regeneration, and neovascularization, hence boosting organ function. MSCs are also capable of migrating to sites of damage or hypoxia and enhancing tissue healing. This can be explained by the release of anti-inflammatory, anti-apoptotic, and trophic substances in the form of nerve growth factors and brain-derived neurotrophic factors (Xie et al., 2021).

Both clinicians and scientists have been interested in furthering stem cell research in cases of degenerative disc degeneration in recent years. It has been demonstrated that stem cells can postpone or even reverse the process of intervertebral disc degeneration. The deterioration of the discs in the spine causes degenerative disc disease, which leads to inflammation and the production of enzymes that further degrade the tissue. MSCs can aid in tissue repair by differentiating into the necessary repair cells and producing cytokines and growth factors that reduce inflammation and promote healing.

Regarding disc degeneration, stem cells serve three fundamental functions. First, stem cells can differentiate into cells resembling intervertebral discs. The regenerative capability of stem cells implanted in IVD consists of their ability to differentiate into nucleus pulposus cells and stimulate the production of new extracellular matrix. Despite the fact that many studies indicate that stem cells die shortly after implantation owing to a lack of nutrition and pH fluctuations, other studies indicate that MSCs and their progenitor cells can survive when injected in large quantities. By secreting chemokines, components, growth factors, and anti-inflammatory mediators via their paracrine pathways, stem cells can boost the viability of resident cells at their implantation sites. Thus, as extracellular matrix secretion rises, the expression of proteins linked with cell senescence falls. Additionally, stem cells can modify the mechanical characteristics of the nucleus

pulposus and lessen the stiffness of the cell and matrix to increase cell survival. Stem cells can postpone the progression of intervertebral disc degeneration by reducing the control of the immune system. Damage to the extracellular matrix is exacerbated in the presence of pro-inflammatory cytokines that mediate inflammatory processes with interleukins, TNF, interferons, prostaglandin E2, and other chemokines. These pathways promote apoptosis, senescence, and autophagy in cells. Stem cells implanted in intervertebral discs can create anti-metabolic mediators, growth factors, and anti-inflammatory cytokines, according to studies (Zhang et al., 2022).

G. Stem Cells for Ligament and Tendon Healing

As the popularity of amateur and professional sports increases, so does the prevalence of injuries. Both conservative therapy and surgical excision and repair have been used to treat tendon injuries. Traditional treatments such as rest, cold, compression, elevation, a brief course of a pain modulator, and anti-inflammatory medications can reduce pain, but they do not result in total healing. The healing process is extremely delayed because of the inadequate vascularization of tendons and ligaments. Healing cannot restore tendon function due to the production of mechanically weaker scar tissue, the danger of ectopic bone formation, and the limited regeneration of fibrocartilage at tendon-to-bone contact (Trebinjac & Gharairi, 2020).

Due to the limits of tendons' self-repair capacities and existing therapies, it is crucial to have alternative tendon regeneration techniques. Similar to mesenchymal stem cells, tendons and ligaments contain a small number of stem cells known as tendon stem/progenitor cells (TSPCs). TSPCs exhibit all of the features of mesenchymal stem cells, including surface markers, the capability for self-replication, and the ability to differentiate into bone, cartilage, and fat. When transplanted, TSPCs create ectopic tissue. The regeneration of ligaments and tendons has been investigated using embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem cells. The most study has been

conducted on mesenchymal stem cells, and the results are positive. MSCs can develop into mesoderm-derived tissues such as ligaments and tendons. MSCs are capable of regenerating tendons due to their ability to differentiate into tenocytes, the cells that compose tendon tissue. In addition, MSCs have a high capacity for proliferation and synthesis, which expedites tissue healing. When MSCs are implanted into injured tendons, they differentiate into tenocytes and generate extracellular matrix, aiding in tissue regeneration. In addition, MSCs possess immunomodulatory properties that are beneficial for reducing tendon inflammation (Lui, 2015).

When injected into torn tendons or ligaments, MSCs can release cytokines and growth factors that encourage the body's natural healing response and recruit necessary cells for tissue regeneration. More study has been undertaken on MSCs for tendon repair than on ligaments. In a prior study, Wang et al. compared the injection of 75 million allogenic mesenchymal precursor cells (MPCs) with hyaluronic acid to the administration of hyaluronic acid in post-ACL reconstruction patients. Patients treated with MSCs reported quicker pain alleviation and functional benefits. Injecting MSCs into the partly torn patellar tendon of rats enhanced tendon repair and increased the mechanical strength of the tissue, according to Yin et al. (Yin et al., 2016).

Centeno et al. reported 29 patients with clinical symptoms and MRI confirmation of grade 1-3 ACL tears who were treated with injections of 2-5 cc of bone marrow concentrate derived from 60–120 cc of whole blood marrow aspirate and combined with platelet rich plasma (PRP) and platelet lysate (PL). At 8.8 months follow-up, 77% of patients demonstrated substantially improved ACL integrity, as measured by T1 MRI ACL signal intensity, according to this study (Lui, 2015). A systematic review of the use of a secretome in tendon and ligament healing by Rhatomy et al. found that an MSCs secretome could enhance tendon and ligament healing in preclinical studies. The paracrine effect of MSCs influence its therapeutic effect on tendon and ligament regeneration. The use of stem cell CM as a treatment for tendon or ligament damage could give an alternative to direct stem

cell therapy that is non-invasive and less complicated. Further research is necessary to comprehend the efficacy and safety of stem cell CM treatment in clinical situations (Rhatomy et al., 2020).

H. Conclusion

Stem cells have demonstrated considerable potential in orthopaedics and trauma surgery for initiating healing processes, compensating for deficiencies, and stimulating the regeneration of tendons, muscles, bones, and cartilage. Stem cell therapy has various advantages, including reduced pain, better function and mobility, and tissue regeneration. The success rate of stem cells for orthopaedic disorders is encouraging. However, its limitation including expensive cost, lack of standardization, and limited availability still have to be solved before its usage can be generalized. Therefore, we suggest further research, develop standardized protocols, combining its approach with tissue engineering, and further explore the stem cell-derived metabolites, such as secretomes and exosomes.

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Chapter 4

Mesenchymal Stem Cells Secretome for Diabetic Wound

Siufui Hendrawan
Jennifer Lheman
David Victorious Lukas
Sukmawati Tansil Tan

A. Diabetes and Its Complications

Diabetic complications can be generalized into two categories, microvascular and macrovascular complications. Microvascular complications are related to complications affecting microvessels such as retinopathy, neuropathy, and nephropathy. On the other hand, macrovascular complications are related to those affecting macrovessels such as coronary artery disease, peripheral artery disease, and stroke (Mauricio et al., 2020; Ohiagu et al., 2021). The macrovascular complications, such as coronary artery disease, had a high prevalence among people suffering from diabetes. Besides that, one of the most common complications of diabetes is peripheral artery disease, which is associated with other diseases, particularly diabetic

S. Hendrawan, J. Lheman, D. V. Lukas, S. T. Tan
Tarumanagara University, e-mail: siufui@fk.untar.ac.id

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peripheral neuropathy. Both these disorders could lead to diabetic foot which usually leads to nonhealing foot ulcers (Mauricio et al., 2020; Ohiagu et al., 2021). There are still many diabetic complications other than those mentioned above.

B. Underlying Mechanism of Diabetic Complications

According to Shi et al. (2018), several studies have shown that hyperglycemic conditions may affect microvascular and macrovascular disruptions. This disease is a major contributor to the development of vascular problems and can mediate the negative effects through many mechanisms (Shi et al., 2018). The mechanisms include increased oxidative stress, mitochondrial dysfunction, excessive cellular apoptosis, and abnormal cellular autophagy, which is caused by long-term diabetic condition and have a potential effect on diabetes complications, whether it is directly or indirectly (Shi et al., 2018). In brief, this excessive production of ROS will increase the synthesis of strong oxidative peroxynitrite, induced damage to DNA, and increased the risks of diabetic microvascular complications throughout several mechanisms (Shi et al., 2017, 2018). The next mechanism is related to cellular and tissue injury in diabetes is cellular apoptosis. Apoptosis is a mechanism for genetically programmed cell death, and it has a major role in the survival of an organisms (Hamzawy et al., 2017). Cell apoptosis related to diabetes is mediated by two substantial forms: stress on endoplasmic reticulum and damage to mitochondria. There are two key pathways that take part in this mechanism, the extrinsic death receptor pathway and intrinsic mitochondrial pathway (Huang et al., 2017; Shi et al., 2018). Another major mechanism involved is autophagy which is another significant way to maintain environmental homeostasis in intracellular (Hamzawy et al., 2017). Being in a hyperglycemic condition for a prolonged period of time, abnormal cellular autophagy could occur. This mechanism is activated when the cell condition is in various stress situations such as lack of essential nutrients and high glucose environment. Autophagy is necessary for cellular functions, the deficiency of this mechanism could lead

to cellular degeneration and disruption of intracellular homeostasis (Hamzawy et al., 2017; Shi et al., 2018). Apart from the mechanisms mentioned above, there are some that believe that growth factors play a role in the development of abnormal growth and impaired regeneration in diabetics (Shi et al., 2018). Thus, it is important to understand the underlying mechanism that is impaired due to this condition.

C. Diabetic Ulcer as One of Serious Concern Complications of Diabetes

Diabetic ulcers are arising as a serious concern due to the complications from amputation cases. The incidence rate of minor and major amputations related to diabetes was 139.97 and 94.82 events respectively, per 100,000 diabetic patients per year. In the case of lower extremity amputations related to diabetes, it is more than two-fold higher in patients with type 1 DM than those with type 2 DM (Ezzatvar & García-Hermoso, 2022). This condition is a result of disruption in all phases of wound healing as a result of diabetes itself (Okonkwo et al., 2020). There are numerous factors that are responsible for the impaired wound healing process in diabetic patients, including impaired growth factor production, angiogenic response, and collagen formation (Fui et al., 2019). Consequently, even slight skin abrasions or scratches tended to develop into chronic wounds in diabetic patients (Avishai et al., 2017).

1. Impaired Wound Healing Process in Diabetes

Normally the wound healing process can be divided into four phases, specifically hemostasis, inflammation, proliferation, and finally the remodeling process (Fui et al., 2019). In brief, hemostasis is the first phase of the process in which the constriction of blood vessels occurs and platelet cells aggregate to clot the wound. The next phase is the inflammatory stage, when inflammatory cells migrate to the wound and blood circulation is increased (4 to 6 days). Then, proliferative

stage, which is when the wound bed is filled with granulation tissue during this phase, followed by angiogenesis which is when the new blood vessels are formed (4 to 21 days). Epidermal cells such as fibroblasts and keratinocytes are proliferated and migrated across the wound. After that, the collagen accumulation happens, then finally the wound edges are contracted. The final phase is remodeling phase which occurs 21 days to 2 years post injury. The tensile strength is increased by collagen crosslinking and the scar is matured (Fui et al., 2019). If there are one or more of these phases impaired, consequently the wound healing process becomes delayed and it will lead to chronic ulcer development.

In diabetic patients with a constant state of hyperglycemia, the endothelial cells lose their integrity and eventually become susceptible to apoptosis and detachment, resulting in impairment of the wound healing process (Okonkwo & Dipietro, 2017; Velnar & Gradisnik, 2018). The study conducted by Okonkwo et al. (2020) also found that there was a decreased amount of functional endothelial cells present in the diabetic skin. There was also impairment in the pruning and refinement process of the capillary bed (Bodnar et al., 2016; Caporali et al., 2017; Okonkwo et al., 2020). Therefore, it leads to the conclusion that skin wounds on diabetic patients are significantly reduced due to neovascularization and pro-angiogenic factor expression after injury (Okonkwo et al., 2020). Moreover, the overproduction of ROS in diabetics will result in cellular damage of endothelial cells (Fui et al., 2019).

Although many comprehensive treatments are currently available, the number of complications related to diabetic wounds, especially diabetic foot ulcers, is still alarming, with the data reported by the IDF showing that 9.1–26.1 million people will develop diabetic foot ulcers (Armstrong et al., 2017; Everett & Mathioudakis, 2018). Hence, advanced treatments for diabetic wounds have been studied intensively in the last decade. Growth factors are known to be involved in every phase of wound healing through their inhibitory or stimulatory effect (Burgess et al., 2021; Fui et al., 2019). Although there is some belief

that in the diabetic condition, growth factors play a major role in repairing tissue, nevertheless, it is still one of the promising targets for diabetic wound healing therapy.

2. Growth Factors That Related to Wound Healing Process

There are some major growth factors that play an important role in the wound healing process. Platelet-derived growth factor (PDGF) is released by platelets as a key factor that functions to increase the infiltration of immune cells, activating macrophages, promoting fibroblast proliferation, and accumulation of the extracellular matrix (ECM). It is involved in the inflammatory, proliferative, and remodeling phases of wound healing (Gardner et al., 2016; Patel et al., 2019). Epidermal growth factor (EGF), which is involved in the proliferation phase, is also released by platelets. It has the function of enhancing cell motility, migration, and cell proliferation (Bai et al., 2016; Fui et al., 2019; Patel et al., 2019; Shin et al., 2022). Transforming growth factor beta 1 (TGF-β1), involved in inflammatory and proliferation phase, it is produced by several cells such as macrophages, fibroblasts, keratinocytes, and platelets. The functions of TGF-β1 are increasing leukocytes and fibroblast migration, promoting angiogenesis, and also stimulating the production of ECM components (Bai et al., 2016; Fui et al., 2019; Mori et al., 2016). Vascular endothelial growth factor (VEGF) is the most known growth factor for its potent angiogenic properties. It increases capillary density and improves the blood metabolism in wounded tissue. It also mediates migration and proliferation of endothelial cells, angiogenesis and tissue granulation during inflammatory and proliferation phases (Fui et al., 2019; Gardner et al., 2016; Patel et al., 2019). Finally, basic fibroblast growth factor (bFGF) is usually highly expressed during late inflammatory stage. This growth factor has the functions to enhance the proliferation of fibroblast, promotes angiogenesis and collagen maturation during the proliferation and remodeling phase of the wound healing (Bai et al., 2016; Fui et al., 2019). Most of these growth factors are secreted by

mesenchymal stem cells, which is why it has become the major focus for advanced therapy in diabetes.

D. Stem Cell-Based Therapy for Diabetes

Recently, stem cells-based therapy has arisen as one of promising alternative treatment to many diseases, including diabetes. Interestingly, mesenchymal stem cells (MSCs) are well known for their regenerative ability and immunomodulatory attributes. MSCs can be found in many perivascular tissues such as bone marrow, adipose tissue, teeth, placenta, umbilical cord, amniotic fluid, and cord blood (Shin et al., 2021). The following are some studies, both pre-clinical and clinical trials, that used mesenchymal stem cells from varied sources to treat diabetic condition such as insulin resistance and control the hyperglycemia condition.

Wharton's jelly derived MSCs and hematopoietic stem cells derived from umbilical cord blood have been analyzed for treatment of type 1 and type 2 diabetes (Bani Hamad et al., 2021). A clinical trial in China used Wharton's jelly derived MSCs on a recently diagnosed type 1 DM patients. This clinical trial was designed as randomized controlled study, and the stem cells were administered via intravenous injection and were combined with insulin administration prior and throughout the follow up period. The dose of implanted MSCs was not disclosed by the authors. The result showed improvement in hemoglobin A1c (HbA1c) levels on the stem cells therapy group, the dosage of insulin administration was significantly decreased, and interestingly, a fifth of patients in the therapy group become insulin-independent, for almost 1.5 years. Moreover, there were no adverse events reported during the study (Bani Hamad et al., 2021; Hu et al., 2013). Other clinical trial was done on type 2 DM patients in China. Wharton's jelly derived MSCs is used and administered twice. First, it was delivered via intravenous injection, then for the second dose it was directly injected through splenic artery using catheter. The first and second dosage was given five days apart. The result showed

there was a decrease in HbA1c and blood glucose levels, moreover the dosage of insulin and other anti-diabetic medication is reduced. Although there are no control group to compare the result in this trial, this result showed that administration of Wharton's jelly derived MSCs can improve the regulation of metabolic pathway and β cell function in type 2 DM patients (Bani Hamad et al., 2021; Liu et al., 2014). The limitation for both studies is the small sample size. Overall, it proved that stem cell therapy was much safer compared to islet and organ transplantation (Bani Hamad et al., 2021). Umbilical cord (UC) derived MSCs are considered a better choice for clinical applications due to its high paracrine potential and it has low immunogenicity (Wang et al., 2018; Xiang et al., 2020). MSC have the ability to repair the cell damage through paracrine mechanisms from several factors such as immunomodulation factors, angiogenic factors, antiapoptotic factors, antioxidative factors, and also cell migration, and targeting and stimulation, although their fundamental and detailed biological mechanism still required further elucidation (Gnecchi et al., 2016; Liang et al., 2014).

Despite its promising results, there are some limitations of this stem cells therapy. These are related to the administration of stem cells, which is via infusion route in most of the studies or via clinical trials regarding type 1 or type 2 DM (Cho et al., 2018). One of the significant challenges is the low survival rate of the engrafted cells. Many transplanted cells eventually will die within hours or days post transplantation (Mitrousis et al., 2018; Sortwell et al., 2000). Numerous efforts have been attempted by researchers to overcome this problem. Pre-conditioning, genetic modification, and mimicking extracellular matrix such as hydrogel have been used to improve the survival of the cells (Li et al., 2016; Zhao et al., 2019). Nonetheless, further strategies and research are needed, including various cells condition and environment, the delivery system, and dosages that must be consider.

E. Secretome Based Therapy for Diabetes

This last decade, researchers were racing to develop cell free therapy derived from stem cells. As described above, stem cells, such as MSCs, have been used in clinical trials to treat diabetes and there are several trials that have proven their positive effects. However, due to some challenges to have the optimal effect from the usage of MSCs, cell-free therapy such as secretome or CM is more preferrable. MSCs from various sources have been known to release numerous paracrine factors that classified as bioactive molecules (Hsiao et al., 2011). These bioactive molecules which secreted into the extracellular space are known as secretome and is secreted by MSCs as a response to specific microenvironment conditions. According to González-González et al. (2020), secretome contains two different components. The first component is a soluble part, mostly comprised of cytokines, chemokines, immunomodulatory molecules, and broad spectrum of growth factors (Madrigal et al., 2014). The second component is a vesicular fragment, consisted of variety type of extracellular vesicles (EVs) (González-González et al., 2020; Teixeira & Salgado, 2020) such as microvesicles (Bruno et al., 2009), microparticles (Kim et al., 2012) and exosomes (Lai et al., 2010, 2015). Various studies on these secreted factors showed that even without the cells, it still has the regenerative ability to repair tissue or organ damage (Pawitan, 2014). This secreted factor can be found in the media where the cells were cultured, therefore the media is called conditioned medium (CM) (Kim et al., 2013).

There are some theories stating that the origin of the MSCs may have a difference in the protein expression. A study conducted by Shin et al. (2021) has done the comparative analysis of the secretome from different sources which are adipose and bone marrow (adult stem cells) and placenta and Wharton's jelly (fetal stem cells). There were plenty of proteins that involved in cellular migration and apoptosis reduction in the secretome derived from adipose, placenta, and Wharton's jelly, but not from bone marrow, though the level is varied between the sources. On adult stem cells, protein secreted by adipose MSCs is associated with the organization such as the development of

cytoplasm, while protein secreted by bone marrow MSCs is related to cellular development, and epithelial-mesenchymal transition. Protein that associated to cell migration and survival were detected similarly on both sources (Shin et al., 2021). Nonetheless, secretome secreted by fetal MSC group was expected to have higher potential than the adult stem cells due to the higher quantity of protein and more diverse proteins they have (Shin et al., 2021).

Secretome or CM has been used in several pre-clinical research particularly for wound healing in diabetes case. In brief, these are some of the studies related to that case. First is an in vitro study using secretome isolated from human adipose tissue-derived MSC has showed its ability to accelerate cutaneous wound healing. The results showed that the epidermal and dermal thickness, vascularized granulation tissue, and dermal collagen layers were increased on the wound treated by the stem cell secretome. The secretome stimulates collagen synthesis and migration of dermal fibroblasts through upregulating the transcription of collagen type I and III. It also may promote wound healing by increasing re-epithelization of the dermal tissue (Park et al., 2018). MSC and MSC-CM accelerated epithelialization, increasing granulation tissue formation. In response to MSC and MSC-CM, dermal fibroblast secrete increased the amounts of collagen type I and alter gene expression (Gnecchi et al., 2016).

We also have done an in vivo study using conditioned medium isolated from human umbilical cord-derived MSCs in diabetes induced rats to observe the wound healing potential. All animal experiments in this study were approved by Institutional Animal Care and Use Committees (IACUC) of the Faculty of Medicine, Tarumanagara University, approval number 001.KEPH/UPPM/FK/IV/2019. The cells were processed at Stem Cell and Cancer Institute Laboratory, Jakarta, Indonesia. The MSCs were cultured under hypoxic condition then the CM was collected. The result showed that this pre-conditioning hypoxic condition could stimulate MSCs to produce higher growth factors such as VEGF, bFGF, and pro-collagen 1 and promote better wound closure in rats. Intriguingly, VEGF was not secreted in CM collected

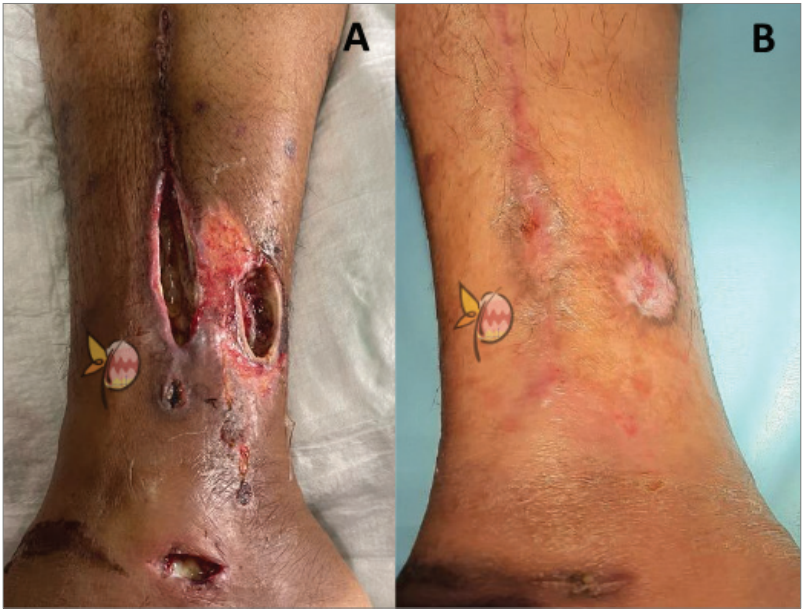
from umbilical cord MSCs that was cultured in normoxic condition. It has been proven that pre-conditioning certain factors such as hypoxia could enhance growth factors secretion. The histopathological analysis on the wound site showed that there is an increase in re-epithelization and also has the largest collagen deposition in the group treated using hypoxic umbilical cord-CM compared to the other group. Therefore, we concluded that the CM collected from umbilical cord MSCs cultured in hypoxic condition has positive effects towards wound healing process based on the result of re-epithelization and collagen formation on the wound site (Hendrawan et al., 2021).

Another in vivo study was done by Saheli et al. (2020) which evaluated the impact of CM collected from human bone marrow derived MSCs for diabetic wound healing in rats. The result showed the healing progress on the diabetic wound treated by CM was improved and comparable to the progress on non-diabetic group. They also found that the inflammation was significantly reduced on day 4 in the group treated with the CM compared to the diabetic control group. Higher expression of EGF and bFGF was also observed on the diabetic wound treated by the CM. Additionally, the collagen density was also increased, the inflammation was repressed, number of fibroblasts and microvessels was significantly elevated on the CM-treated group when compared to the diabetic wound. Therefore, this study also demonstrated that administration of MSC-CM has the potential to effectively improve the quality of healed wounds in chronic diabetes condition, which mainly through the modulation of fibroblast behaviors (Saheli et al., 2020).

Even though numerous researches were done using secretome or CM in relation to wound healing, especially diabetic wound, however, there are still only a few pre-clinical trials using CM or secretome systemically to treat hyperglycemic condition and other diabetic complications. We have done a pilot study to see the effect using CM from hypoxic human umbilical cord MSCs via intravenous injection on diabetic induced rats. The pilot study was approved by IACUC of PT. Bimana Indomedical, Bogor, ethical approval number R.02-21-IR.

The CM was injected intravenously through rat tail vein and the blood glucose concentration was monitored for 1 month. The results showed that the insulin concentration was decreased in CM group and was comparable to the normal rats. Based on this result, it suggested that the administration of CM could reduce the hypersensitivity of β cells. There were no side effects observed during the study (unpublished data) (Hendrawan et al., 2021; Tan et al., 2021).

We also performed a clinical study (number NCT04134676), in which we evaluated the therapeutic potential of CM on chronic ulcer wounds especially on diabetic patients. The ethical clearance for this study was obtained from Human Research Ethics Committee, Institute of Research and Community Engagement of Tarumanagara University, number 1007-Int-KLPPM/Untar/VI/2020. Umbilical



Notes: (A) Before treatment and (B) After treatment

Photo: Sukmawati Tansil Tan (2021)

Figure 4.1 Representative Image of Diabetic Chronic Ulcer Wound Treated with MSC CM

cord was obtained with the parental consent and was processed at Tarumanagara Human Cell Technology Laboratory, Jakarta, Indonesia. The CM was collected from MSC cultured under hypoxic condition (Figure 4.1a). The CM and other active ingredients were mixed in the form of 10% gel for topical use. An ample amount of the gel was applied to the wound. The results showed that the width and length of the wound decreased. Moreover, the bed of wounds is improved after 2 weeks post treatment (Figure 4.1B). The wound was treated for one month. There are no adverse effects observed in this study. Overall, the study showed that the topical administration of 10% gel CM can effectively enhance wound healing, in particular diabetic chronic ulcers (Tan et al., 2023).

F. Future Prospect and Challenge Against Secretome Wide Clinical Application

Stem cell-based therapy has emerged as a prominent alternative therapy to various degenerative diseases (Park et al., 2018). However, there are numerous challenges for clinical application of this therapy. The process of manufacture, sources, cell culture protocols, level of expansion and status of the cells can influence the therapeutic effectiveness of MSCs. Until now, allogenic or autologous MSCs have been used in clinical trials, while xenografts of MSCs are only applied in pre-clinical studies (Shin et al., 2021). The concerns are delivery route and dosage of the MSCs. Viable cells usually were delivered to the body via injection or catheter. However, the data showed that injection of live cells through a syringe needle can reduce the cell viability below 32% and it could cause irreparable damage to the cell membrane. It could also lead to a raise of an immune response that can be harmful for healing process (Ahangar et al., 2020). Moreover, there are side effects that are associated to MSC administration such as transient fever, constipation, and fatigue. Neither serious adverse events nor mortality were discovered across the clinical studies (Wang et al., 2021).

On the contrary, secretome provides an option for cell-free therapy with lower immunogenicity reaction. Secretome has the advantage that it can be prepared ahead in larger quantities and immediately become available for application (Xia et al., 2019). Despite the fact that it is easier to produce, handle, and store compared to the viable cells, it also has several drawbacks and challenges to bring it to bedside application (González-González et al., 2020).

To date, there is still no clinical trials have been registered in the clinicaltrials.gov that used the mesenchymal stem cells secretome or conditioned medium, whether to treat diabetic condition such as to control the hyperglycemia or to treat the diabetic complications cases. In brief, here are some of the challenges with secretome especially secretome from MSCs as therapeutic product. First, the characterization of secretome is needed. Due to the composition of secretome, it has become highly challenging to define specific function of each components and quantify the activity (Ahangar et al., 2020; Vizoso et al., 2017). Secondly, the inconsistency during the preparation of secretome from the MSCs. It is well-known that there are many factors that could affect the quality and efficacy of the secretome. Health condition and age of the donors, also the methods for isolation and culture the MSCs, are some of the factors that must be considered. The donor should be strictly screened and free from hepatitis B virus and human immunodeficiency virus (HIV) (Ahangar et al., 2020; Lukomska et al., 2019). The source of the MSCs is also one of the hurdles, some of it possibly due to ethical issue. For example, the usage of human fetal which obtained from the abortion procedure, although it has unique properties, has ethical issue for clinical application. Conversely, human umbilical cord should be more suitable as source of MSCs because it was categorized as clinical waste, therefore, there are no ethical issue to use the umbilical cord tissue (Wang et al., 2023). The other challenges regarding inconsistency are the heterogenicity of the MSCs, number of cells, and the interval of time. The most crucial part of the challenges is to produce the secretome under pharmaceutical standard and in the Good Manufacturing Practice (GMP) certified

facility. The secretome production under good manufacturing protocols can improve the consistency from one batch to another and importantly the efficacy of the secretome can be reproducible (Ahangar et al., 2020; De Sousa et al., 2016). The other concern part of secretome application is the potential side effect of the secretome administration. Despite the fact that there are only a few reports regarding the negative effects or even adverse events of secretome, there is always a risk that potentially happens when administering foreign substance. One of the problems is the immunosuppressive properties that has been reported in some studies (Zhao et al., 2016). Therefore, there is probability that the usage of secretome could cause immunodeficiency and poses risk to an infection (Bascones-Martinez et al., 2014). Hence, the optimum dosage for secretome administration should be clearly specified to have the balance of efficacy and safety of this secretome based treatment (Ahangar et al., 2020). Another concern regarding the instability and half-life of the protein contained in the secretome could be overcome by pre-conditioning the cells to increase the paracrine activity and production of the cells (Park et al., 2018). The alteration, namely hypoxia, inflammatory stimulus, or even the usage of bioreactors on preconditioned cells, was also reported to be related to increases the therapeutic potential of secretome (Pinho et al., 2020). We have proved that pre-conditioning such as culturing the cells in a hypoxic condition will increase the growth factor production in the secretome compared to the cells culture in normal condition (Hendrawan et al., 2021). Besides the challenges, there are concerns such as the route of administration, dosage, and duration of secretome application that need to be determined and standardized. The most common route of administration is topical for wound healing treatment (Fui et al., 2019). For other therapy, there is pre-clinical trial that used injection (intramuscular, intravenous) as the delivery route. However, there is still no clinical trials for diabetes that administered secretome as its therapy. Related to dosage, it will become one of the difficult challenges to calculate the generalized

dosage and duration needed for diabetes related disease. Although there is a study that has demonstrated the repeated administration of secretome can increase the duration of secretome effects as we have described above, the available data is still very limited. Furthermore, for wider clinical application, it is necessary to apply the precautionary principle and based on scientific evidence on safety and efficacy. It is also necessary to increase the education of the general public on how to interpret and apply these new findings. In Indonesia, the support from the government is obvious and have already regulated the provision of stem cell services which has all been stated in Indonesian Minister of Health Regulation Number 32, 2018 (Permenkes No. 32, 2018). However, there are still limited GMP certified facilities that has been established in the country. Limited budget is also one of the biggest concerns, which is why there is still a long way to go to bring the secretome to bedside application widely in Indonesia. Hopefully, the government concern to decrease the number of diabetes related complications could ease the way of secretome clinical application in Indonesia. In conclusion, there are numerous treatments for diabetes; nonetheless, the complications of this disease are still happening.

There are evidences that in most of the pre-clinical and clinical trials that used stem cell-based therapy which showed positive results, especially regarding diabetic wound healing treatment. While there are many challenges, this therapy is highly potential and very promising as alternative therapy for diabetes and its complications in translational medicine. New strategies are needed for overcoming limitations of stem cells and its secretome to be applied in a broad field of disorders, such as the use of encapsulated stem cells (Freimark et al., 2010) and nanotechnology (Zaghary et al., 2021). Moreover, cooperation among all stakeholders is essential to accelerate clinical applications. Good clinical trials to prove safety and actual efficacy of stem cell therapy are required to rush application and development of commercialized products.

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Chapter 5

Mesenchymal Stem Cells and Their Conditioned Medium: For Skin Aging

Winawati Eka Putri
Cita Rosita Sigit Prakoeswa

A. Introduction

Skin is an organ that acts as a barrier to the human body and is one of the most commonly observed indicators of aging. Aging is a complex inevitably process happens to all living things. It occurs naturally but can also be accelerated by various factors both intrinsic and extrinsic. Extrinsic factors like ultraviolet radiation can cause skin damage, which results in the appearance of signs of aging or commonly referred to as photoaging. Photoaging overlaps with intrinsic aging. Some common signs of photoaging include brown spots, wrinkles, broken capillaries, decreased elasticity, and uneven skin texture. The severity of photoaging damage depends on how much the skin is protected from ultraviolet light. Various efforts can

W. E. Putri, C. R. S. Prakoeswa
Universitas Nahdlatul Ulama Surabaya, e-mail: winawatiekaputri@gmail.com

Putri, W. E., & Prakoeswa, C. R. S. (2025). Mesenchymal stem cells and their conditioned medium: For skin aging. In B. Supartono, & A. Noviantari (Eds.), *Discovering the miracle of stem cells* (97–115). BRIN Publishing. DOI: 10.55981/brin.1128. c1299, E-ISBN: 978-602-6303-50-9

be made to reduce the signs of photodamage on the skin, such as the use of pharmacotherapy, both systemic and topical, and, recently, the use of stem cells. The application of mesenchymal stem cells and their conditioned medium (CM) provide promising and effective treatment to rejuvenate aged skin.

B. Mesenchymal Stem Cells (MSCs)

Since the discovery of stem cells (SCs) in the medical world, many studies have been conducted to find therapy for disease using stem cells, including in dermatology and plastic surgery, because of their ability to repair and rejuvenate various tissues (Bashir et al., 2019; Ogliari et al., 2014). Stem cells are a unique cell population of undifferentiated cells. Stem cells have three characteristics, including the ability to divide, the ability to regenerate, and plasticity (the ability to produce other types of cells, different from the cell of origin) (Hasegawa & Ikeda, 2017; Rantam et al., 2014). Stem cells can generally be found in various body tissues, from embryonic to adult. Stem cells are classified into embryonic and adult stem cells (Hasegawa & Ikeda, 2017; Ogliari et al., 2014; Prodinger et al., 2017; Rantam et al., 2014). Embryonic SCs are found in the blastocyst, while adult SCs are found in the forming fetus and play a role in repairing more specialized tissue damage. Based on their origin and ability to differentiate, adult SCs are divided into hematopoietic and mesenchymal stem cells (MSCs) (Hasegawa & Ikeda, 2017; Ogliari et al., 2014; Rantam et al., 2014).

Mesenchymal stem cells are progenitor cells of the mesoderm. The name was proposed by Arnold Caplan in 1991 for its ability to differentiate into more than one cell that forms connective tissue. Mesenchymal stem cells are identified based on the minimum criteria given by the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) in 2005: (1) adherent cell plasticity with self-renewal ability; (2) expression of cluster of differentiation 90 (CD90), CD73, CD105, and CD36, and no expression of CD11b, CD45, CD31, and CD106; (3) in vitro differentiation potential into osteogenic, adipogenic, and

chondrogenic lineages (Andrzejewska et al., 2019; Fitzsimmons et al., 2018; Hasegawa & Ikeda, 2017; Hu et al., 2018; Lavoie & Rosu-Myles, 2013; Trzyna & Banaś-Ząbczyk, 2021). Mesenchymal stem cells are the most researched adult stem cells for clinical trials in various diseases. These stem cells have immunomodulatory, anti-inflammatory, low immunogenicity, and high immunosuppressive properties that can be effective as therapy in autoimmune and inflammatory diseases (atopic dermatitis, psoriasis and lupus erythematosus, vitiligo and alopecia areata) (Baldari et al., 2017; Hasegawa & Ikeda, 2017). The use of MSCs for tissue regeneration is possible due to the trophic, paracrine, and immunomodulatory properties of these SCs (Damayanti et al., 2021; Pokrovskaya et al., 2020). The ability of MSCs in terms of cell proliferation and differentiation, paracrine signaling, and tissue repair depends on the donor's age, patients with diabetes, obesity, and cardiovascular disease (Baldari et al., 2017).

There are various GFs, such as EGF, b-FGF, TGF-b, and growth differentiation factor (GDF)-11 which play roles in rejuvenation. In addition, MSCs also have antioxidant activity that can increase the levels of endogenous antioxidants, which provides protection to dermal fibroblasts and keratinocytes against oxidative stress (Bashir et al., 2019). Mesenchymal stem cells can produce angiogenesis through VEGF, while the regeneration process can be produced by GFs that have anti-apoptotic effects, including HGF, insulin-like growth factor-1 (IGF-1), VEGF, Cytokine-Induced Neutrophil Chemoattractant-3 (CINC-3), TIMP-1, TIMP-2, osteopontin, growth hormone (GH), bFGF-BP, brain-derived neurotrophic factor (BDNF), TGFα, HGF, EGF, NGF, IGF-binding proteins-1 (IGFBP-1), IGFBP-2, macrophage colony-stimulating factor (M-CSF). Growth factors from MSCs can also reduce tissue fibrosis during regeneration, such as keratinocyte growth factor (KGF), HGF, VEGF, angiopoietin-1, stromal cell-derived factor-1 (SDF-1), IGF-1, EGF, HGF, NGF, and TGFα (Andrzejewska et al., 2019; Harrell et al., 2018). Mesenchymal stem cell sources can be isolated from various tissues such as bone marrow (BM-MSCs), umbilical cord (UC-MSCs), placenta (P-MSCs), umbilical cord

blood (UCB-MSCs), amniotic fluid (AF-MSCs), and adipose (ASCs) (Andrzejewska et al., 2019; Hasegawa & Ikeda, 2017; Hu et al., 2018).

Bone marrow mesenchymal stem cells have been widely used for wound healing (Huynh et al., 2022; Karina et al., 2021; Revilla & Mulyani, 2020; Yonghong et al., 2022). These cells acts to shorten wound healing time, increase VEGF expression, and also increase the number and density of blood vessels in the wound area. (Karina et al., 2021; Yonghong et al., 2022). Epidermal growth factor and epidermal growth factor receptor (EGFR), which play a role in keratinocyte proliferation and migration in wound healing, were reported to increase by Revilla and Mulyani (2020). However, due to their disadvantages of being invasive and associated with complications during BM-MSC source collection, like infection and bleeding, BM-MSC is slowly being replaced by other SCs such as UC-MSC, UCB-MSC and other ASCs (Fitzsimmons et al., 2018; Hasegawa & Ikeda, 2017; Hu et al., 2018).

Umbilical cord-derived MSCs show promising therapeutic effects due to their immunological compatibility, long-term survival, high differentiation potential, and easy manufacturing process. In vitro and in vivo studies for wound healing showed promising results using UC-MSC (Dehkordi et al., 2019). In vivo studies show that UC-MSCs regulate SOD and MDA levels also Col-1 and VEGF expression in aging skin. An in vitro study shows UC-MSCs work by performing cell migration, inhibiting ROS production, and reducing oxidative stress expression through paracrine-mediated autophagy inhibition in aging human fibroblasts (Li et al., 2022). These SCs also increase collagen and fibroblasts in photoaging (Kencanawati et al., 2021).

Adipose stem cells are pluripotent cells that can differentiate into various cell types (Dehkordi et al., 2019; Fitzsimmons et al., 2018; Hasegawa & Ikeda, 2017; Hu et al., 2018). The collection site and type of adipose tissue may affect the proliferation, endocrine function, gene expression, surface antigens, and differentiation potential of ASCs (Trzyna & Banaś-Ząbczyk, 2021). The ASCs are easily obtained, easier to generate, more abundant than other types of MSCs, and have fewer ethical controversies than BM-MSCs (Dehkordi et al., 2019; Hu et

al., 2018). This type of SC is used as a therapy for various diseases due to its paracrine and autocrine properties and by enhancing the recruitment of endogenous precursors (Trzyna & Banaś-Ząbczyk, 2021). Adipose stem cells produce a series of GFs, such as VEGF, bFGF, TGF-β1, TGF-β2, HGF, KGF, platelet-derived growth factor AA (PDGF-AA), and placental growth factor (PGF) (Trzyna & Banaś-Ząbczyk, 2021; Zhang & Duan, 2018).

C. Mesenchymal Stem Cell-Conditioned Medium (MSC-CM)

The development of mesenchymal stem cell-conditioned medium was carried out due to the limitations of MSC in terms of low viability and mode of transport (Andrzejewska et al., 2019; Damayanti et al., 2021; Dehkordi et al., 2019; Harrell et al., 2018; Pokrovskaya et al., 2020). The advantages of MSC-CM are that it is easy to obtain, more economical, and can be produced, packaged, and transported faster (Yang et al., 2021). The primary mechanism of action of MSCs is its paracrine effects of various GFs or cytokines (Damayanti et al., 2021). There are several approaches to optimize MSC-CM, also called preconditioning, including modulation of culture conditions (hypoxia or anoxia), 3D culture, the addition of trophic factors (GFs, cytokines or hormones), lipopolysaccharides and other pharmacological agents. This preconditioning causes the number of MSCs to multiply and produces immunomodulatory or immunosuppressive, anti-apoptotic, pro-angiogenic, and trophic effects (Baldari et al., 2017; Schäfer et al., 2016; Seo et al., 2019).

Mesenchymal stem cell-conditioning medium or secretome consists of GFs, cytokines, chemokines, ECM components, proteins involved in the adhesion process, enzyme activators or inhibitors (Andrzejewska et al., 2019; Harrell et al., 2018; Pokrovskaya et al., 2020). The paracrine activity of MSCs is also associated with their ability to produce extracellular vesicles (EV), including exosomes, microvesicles, and apoptotic bodies. The biological activity of EVs is comparable to that of MSCs (Andrzejewska et al., 2019). Damayanti

et al. (2021) reviewed MSC-CM in the field of dermatology and reported that MSC-CM is used for wound healing, photoprotection, hair growth, and also as an antimicrobial in skin wounds. A systematic review by Putri, Endaryanto, Rantam, et al. (2021) reported that eleven clinical or in vivo studies using MSC-CM showed improvements in clinical outcomes with or without histological examination in skin aging.

1. Bone Marrow Stem Cell-Conditioned Medium (BMSC-CM)

Similar to its SC origin, BMSC-CM has also been widely used for wound healing (Aryan et al., 2018; de Mayo et al., 2017). Balasubramanian et al. (2017) found that most factors in BMSC-CM play a role in fibroblast proliferation (FGF-7/KGF, PDGF), angiogenesis (VEGF, HGF, IGF-1, Ang-1), cell migration (SDF-1α, M-CSF) and epithelialization (TGF-β1, FGF-7/KGF, laminin, TIMP-1, and TIMP-2), and anti-inflammation (PGE-2).

2. Amniotic Fluid Stem Cells-Conditioned Medium (AFSC-CM) and Amniotic Membrane Stem Cell-Conditioned Medium (AMSC-CM)

Previous studies indicated various amniotic fluid stem cells-conditioned medium utilizations such as reduced alzheimer disease-like pathologies in the human neuronal lineage, accelerated cutaneous wound healing, and supressed breast cancer cell (Hasanpour et al., 2022; Jun et al., 2014; Pashaei-Asl et al., 2022). Study by Yoon et al. (2010) showed that AFSC-CM contains high levels of growth factors, cytokines, and chemokines, such as TNF-α, VEGF, TGF-β, Leptin, IL8, and IL-6.

Several clinical studies using AMSC-CM have been conducted. One of them is for acne scars conducted by El-Domyati et al. (2019). They reported that there was an improvement in acne scars using micro-needling and AMSC-CM and an improvement in the shape of collagen and elastin fibers.

3. Chorion-Derived Stem Cell Conditioned Medium (CDSC-CM)

Chorion-derived stem cell has similar functions to ASCs, namely promote human fibroblast growth and secretes growth factors that affect wound healing (Kim et al., 2015). A study by Kim et al. (2015) demonstrated that CDSC secretes various growth factors, such as IL-6, IL-8, MCP-1, and regulated upon activation, normal T cell expressed and presumably secreted (RANTES) growth factors that were measured in higher concentration in CDSC-CM.

4. Umbilical Cord Stem Cell-Conditioned Medium (UCSC-CM) and Umbilical Cord Blood Stem Cell-Conditioned Medium (UCB-CM)

Study on wound healing conducted by Sunarto et al. (2020) using Wistar rats showed that topical UCSC-CM gel from rats could increase VEGF levels from day three of therapy, meanwhile a decrease in VEGF levels was reported on day nine. Collagen density assessed by Masson's trichome staining was reported to increase significantly. Studies on UCSC-CM in skin aging conducted by Liang et al. (2022) reported that the most abundant GF in UCSC-CM was EGF, followed by VEGF-A, HGF, FGF-2, VEGF-D, and PDGF-BB. In addition, a decrease in melanin index, UV spots, brown spots, wrinkles, pores, and increased skin elasticity were also obtained. Kim, Kim, Kim, et al. (2020) reported that the number of areas with micro crusts and erythema was reduced on patients' cheeks post fractional CO_2 laser after using UCSC-CM.

5. Adipose Stem Cell-Conditioned Medium (ASC-CM)

One of the strategies to optimize ASC is to use CM or secretome with a more specific form, namely EV (Trzyna & Banaś-Ząbczyk, 2021). The in vitro study by Guo et al. (2020) reported that the storage life of ASC-CM is less than 13 weeks. The study by Sun et al. (2014) created a hypoxic environment for ASCs by using an oxygen absorber, that caused the oxygen concentration to be less than 1% and the carbon

dioxide concentration to be around 20%. The hypoxic environment exposed to ASCs resulted in CMs containing increased levels of VEGF and/or HGF, as well as GF and other differentiation factors, such as TNF-α, FGF, and EGF (Bertozzi et al., 2018). Studies by Moon et al. (2012) and Son et al. (2015) found that ASC-CM contained HGF, FGF-1, GM-CSF, IL-6, VEGF, and TGF-β3. A subsequent study reported highest concentration of PDGF in ASC-CM (Dubey et al., 2018). PDGF-AA was also reported became a major component of ASC-CM. PDGF-AA, an isoform of PDGF, is bound to the PDGF-α receptor. It increases dermal fibroblast proliferation and elastin expression (Guo et al., 2020). Although not in high amounts, ASCs also secrete GDF-11, which can promote proliferation, cell differentiation, migration, and secretion of ECM, which is important for the repair of skin aging (Mazini et al., 2020).

Studies on wound healing using ASC-CM have been conducted (Alinda et al., 2022; Lee et al., 2014; Moon et al., 2012; Sun et al., 2014). The ability of ASC-CM in cell adhesion and migration plays a role in wound healing and tissue remodeling (Moon et al., 2012; Sun et al., 2014; Xu et al., 2014). These CM also increased the expression of type I collagen, angiogenesis as well as circulating SC capture. Skin graft studies from the skin of C57BL/6 mice transferred to BALB/c mice using ASC and ASC-CM injection showed a significant decrease in proinflammatory cytokines (IL-6). Both cytokine gene expression of interferon-λ (IFN-λ), IL-2, and TNF-α was also decreased (Lee et al., 2014). On histopathologic examination, Lee et al. (2014) also found fewer macrophages and decreased VEGF were seen after ASC or ASC-CM therapy.

D. Application and Risks in Skin Aging

The widely used MSCs and their conditioned medium administration method for skin aging treatment is subcutaneous injection. The use of microneedle as tool to apply MSC-CM was also reported in some studies. Various treatments utilizing stem cell-conditioned medium have resulted in improvements in aging skin. Alhaddad et al. (2019) used

BMSC-CM cream from red deer on photoaging patients and reported improvements in photoaging. An in vitro study by Balasubramanian et al. (2017) reported antiaging potential in UVB-irradiated human fibroblasts using BMSC-CM. These CM improved collagen, elastin, and hyaluronic acid levels and reduced ROS compared to the control. Another study, both in vivo and in vitro, by Amirthalingam et al. (2019) reported the formation of cyclobutene pyrimidine dimers (CPD), which cause primary lesions in UV-irradiated DNA, can be prevented by BMSC-CM. They showed that these CM extensively protected mouse skin from toxic UVB irradiation.

The in vitro study by Huh et al. (2014) used human amniotic fluid stem cells-conditioned medium on UVA-irradiated dermal keratinocytes and fibroblasts. The study reported increased proliferation of dermal keratinocytes and fibroblasts. The study also showed decreased expression of MMP-1 and increased procollagen 1A. This CM can repair cell damage caused by UVA. Parrado et al. (2019) showed that AMSC-CM increased CAT and decreased MDA while also blocking the cell cycle. This may delay premature aging due to oxidative stress.

A study by Li et al. (2016) reported that chorion-derived stem cell conditioned medium (CDSC-CM) contains EGF, TGF-β, IL-6, and IL-8. They also reported that a CDSC-CM could increase proliferation in UVB-irradiated keratinocytes. In addition, this CM increased the number of cells in S and G2/M phases and cell migration and myeloid cell leukemia-1 (MCL-1) protein and modulated the extracellular signal-regulated kinases (ERK) 1/2 signaling pathway. Decreased ROS and DNA damage were also reported in keratinocytes treated with CDSC-CM.

The in vitro study by Kim et al. (2018) using HDF showed that topical UCB-CM exhibited anti-wrinkle effects and significantly increased skin density. The study found that the anti-wrinkle effect was due to increased cell migration and the synthesis of type I and type III collagen. The increase was higher in UCB-CM than in HDF-CM and ASC-CM. They also reported that the secretion of GDF11

(rejuvenation factor) was highest in UCB-CM. Another in vitro study by Dewi and Sandra (2019) used mice fibroblasts that were given UCB-CM and then irradiated with UVB. The use of UCB-CM before UVB irradiation can significantly reduce the percentage of cell apoptosis.

Adipose stem cell conditioned medium is also used as a therapy for skin aging (Kim, Kim, Lee, et al., 2020; Lee et al., 2021). The use of ASC-CM produces a protective effect against skin damage caused by ROS in the form of increased SOD and GPx activity in dermal fibroblasts (Hong et al., 2019; Park & Kim, 2010; Widowati et al., 2022; Xu et al., 2014). This mechanism upregulates Nrf2 expression so that the expression of antioxidant enzymes such as SOD-1 also increases. The ASC-CM can increase the migration and proliferation of keratinocytes and fibroblasts by reducing the number of cells in the S and G2 phases and increasing the number of cells in the G1 phase therefore increasing dermis thickness (Moon et al., 2012; Noverina et al., 2019). Study by Xu et al. (2014) and Putri et al. (2022) reported that dermis thickness increased significantly after the use of ASC-CM. A decrease in epidermal thickness was also found (Putri et al., 2022). Adipose stem cell conditioned medium was given by Kim, Kim, Lee, et al. (2020) to middle-aged women while Putri, Endaryanto, Tinduh, et al. (2021)'s was given to photoaging model Wistar rats. They reported similar improvement in skin moisture and transepidermal water loss (TEWL). Kim, Jung, et al. (2020) also found that melanin, erythema on the cheeks, and eye wrinkles were decreased.

Currently there were no reports on MSC-CM negative effect on its application, but there was adverse effect reported by Prakoeswa et al. (2019) in the form of slight erythema and urticaria due to microneedle used to administer MSC-CM.

E. Conclusion

The use of mesenchymal stem cells and their conditioned medium as a skin aging therapy continues to attract researchers' attention. Recent studies have shown positive results about their use on skin aging.

Based on recent studies, MSCs and MSC-CM proved safe to use. The limitation faced is the absence of standardized procedures in making stem cells conditioned medium and the high cost of production. Further studies are needed on the appropriate manufacture and administration technique of MSCs so that they can provide maximum benefits for treating skin aging.

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Chapter 6

Stem Cells for Acute Myocardial Infarction: Safety and Efficacy

Teguh Santoso, Idrus Alwi, Cynthia Retna Sartika, Cosphiadi Irawan, Dewi Wulandari, Ika Prasetya Wijaya, Eka Ginanjar, Elizabeth Merry Wintery, Mohamad Syahrir Azizi, Aw Tar Choon, Bayu Winata Putera, Yanni Dirgantara, Angliana, Ditta Kalyani Devi, Nadya Karina, Rima Haifa, Nabilla Farah Naura, Billy Yosua Costantin Pongajow, Marsya Nilam Kirana, & Atikah Anwar Hasibuan

A. Introduction

ST-segment elevation myocardial infarction (STEMI) is a leading cause of subsequent mortality worldwide. Rapid reperfusion of infarct-related arteries, either with thrombolytic therapy or primary percutaneous coronary intervention (PCI) with stent implantation, has been demonstrated to improve the prognosis of patients with STEMI significantly. However, fewer than 50% of patients with STEMI currently achieve adequate reperfusion before irreversible damage to the supplied myocardial tissue occurs. It is known that

T. Santoso, I. Alwi, C. R. Sartika, C. Irawan, D. Wulandari, I. P. Wijaya, E. Ginanjar, E. M. Wintery, M. S. Azizi, A. T. Choon, B. W. Putera, Y. Dirgantara, Angliana, D. K. Devi, N. Karina, R. Haifa, N. F. Naura, B. Y. C. Pongajow, M. N. Kirana, A. A. Hasibuan

Medistra Hospital, e-mail: tsantoso@cbn.net.id

Santoso, T., Alwi, I., Sartika, C. R., Irawan, C., Wulandari, D., Wijaya, I. P., Ginanjar, E., Wintery, E. M., Azizi, M. S., Choon, A. T., Putera, B. W., Dirgantara, Y., Angliana, Devi, D. K., Karina, N., Haifa, R., Naura, N. F., Pongajow, B. Y. C., Kirana, M. N., & Hasibuan, A. A. (2025). Stem cells for acute myocardial infarction: safety and efficacy. In B. Supartono, & A. Noviantari (Eds.), *Discovering the miracle of stem cells* (117–142). BRIN Publishing. DOI: 10.55981/brin.1128.c1300, E-ISBN: 978-602-6303-50-9

myocardial necrosis starts rapidly after coronary occlusion, usually before reperfusion can be achieved. Since adult cardiac myocytes cannot routinely re-enter the cell cycle and proliferate, the heart's capacity for self-repair to compensate for the massive loss of cardiac myocytes is limited. The loss of viable cardiac myocytes during STEMI subsequently initiates a process of adverse left ventricular (LV), characterized by mechanical expansion of the scarred, infarcted myocardium, followed by progressive LV dilatation and dysfunction, culminating in heart failure and death. Additionally, failed reperfusion can also lead to potential ischemia-reperfusion (I/R), which can further damage cardiac myocytes through ferroptosis activity within the cells due to increased iron accumulation during ischemic myocardium, leading to an increased myocardial oxidative stress and cell death (Song et al., 2020).

Furthermore, increased oxidative stress from I/R could induce high levels of Interleukin-18 (IL-18) and potentially aggravate inflammation and tissue damage, leading to more acute coronary syndromes, myocardial dysfunction, and eventual heart failure (Huang et al., 2020). Indeed, in chronic heart failure and after myocardial infarction, most of the compensatory gain in myocardial mass results from hypertrophy rather than cell division (Kalra et al., 2018). Various pharmacological agents have been demonstrated to reduce early mortality rates and the risk of further heart attacks. However, especially in the case of large areas of infarction, other treatments for the regeneration of damaged cells after STEMI are deemed necessary (Botleroo et al., 2021). Cell therapy has emerged as a promising alternative strategy for the regeneration of cardiac cells. The rationale for cell therapy to be administered after STEMI derives from the assumption that given the insufficient regeneration in the injured heart tissue, those cells are expected to be able to replace or repair damaged vascular and cardiac tissue, or even secrete exosomes targeting specific mechanisms causing myocardial cell damage and inhibiting those mechanisms to prevent further damage of the heart tissue (Fisher et al., 2015; Seitz et al., 2019).

B. Factors that Influence the Succes of Stem Cell Therapy

The success of stem cell therapy for clinical use remains to be validated and many issues must be elucidated. Those issues, among others, are selection of appropriate types and number of stem cells, routes of administration, assessment of response to cell therapy, survivability rate due to site environment stresses as well as regulatory, and ethical issues (Amiri et al., 2015; Satessa et al., 2015).

1. Source

Mesenchymal stem cells (MSCs) are a choice of cell therapy due to multipotent factors, survival ability, plasticity, engraftment, paracrine activity, and low immunogenic potential (in allogeneic use). The cellular mechanism of MSCs therapy in cardiac regeneration is still unclear, but is thought to be through several mechanisms, including inflammatory response and angiogenesis, antifibrotic, differentiation of stem cells into new cardiomyocytes, and autophagy processes. Many clinical trials have been conducted to investigate the efficacy of MSCs derived from various tissue types. MSCs can be isolated from bone marrow, adipose tissue, and umbilical cord. Adult allogeneic MSCs derived from bone marrow are the type most widely used in the treatment of cardiovascular disease. However, many researchers are turning to use mesenchymal stem cells from the umbilical cord (UC-MSC) because their advantages include being easy to obtain without invasive procedures, having a primitive phenotype, and having long telomeres so the potential for proliferation and differentiation is better. The study by Gao et al. (2015) reported that post-transplant UC-MSC clinical events were not significantly different compared to the placebo group but death after 18 months of follow-up was found in the placebo group, whereas in the UC-MSC group, there were no deaths, recurrence of myocardial infarction, thrombosis, cerebral infarction or arrhythmias. Another study conducted on humans reported that there was a decreased scar size, an improvement in tissue perfusion, and an

increase in regional function after being injected with mesenchymal stem cells via intramyocardially (Mahmud et al., 2022).

Some preclinical studies have demonstrated that UC-MSC expressed cardiac-specific molecules, enabling them to readily differentiate into cardiomyocyte-like and endothelial cells in vitro (Jung et al., 2017). Paracrine effects exerted by UC-MSC may also enhance vascular regeneration and cardiomyocyte protection, such as through the upregulation of various types of exosomes in the form of miRNAs, which was shown to inhibit cardiomyocyte apoptosis, reducing the fibrotic area, inhibiting ferroptosis and improving the cardiac function (Bartolucci et al., 2017; Huang et al., 2020; Song et al., 2020; Zhu et al., 2020). From a genetic standpoint, several studies state that MSCs maintain genetic stability throughout the entire in vitro culture phase. A study by Zamani et al. (2022) showed that in different passages of culture, there was no significant abnormality was observed in the karyotype and morphology of MSCs. Based on the explanation above and in attempting to enhance the effects of stem cell therapy, we choose to use allogeneic umbilical cord mesenchymal stem cells (UC-MSC) for this study. Allogeneic mesenchymal stem cell therapy is confidently secure for administration, fortified by a meticulous donor selection process and an abundance of research findings consistently reaffirming its safety. The allogeneic properties of MSCs offer the possibility of early administration as an "off-the-shelf" product and they can be subjected to higher quality control than autologous products (Crisostomo et al., 2015).

2. Route of Administration and Dose

The delivery method of stem cells also plays a crucial aspect in therapeutic efficacy. Intracoronary and intramyocardial routes are two routes that have been mainly used (Hénon, 2020). Based on the Transplantation of Progenitor Cells and Regenerative Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) study on 20 patients who were administered stem cells via intracoronary using a stop-flow

technique with an over-the-wire balloon, it was reported to be safe, without experiencing thrombotic complications, arrhythmias, or other cardiovascular events. A study by Zhang et al. (2021) used bone marrow MSCs via intracoronary and showed no adverse reactions, such as stent thrombosis, recurrence of myocardial infarction, arrhythmia, tumor, and myocardial fibrosis. In his study, an ultra-long guide wire was inserted, followed by the insertion of a balloon catheter distal to the stent. Then, the ultra-long guide wire is removed, and the balloon catheter is inflated with pressure until there is no blood flow to the distal target vessel (balloon inflation period). A study by Kim et al. (2018) provides bone marrow MSCs in patients with acute myocardial infarction via intracoronary. The results reported that there was no adverse event with significant improvement in LVEF 4-month and 12-month follow-up. Based on the explanation above, it can be concluded that intracoronary administration is feasible and safe. In this study, we used a dose of 50 million cells as previously reported experience with this number of large-size stem cells after STEMI has been safely reported for use (Bobi et al., 2017).

The intravenous route is also used for therapy, but only in a few trials and is most often used in experimental small-animal studies (Hénon, 2020). The intravenous route is the most convenient and least invasive. It can be used because of the preponderance of physiological homing signals of stem cells to migrate toward the site of injury. Strategies of intravenous infusion are particularly appropriate for recently infarcted as well as reperfused myocardium. The study of Charles et al. (2020) administering exosome MSCs intravenously to pigs showed a reduction in infarct size of ~30%–40% in the exosome group on day 28, with a value of infarct size (5.96 ± 0.99% for exosome group vs. 10, 23 ± 1.02% for control, $p < 0.01$). In a review article by Razeghian-Jahromi et al. (2021), doses of 0.5, 1.6, and 5 million cells/kg were shown to be safe for intravenous administration. Therefore, for intravenous administration in this study, we used a dose of 2×10^6 cells/kg.

3. Preparation of Stem Cells and Timing of Administration

The stem cells that are used for therapy must meet the requirements for clinical application. However, the lack of standardized methods for isolating, expanding, and validating stem cells presents significant obstacles and challenges to producing high-quality stem cells.

For this study, the stem cells were provided by PT. Prodia StemCell Indonesia and the Ethics Committee of the Faculty of Medicine, University of Indonesia (No. 245/UN2.F1/ETIK/PPM.00.02/2019), with regards to the protection of human rights and welfare in medical research has reviewed and approved the research protocol and information of the potential patients. The baby's umbilical cord was taken aseptically from a healthy donor who had gone through a screening panel for HBsAg, HCV, Anti-HIV, TPHA, Anti-CMV IgG, Anti-Toxoplasma IgG, and clinical evaluation of disease factors. The baby's umbilical cord will be taken by cutting it about 10 cm long. The umbilical cord obtained was mixed into growth media containing 10% DMEM and HPL (human platelet lysate), then incubated at 37°C and 5% CO^2 for approximately 3 weeks. Every 3–4 days, the medium is replaced, until the cells reach a confluence of about 80%. The stem cells are then expanded until passage 5 and proceed towards quality control before use including identity, viability, purity, potency, and stability. Identity testing includes morphology, immunophenotyping, and differentiation ability of MSCs. MSCs have a spindle-shaped morphology, like fibroblasts, can express CD90, CD73, CD105, and Lin Negative, and have the ability to differentiate into adipocytes, chondrocytes, and osteocytes. Purity testing includes the detection of endotoxin and mycoplasma. Endotoxin and mycoplasma detection results must show negative results. Potency testing includes the ability of the SPM to produce paracrine effects. Stability testing includes karyotype examination. For viability, the minimum criteria are >80%.

For timing administration, the optimal timing of injection is best before scar formation in the ventricular wall. However, too early of

administration should be avoided as physiological mechanisms at that time are conducive for cells to migrate into an inflammatory microenvironment, which makes it unfavorable for cell survival and reduces its efficacy. A potential option to circumvent this issue of cell survivability and efficacy loss during early administration is by employing additional strategies that strengthen the stem cell during transplantation. Several available strategies include the pre-treatment of stem cells with serum deprivation (SD), hypoxia, some pharmacological agents, or even cryoprotective factors (Baldari et al., 2017). In a study done by Alijani-Ghazyani et al. (2020), overexpression of the cryoprotective factor Lipocalin 2 (Lcn2) is able to increase MSC survivability by acting as an antioxidant, anti-apoptotic, and anti-inflammation for the stem cells. Furthermore, the potential loss of homing mechanism of the stem cell during transplantation into STEMI is improved through Lcn2, as the factor is shown to increase stem cell adhesion capability and provide stress protection. Various other reported benefits of Lcn2 assisting in MSC transplantation in STEMI are mitigating LV remodeling, alleviating I/R-induced tissue damage, and reducing apoptotic cells due to oxidative stress. Although Lcn2 has shown promising novel outcomes, the proper mechanism of Lcn2 protective mechanism is still being researched and its effects are known mostly in non-clinical studies, though there are other experimental clinical studies on different diseases (Alagesan et al., 2022; Hermann et al., 2019; Roudkenar et al., 2018). The study by Yang et al. (2023) reported that the best time for MSCs transplantation was 1 week after infarction. Therefore, for the injection timing in this study, we chose between 6 and 7 days after STEMI.

C. Study Treatment and Follow-Up Results

In this study (number KET-245/UN2.F1/ETIK/PPM.00.02/2019), we reported four male patients with STEMI marked with symptoms, such as chest pain, cold sweat, and shortness of breath. The patient received either an intracoronary or intravenous umbilical cord-derived mesenchymal stem cells (UC-MSCs) injection. Administration via

intracoronary was administered by a stop-flow technique via an over-the-wire balloon catheter positioned within the stent portion while the intravenous route is injected directly into the vein using a syringe. All the written informed consent of patients was recorded, and this study was funded individually by the patient and with the assistance from PT. Prodia StemCell (ProSTEM), Jakarta, Indonesia.

1. Injection of UC-MSC

Case 1:

A 48-year-old male patient was admitted because of anterior STEMI. Left ventricular ejection fraction (LVEF) examined by MRI was 39%. One week after PCI, the patient was treated with around 50×10^6 UC-MSCs via the intracoronary route. There was no adverse event at two weeks post-transplantation.

Case 2:

A 45-year-old male patient was diagnosed with anterior STEMI and hypertension. Echocardiogram showed severe hypokinesis of the apical lateral and mid-septal wall with an LVEF of 45%. The LVEF was also 44% by MRI. After undergoing successful PCI, the patient was treated with intravenous UC-MSCs with a dose of 2×10^6/kg weight body (214×10^6) one week later. There was no adverse event at two weeks post-transplantation.

Case 3:

The patient was a 54-year-old male with acute anterior STEMI, hypertension, and diabetes mellitus. LVEF examined by MRI and echocardiogram was 20% and 24%, respectively. One week after PCI, the patient was treated with an intravenous infusion of 152×10^6 (2×10^6/kg weight body) of UC-MSCs. There was no adverse event that happened 2 weeks following the transplantation.

Case 4:

A 78-year-old male patient was diagnosed with anterior STEMI. On echocardiogram, the LV was grossly normal in size, and there was moderate to severe septal hypokinesis. The patient then underwent

Table 6.1 Summary of Case Illustration

Subject	Age	Gender	Diagnose	Intervention	Result			
					LVEF (%)		6MWT (m)	
					Before	After	Before	After
Case 1	48-year-old	Male	Anterior STEMI	Intracoronary	39	40	522	562
Case 2	45-year-old	Male	Anterior STEMI and hypertension	Intravenous	44	52	500	390
Case 3	54-year-old	Male	Acute anterior STEMI, hypertension, and diabetes mellitus	Intravenous	20	42	453	578
Case 4	78-year-old	Male	Anterior STEMI	Intracoronary	35	40	336	410

PCI and after the course of one week, was injected with 50×10^6 UC-MSCs via intracoronary route. The summary of case illustration is shown in Table 6.1.

2. Left Ventricular Ejection Fraction (LVEF)

The left ventricular ejection fraction (LVEF)–calculated as the stroke volume (end-diastolic volume minus end-systolic volume) divided by the end-diastolic volume–remains the main driver for categorizing heart failure (HF) and it is a cornerstone in all randomized clinical trials for patients with HF. Although LVEF has many acknowledged limitations, it remains key for the classification, stratification, management, and surveillance of HF during follow-up because it is easy to obtain and non-invasive. LVEF is a pivotal measure for managing HF by HF specialists and general cardiologists, but beyond cardiologists, it is well known and understood by a majority of internists, general practitioners, and geriatricians. Left ventricular ejection (LVEF) was examined before therapy, 6 months, and 12 months after therapy using MRI and echocardiogram. In CASE 1, LVEF examined by MRI was 39% and LVEF examined by echocardiogram was 38%, as a baseline. After six-month post-transplantation, an MRI examination showed a stable LVEF at 38%. At twelve months of observation by MRI, the LVEF remained unchanged at 37%. Echocardiogram before stem cells treatment showed septal and apical akinesis; with LVEF at 38%. Six

months of observation by echocardiogram showed an LVEF value of 40%. At 12 months, post-transplantation showed left ventricular dilatation and LVEF of 40%. In CASE 2, the echocardiogram showed severe hypokinesis of the apical lateral and mid-septal wall with LVEF of 45%. The LVEF was also 44% by MRI. Magnetic resonance imaging at 12 months demonstrated improvement of LVEF to 52%. Defects of the perfusion area and viability area were stable. Echocardiogram LVEF improved to 50% at 6 months. In CASE 3, LVEF examined by MRI and echocardiogram was 20% and 24%, respectively. The LVEF shown by MRI had increased from 20% to 37% in 6 months, and 42% in 12 months whereas the LVEF result shown by echocardiogram had increased from 24% to 37% in 6 months, and 45% in 12 months. In CASE 4, MRI showed LVEF improvement from 35% at baseline, 41% at 6 months, and 40% at 12 months after transplantation. At the same time, LVEF examined using an echocardiogram showed an improvement from 45% at baseline to 51% at 6 months, and 52% at 12 months (Figure 6.1).

Based on the result of our study, the overall LVEF was significantly higher in using the echocardiography method of measurement compared to that from MRI. Significant change of LVEF value through stem cell injection occurs mostly during the six months interval compared to the final 12 months, in which the value either increases or decreases significantly (MRI-6 months: +10.3; n=3; ECHO-6 months: +6.5; n=4), with CASE 1 being an exception for MRI, where the value consistently dropped across the 6–12-month intervals (MRI-6 months= -2). Prominent LVEF difference was also noticed between the measurement method, with MRI measuring lower LVEF compared to echocardiography. CASE 2 was an exception to these differences as the LVEF was higher in MRI compared to that of echocardiography (MRI-6 months: 52 > ECHO-6 months: 50).

In terms of scan accuracy, LVEF results from MRI scans seem to produce the most accurate results. In contrast, although echocardiographic measurements produce higher LVEF results, there is very low accuracy in its evaluation due to input results on

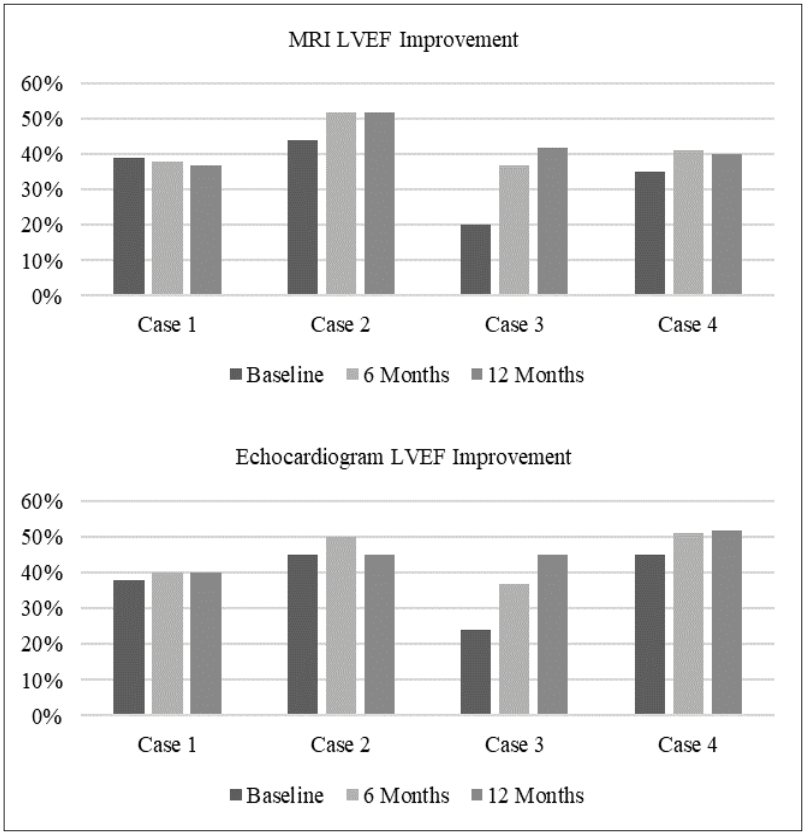


Figure 6.1 LVEF examined by MRI and echocardiogram.

the measurement having high heterogeneity and being subjective to each radiologist. Furthermore, echocardiography results have two cases identified as STQA (status quo ante) which has no significant or identifiable increase across the LVEF result measurement (CASE 1 and CASE 4), while MRI only has one case that is STQA (CASE 1), with the rest of the cases having a significant increase across the LVEF result. Additionally, using the heart failure guideline range (Heidenreich et al., 2022), categorical assessment of LVEF value is possible through the accurate result of MRI. From this, cases one to four was determined as reduce (n=<40; CASE 1: 39–37), mildly reduce

to preserve (n=40–50–n=>50; CASE 2: 44–52), and reduce to mildly reduce heart failure for both case three and four (n=<40–n=40–50; CASE 3: 20–42; CASE 4: 35–40).

Since there are limited participants in this case study, the discussion focus on stem cell changes, effects, and potentials towards myocardial infarction will be directed more towards individual case assessment, through various significant and interesting points within the MRI and ECHO scans data. Referring to MRI scans (Figure 6. 1), CASE 1 was shown to have no significant changes in allogeneic-umbilical cord mesenchymal stem cell (UC-MSC) treatment during the patient's LVEF across the 12 months of observation. According to the patient's MRI scans, the infarct size of the patient greatly exceeded the standard infarct size with a value that of >75%. This might suggest the decreased efficacy of the stem cell treatment leading to no significant LVEF changes during observation. Furthermore, the infarct size of other cases is only around the ≥50% range, hence the small increases in LVEF can be seen within the MRI scans data (Lee et al., 2020).

3. Quality of Life (Six-Minute Walking Test)

The six-minute walking test (6MWT) is a simple test that requires no specialized equipment or advanced training for physicians and assesses the submaximal level of functional capacity of an individual while walking on a flat, hard surface in a period of 6 min (6-minute walk distance; 6MWD). The 6MWT may be used as a tool for the measurement of a functional status of a patient especially in the case of advanced diseases with multiple comorbidities who cannot perform more complex exercise tests, such as patients with HF, chronic obstructive pulmonary disease, or cystic fibrosis. The prognostic role of 6MWT in terms of morbidity and mortality has been evaluated especially in patients with pulmonary arterial hypertension and in HF populations. The 6MWT should be performed preferably indoors, on a flat, straight, hard-surfaced corridor usually at least 30 m long. The 6MWT has been extensively used in various clinical studies in

the assessment of response to interventions in patients with HF as a measure to evaluate the effect of the treatment on a patient's functional status. It is considered to be an easy, widely available, and well-tolerated tool, yet with a questionable role in patients with HF, in contrast to populations of pulmonary arterial hypertension in whom 6MWT has been established as an important endpoint in clinical studies that led to therapy approval. The six-minute walking test was examined before therapy and at 2 weeks, 3 months, 6 months, and 12 months after therapy. In CASE 1, the 6-minute walking test results at baseline, 2 weeks, and 3, 6, to 12 months after injection were 522 m, 549 m, 561 m, 567 m, and 562 m respectively. In CASE 2, the 6-minute walking test results at baseline, 2 weeks, and 3, 6, to 12 months after injection, were 500 m, 518 m, 549 m, 593 m, and 390 m respectively. In CASE 3, the 6-minute walking test result had improved significantly from 453 m at baseline, 500 m at 2 weeks, to 639 m at 6 months. Unfortunately, it declined to 578 m at 12 months of observation. In CASE 4, the 6-minute walking test results at 2 weeks and 3, 6, to 12 months after injection were 336 m, 408 m, 324 m, and 410 m respectively (Figure 6.2). Overall, CASE 4 has the lowest recorded peak distance at 410m as opposed to other peaks of other cases (CASE 1: 567; CASE 2: 593; CASE 3: 639). One possible explanation for this decrease might be

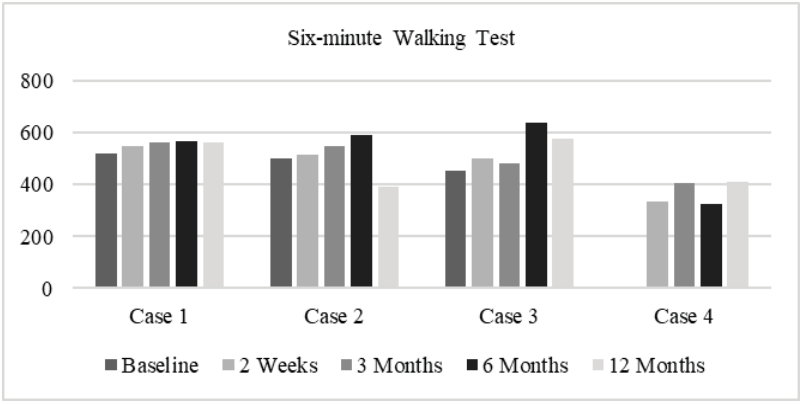


Figure 6.2 Quality of Life Assessment

that the subject in CASE 4 is significantly older than the subjects in the other cases (CASE 4, Age: 78), which generally makes it difficult to cover long distances due to lower stamina and physical conditions associated with old age.

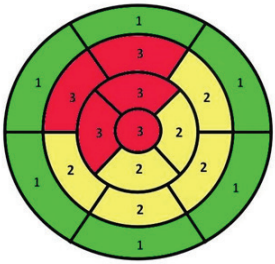
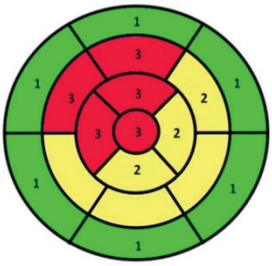
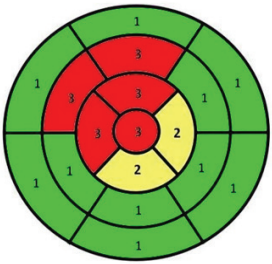
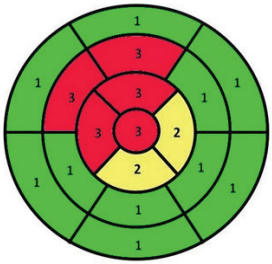
The result of the study is the same as the study by Bartolucci et al. (2017). Bartolucci's study used UC-MSC in patients with chronic stable heart failure with reduced ejection fraction (HFrEF) and showed that there was an improvement in left ventricular function, functional status, and quality of life in patients.

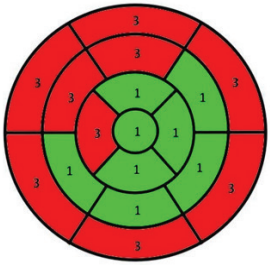
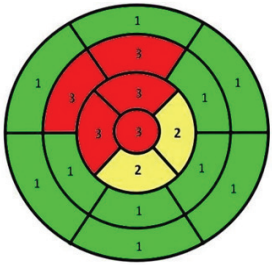
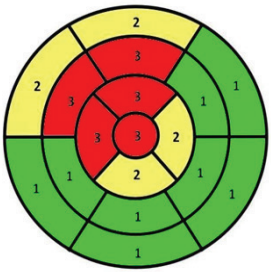
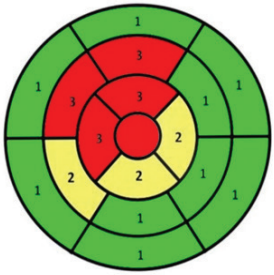
4. Regional Wall Motion Abnormalities

Additionally, the visualization of regional wall motion abnormality (RWMA) echocardiographic scans involves a quantitative assessment using scoring methods or an index, such as the wall motion score index (WMSI), which is calculated by dividing the sum of the score of each segment between the number of visualized segments (Gurunathan & Senior, 2017) and analyzed by two independent expert echocardiographers. In CASE 1, RWMA was consistent with previous anterior myocardial infarction, right ventricular contractility, and the valves were normal. No significant improvement of the WMSI value in the 12 months of observation seen in Table 6.2 can be found. Similarly, this can be said for CASE 2 and CASE 4, in which their WMSI value seen in the RWMA 17-segment model showed no improvement from the pre-treatment model scan towards the post-treatment scan (12 months; Table 6.2). Interestingly, only CASE 3 was shown to elicit significant improvement of its WMSI in the RWMA model, in which previous basal segments (anterior, anteroseptal, inferoseptal, inferior, inferolateral, anterolateral) that is labeled akinesis contractility improved into hypokinesis in the basal-anterior and anteroseptal segments, as well as into neurokinetic in the inferoseptal, inferior, inferolateral, and anterolateral segments, indicating a positive change through the stem cell treatment. While

the basal segments experienced improvement, the same cannot be said for the apical segments (anterior, septal, and lateral), in which the segments regressed into hypokinetic and akinesis contractility. The reasoning behind these changes is still being understood, however, the gradual loss of stem cell during transplantation on the injury site due to an inflamed environment is a potential cause for the gradual regression of interior apical segments, since the septal segments is further outside and reachable first by the stem cells allowing foremost repair (Alijani-Ghazyani et al., 2020; Kim et al., 2018).

Table 6.2 Regional Wall Motion Abnormalities (RWMA) and Wall Motion Score Index (WMSI) Result

Subject	Pre-treatment (Baseline)	Post-treatment (12 Months)
Case 1		
Case 2		

Subject	Pre-treatment (Baseline)	Post-treatment (12 Months)
Case 3		
Case 4		

D. The Role of Mesenchymal Stem Cells: Mechanism of Action

Studies suggest that MSC treatment benefits may be due to paracrine signaling. Paracrine signaling may play a role in promoting cardiomyocyte survival and increasing cardiomyocyte proliferation by inhibiting apoptosis. This statement is supported by a study in rats which proves that proteins such as VEGF, PDGF, IGF-1, and IL-1b secreted by MSCs can prevent cardiomyocyte apoptosis.

The role of MSCs in cardiac repair is also elicited through immunomodulatory and anti-inflammatory actions. When stem cells are injected into the myocardium, they suppress the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and monocyte chemoattractant protein-1, which reduces the inflammatory response to damage. The tissue inhibitor of metalloproteinase-1 and post-MI elevations in the expression of collagen-I and collagen-III have both

been demonstrated to be inhibited by MSC transplantation. The proliferation of cardiac fibroblasts and the generation of collagen-I and collagen-III from these cells are both severely inhibited by the paracrine compound secreted from MSCs. Thus, MSCs also play a role in preventing post-infarction remodeling.

Another important effect of MSCs for cardiac repair is inducing neovascularization. This process involves proteins, such as VEGF, FGF, and HGF (Bagno et al., 2018; Guo et al., 2020; Heinen et al., 2019; Hodgkinson et al., 2016).

E. Limitation of Study

The limitations of this study are the small number of participants and there are several factors identified through this study that can be attributed to the decrease and increase of the various assessments of STEMI patient functionality, such as LVEF value, WMSI scores, and the 6MWT. Infarct sizes as a factor influencing non-significant change in LVEF value (CASE 1) and old age affecting stem cell efficacy and homing properties, as well as reducing the distance traveled during 6MWT, is shown to be the noticeable factors affecting results in this study.

Furthermore, other factors affecting results in this study are speculated to be the injection route, in which both IV and IC injection methods differ in their results. As seen in Figure 6.1, two of the cases that experienced an increase in LVEF value are CASE 2 and CASE 3, while those that do not are CASE 1 and CASE 4. These two cases were given two different methods of stem cell injection, in which CASE 1 and CASE 4 experienced intracoronary injection (IC), while CASE 2 and CASE 3 experienced intravenous injection (IV). This result implies that cases that were given IC stem cell injection produced lower results, while those given IV injection experienced an increase. In many clinical studies, both IV and IC stem cell injections have been shown multiple times to yield significant and safe results in improving LVEF values, PET, or RWMA during in vivo studies on STEMI (Bartolucci et al., 2017; Gao et al., 2015; Peng et al., 2019).

Additionally, a systematic review by Lalu et al. (2018) of stem cell therapy on AMI confirms this same positive trend of both IV and IC injection methods being safe, effective, and viable for use.

At their core, IC and IV differ only in their injection route, which is through the direct path to the bloodstream for intravenous infusion and intracoronary administration through the direct path of the coronary veins (Lalu et al., 2018). Furthermore, both injection methods also seem not to produce any significant difference in their method of injection, aside from the fact that IV stem cells can get trapped during development in lung airways, such cause did not occur in this experiment, as our IV patients produced the significant increase (Liesveld et al., 2020; Schlundt et al., 2015). Although this is not significant to cause a large change in LVEF value, potential reasons that can be accounted for are additional statuses found in the intracoronary injection cases (CASE 1 and CASE 4). CASE 1 is affected with a larger infarct size that of >75%, requiring perhaps more stem cell dosage, and CASE 4 has additional affecting factors such as old age, which can attribute to influencing UC-MSC homing property, to which the stem cell injected might home in onto other organs that requires stem cell, and not towards the myocardial infarction site alone, thus requiring more stem cell dosage. Furthermore, this occurrence is likely to occur, as MSC homing efficiency is reported to be poor (<10%) in many various imaging studies, as well as previously said, that stem cells have the potential in getting trapped in lung pathways (Krueger et al., 2018; Liesveld et al., 2020). Another interesting instance in CASE 4 condition is that the patient was also diagnosed with a multi-vessel type blood vessel, as opposed to other cases that are only diagnosed with a single vessel, which again might suggest that the stem cell requirements for the treatment could potentially be higher than what is used. This factor could also be assisted in explaining the ineffective homing mechanism of MSCs coupled with old age, which makes the stem cell open to home into more areas that are needed.

Another potential factor is through the blood vessel type disease, which is a multi-vessel or single vessel, in which the multi-vessel type

was present in CASE 4, where most of the LVEF and 6MWT data was lower than other cases, potentially contributing as an affecting factor. As for case series with an improvement (CASE 2 and CASE 3), both cases have lower infarct size compared to CASE 1 with around ≥50% infarct size and are considerably younger in contrast with CASE 4, as well as having single vessel type. Therefore, implying a correlation between age, infarct size, and blood vessel type as factors affecting STEMI treatment using UC-MSC.

This limitation of the study can be improved in the future by choosing the best route of administration, doses, and timing of transplantation. The retention of stem cells also can be achieved by exercise training (ET). A study by Souza Vieira et al. (2020) showed that ET improves myocardial microenvironment for stem cell transplantation.

The findings of this study suggest that UC-MSC therapy has the potential to significantly improve clinical outcomes in AMI patients. This could lead to reduced mortality rates, fewer complications, and a better overall prognosis for individuals suffering from heart attacks. The observed reduction in myocardial damage following UC-MSC treatment highlights the possibility of preserving cardiac function and limiting post-AMI heart failure. This implies that UC-MSC therapy may represent a valuable addition to the standard treatment protocol for AMI. Moreover, the sustained improvement in left ventricular function seen in our study could translate into long-term benefits for AMI patients. This implies that UC-MSC therapy might contribute to enhanced cardiac rehabilitation and a better quality of life for survivors. Furthermore, our research underscores the importance of patient-specific approaches in AMI therapy. The identification of patient characteristics associated with a positive response to UC-MSC treatment suggests the potential for personalized medicine, where individuals can be stratified for the most effective treatment strategies. Combining UC-MSC therapy with other innovative treatments, such as gene therapy or cardiac rehabilitation programs, may further enhance outcomes. These findings imply that AMI treatment protocols

may evolve to incorporate a multi-modal approach for optimal results. Additionally, the observed safety and low incidence of adverse effects in UC-MSC therapy indicate that this treatment modality holds promise for broad application in clinical settings. Further studies should explore the long-term safety of UC-MSCs and determine the ideal dose and delivery method. Our results highlight the need for additional research to elucidate the precise mechanisms by which UC-MSCs confer their benefits in AMI. Future investigations can focus on optimizing cell dosing, timing of administration, and long-term outcomes to refine the clinical application of UC-MSC therapy.

F. Conclusion

The case series has successfully identified factors that may influence the outcomes of allogeneic umbilical cord mesenchymal stem cell (UC-MSC) treatment for STEMI. However, further research is needed to draw more definitive conclusions. The study's limitations include a small sample size of only four patients, making it challenging to generalize the results, despite the observed significant improvements. Additionally, incomplete radiological data and variations in results across different medical institutions introduced heterogeneity to the study, complicating the interpretation of similar results. To obtain more robust and compelling results, future research should involve longer follow-up periods and larger patient cohorts, specifically focusing on younger or early-stage STEMI patients. In conclusion, our study demonstrates that using allogeneic UC-MSCs as an adjunct treatment for anterior STEMI is safe and well-tolerated, offering promise for STEMI patients. However, additional research is essential to build upon these findings and address the identified limitations.

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Chapter 7

Stem Cell Based Therapies for Neurological Disorders

Somia Gul
Saba Majeed
Aisha Aziz

A. Introduction

Stem cell therapy is the only potential regenerative treatment that provides complete treatment for neurodegenerative diseases. The currently available treatments, either neurosurgical or pharmacological, are not efficient in treating the progression of neurodegenerative diseases. Stem cell therapy aids neuronal regeneration which modifies aberrations occurring in neuronal circuitry. In this chapter, stem cells therapy for different neurodegenerative diseases with their uses and clinical applications are discussed.

B. Stem Cells

Stem cells were originally generated from the German word *Stammzelle*, which was coined by German biologist Ernst Haeckel.

S. Gul, S. Majeed, A. Aziz

Jinnah University for Women, e-mail: SomiaGul@juw.edu.pk & drsomi1983@yahoo.com

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Stammzelle means a type of cell that produces other cells. One of Haeckel's theories describes a stem cell as a fertilized egg that generates many cells. Following that theory, embryonic cells and bone marrow cells are also named as stem cells by various researchers due to both differentiating into more specialized cells.

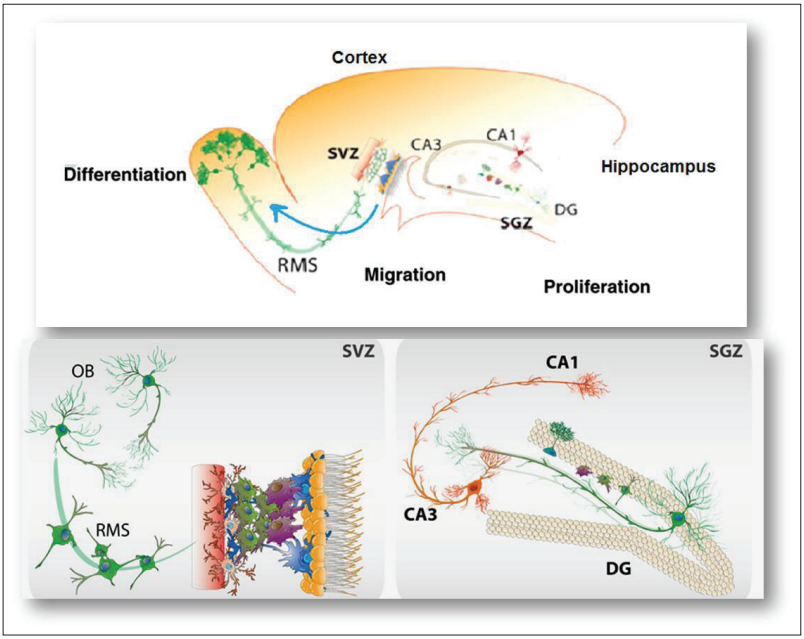
In recent research, stem cell has evolved as an extensive term and is defined as the cells having potency for regenerating and stimulating cells of various phenotypes. Stem cell biology has now been centralized in drug discovery for treatment of many diseases. Therapeutic approaches based on stem cells reflect a new pathway for management of chronic and persistent diseases. The definitive approach of stem cell-based therapy is to potentiate the body's regenerative capability through regulation of tissue homeostasis and regeneration.

On the basis of origin, stem cells are classified into mesenchymal, epithelial, hematopoietic, embryonic pluripotent, and neural stem cells. Human pluripotent stem cells, further include human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), have been employed in preclinical studies of neurologic diseases.

Research advancements have shown great potentials of neural stem cell-based therapies as regenerative medicines for treating various neuronal disorders. Neural cells are differentiated into cells, namely oligodendrocytes and astrocytes. Meanwhile, progenitor cells are based on limited differentiation, just like neuroblasts which can only be differentiated into neurons. Similarly, glial or oligodendrocytes progenitor cells can be developed into either astrocytes or oligodendrocytes.

C. Neural Stem Cells Niche in Brain

The process of neurogenesis is conducted in the neurogenic region of the brain. The two neurogenic zones involved in this process are the subventricular zone (SVZ) and the dentate gyrus (DG) of the hippocampus (Figure 7.1). In addition to these areas, there are also



Source: Adapted from DeHamer et al. (1994)

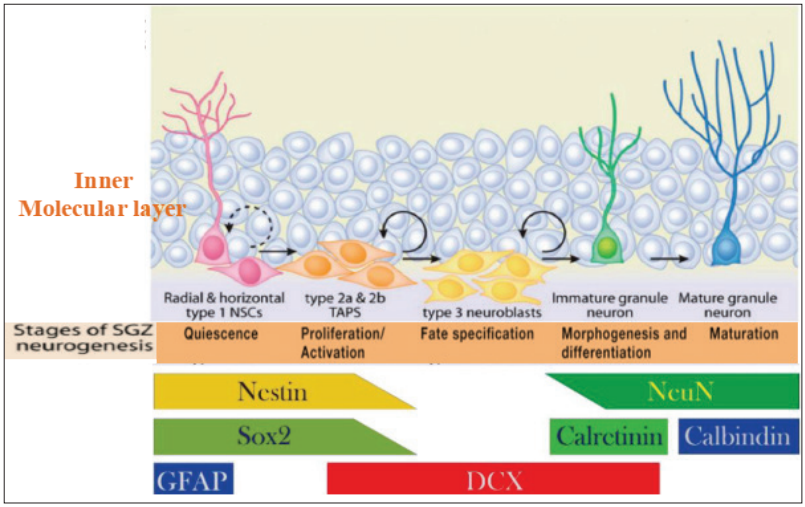
Figure 7.1 Neurogenic niches of the brain include the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG).

other distinct brain regions, such as hypothalamus, cerebellum, cortex, and striatum, where new neuronal cells are produced.

However, the highest proliferation of neurons has been observed in the SVZ area in the mammalian and human brains. An average of 40,000 cells per day are produced in the SVZ region of the brain. The new neuronal cells travel from the SVZ region to the olfactory bulb (OB), where they differentiate into interneurons.

1. Neuronal Development and Survival of Stem Cells

The subgranular zone of the adult hippocampus possesses radial glial cells which act as the neuronal precursor cells (NPCs). These cells are further differentiated into Type-1, Type-2, and Type-3 cells (Figure 7.2).



Source: Adapted from Hsieh (2012)

Figure 7.2 Stages of neuronal development from neural stem or progenitor cells to mature neurons expressing biomarkers.

a. Type 1 Cells

Type 1 cells are uneven and triangular in shape, exhibit a long apical process raised from the granular cell layer. These are diffused into the inner molecular layer, where they develop numerous tiny processes. The type 1 cells express both *Nestin and Glail* fibrillary acidic protein (GFAP) biomarkers which will be further discussed later in this chapter.

b. Type 2 Cells

Asymmetrical division of neural progenitor cells gives rise to one neuronal stem cell (NSC) and one daughter type 2 cells. This daughter cell undergoes a frequent symmetric cell division and produces two differentiated or two symmetrical daughter cells, before acquiring its terminal fate and differentiation. These daughter cells are further termed as a "transit-amplifying cell". These cells are expressed by a protein biomarker namely nestin. They have distinct short processes

with dense nucleus and irregular shape. They have a swift and high proliferation rate.

The neural stem cells divide asymmetrically in constant manner, and produce differentiated astrocytes with self-renewing capacity. The NSCs are thought to be depleted after short span of time. However, recent research has shown that the NCSs can multiply and increase their population besides having self-renewing capability. It is an established fact through studies that NSC located in adult hippocampal niche have capability to generate various cellular lineages and resulting populations can survive for longer period of time. Additionally, the existence of an intermediate progenitor cell is capable to undergo several symmetric divisions and tends to produce transit-amplifying cell which are serving as NSCs or neurons. These pathways of lineage development can be influenced by functional alterations in the neurogenic and non-neurogenic niche in different animals.

c. Type 3 Cells

In most cases, transit-amplifying cells are differentiated and give rise to type-3 cells, which express doublecortin protein (doublecortin is found in differentiating and migrating neurons. It is a microtubular associated protein). These cells show negative expression for nestin. These cells also express a polysialylated form of neural cell adhesion molecule (PSA-NCAM). PSA-NCAM is a migrating and developing neuronal marker.

Type-3 cells can proliferate and amplify to the next phase, continuing to express a biomarker called as Distinct feature of double cortin (DCX) and convert to postmitotic immature granule neurons. DCX marks neurons in postmitotic stage, which are still having some neurons with characteristic of synaptic plasticity. Synaptic plasticity is described as the neuronal ability to strengthen their connections. It is an important process for brain development and regeneration.

Type 2 and type 3 cells are deficient in functional synapses, but are stimulated by GABA-nergic signals from surrounding interneurons.

A high proportion of stem cells reach type 2 and 3 neuronal phases after two to three days of division.

D. Progressions of the Adult Granule Neurons

Adult granule neurons transiently express a protein biomarker called Ca2+-binding protein calretinin in the early post-mitotic phase. They are also characterized by neuronal marker Neuronal Nuclei (NeuN) found in the postmitotic stage. At this stage, cells retain their round or triangular shape nucleus similar to the morphology of type 3 cells. This phase is followed by the rapid axonal extensions targeting CA-3 region, which is found in the hippocampus and regarded as a pacemaker of hippocampal region. The axonal connections become clearly visible, within three to five days of division.

The visibility of apical dendrites at initial division takes 48 hours and is retained in type 3 cells and early mitotic cells. The outgrowth and projections rise from newly born neurons, at the rate of 15 μm per day during first three days. However, the length of projections grows double approximately from four to five days. The release of inhibitory GABAergic signals from hilar cells, which is located in dentrate gyrus region of brain, facilitates synaptic networks towards immature granular neurons. These networks are further propagated from axonal projections of hilar cells adjoining dendrites and are located in deep molecular layer. A larger proportion of new born neurons are recruited for their functional integration or elimination following assortment processes.

The phases of stem cells development indicate that most of expansion processes takes place at the stage of precursor cells, although final differentiation and thereby the fate specification, for long term neuronal survival, occurs primarily in postmitotic phase. Majority of new cells of the population multiplies significantly during the early period of proliferation and amplification. Afterwards, these cells decline immensely—more than 50%—before acquiring stability within seven-fourteen days. This state continues over longer period of time, from several days to years.

E. Regenerative Role of Adult Neuronal Stem Cells

The main emphasis of modern stem cell research is to recognise the contribution of newly produced neurons in brain function and its efficient integration in brain circuit. Experimental approaches anticipate that progenitor or adult neuronal stem cells are not only produced throughout life, but also play an important role to develop the functional brain networking. The generation of adult NSCs and endogenous neurogenesis opens the new pathways to treat the neurological disorders.

Emergent understanding about the characterization of neural stem cell provides novel therapeutic targets based on stem cell therapy and are aimed to restore the functional deficit in CNS disorders. Comparable to adult stem cells, which are basically repairing various tissues and organs, the adult NSCs are likely to play an important role to replace the dead cell after injury and repairs the damage brain tissue. The capability of adult NSCs to regulate the brain homeostasis may protect the physiological functions against depression, trauma, and anxiety from neuronal defects.

F. Neuronal Stem Cell Generation in the Hippocampus

Hippocampus has a vital role in incorporating information into memory and generating new neurons. Moreover, it is also involved in pathophysiology of mood disorders. Hippocampal volume is being reduced in psychiatric conditions like depression. The production of neurons in the hippocampal region is termed as hippocampal neurogenesis. Stem cell generation in the hippocampus has been studied in non-human primates, rodents, and humans.

In the dentate gyrus (DG) of hippocampus, the subgranular zone (SGZ) possesses neural precursor cells which produces newborn neuronal cells. These cells move towards the granular layer and there they mature to granule neurons. The axons of these cells are projected to pyramidal cell layer of CA3 region, while the dendrites

are projected to molecular layer. In the following months, these cells are going to form further synaptic connections. Young neurons show distinctive properties like a lower threshold for excitation and longer period potentiation. Moreover, the new neurons are significant for hippocampal dependent learning and pattern separation. Neurogenesis in the dentate gyrus of hippocampus occurs in form of clusters linked with blood vessels.

1. Regulation of Hippocampal Neurons

Advance development of neuronal progenitor stem cells in hippocampus is influenced by various extrinsic and intrinsic behavioural, as well as molecular factors. These factors regulate all phases of neurogenesis. Enhancement factors of neurogenesis involves exercise, enriched environment, and learning. Mood-modulating antidepressant drugs, like fluoxetine, also increase the patterns of neurogenesis.

Factors which are likely to reduce hippocampal neurogenesis include different types stress, aging, and various other diseases such as Alzheimer's disease and Parkinson. In addition to these factors, numerous proteins, endogenous stimuli, hormones neurotransmitters, and epigenetic markers have an impactful role in regulating proliferation, differentiation, or fate determination of new born neurons.

The neural stem cells residing in SGZ are essential to modulate the hippocampal growth for memory formation and to maintain neural plasticity throughout life. Additionally, it has a substantial role in the regulation of mood. SVZ neural stem cells play a significant role in the maintenance of olfactory lobe (OB) structural morphology and conserves the high cellular turnover. Continuous addition of new neurons has promising roles in the development of olfactory circuits influencing sensory organs to adapt the behavioural alterations. Impaired memory and reduced ability to perform different tasks is a serious consequence of disruption in hippocampal neurogenesis.

2. Therapeutic Implications of Hippocampal Neurogenic Stem Cell Therapy

Neurogenesis in the hippocampus through neuronal stem cells differentiation supports hippocampal circuits repair. It also facilitates cognitive and emotional behavior patterns, regulated by the hippocampus. Distortions on adult hippocampal neurons are being associated with the dementia disorder called as Alzheimer's disease (AD). Stem cell therapy, including cell engraftment, reprogramming with glial neurons, and regenerating adult hippocampal neurons, has compensated degenerated neuronal circuits and emerged as potential treatments for recovering neuronal loss. Transplantation strategy of NSCs may also recover the neuronal loss by attenuating homeostasis of brain tissue and producing a synergistic effect with neuroprotective drugs and other therapies.

G. Potential Risks Associated with Stem Cell Therapy

Advanced clinical outcomes of stem cell therapy for neurological diseases are also associated with potential risk factors. These risk factors should be analyzed critically for better clinical treatment with stem cell products. These risk factors are dependent on stem cells types, in vitro culturing of cells, route of administration, intended location, proliferation, differentiation, treatment irreversibility, and survival time frame of engrafted cells. The potential identified risks in clinical and animal studies include allergic reactions, unwanted immune responses, and tumor formation.

The use of human embryonic stem cells (hESCs) are limited due to two major risk factors, including the risk of immunological rejection because hESCs are isolated from pre-implantation blastocysts, and ethical issues due to the human embryonic cells' destruction. The transplantation procedures with stem cells are often invasive and produce severe complications, especially in elderly and paediatric patients.

Human-induced pluripotent stem cells are also associated with potential risks of cardiac arrest to patients, arrhythmia, and risk of cancer formation. Bacterial infections and inflammation are also one of the crucial risk factors which cause mortality of patients undergoing stem cell-based therapy.

H. Safety Evidences Associated with the Administration of Stem Cells

Various clinical trials have established the results of efficacy and safety of mesenchymal stem cells for treatment of diseases, such as haematological malignancies, acute myocardial infarction, chronic heart failure, and acute respiratory distress syndrome. Large clinical trials with better outcomes are still needed to market stem cell-based products on large scale.

Safety and efficacy of mesenchymal stem cells (MSCs) treatment has been modulated at advanced stages through recent studies. These cells have demonstrated protective effects with no adverse effects in acute respiratory distress syndrome (ARDS) patients in small clinical trials

Diabetes is a serious medical health problem leading to various other diseases. Bone marrow-derived mesenchymal precursor cells (MPCs) have been evaluated for feasibility and tolerability, in type 2 diabetes patients. There were no serious adverse effects including serious hypoglycemia. There was no formation of donor-specific anti-HLA antibodies.

Stempeucel are allogeneic mesenchymal stromal cells (MSCs) derived from bone marrow of healthy, adult volunteers. Its safety and efficacy profile has been established through phase I/II randomized, double-blind, single-dose study in myocardial infarction patients. There was a great improvement in ejection fraction outcomes compared with the placebo group, with no serious adverse effects.

Intravenous administration of Cx611 which is a preparation of allogeneic expanded adipose-derived stem cells (eASCs) in patients

with refractory rheumatoid arthritis (RA) has proven to be safe and tolerable. The effects of eASCs were associated with none of the dose-related toxicity and have shown promising clinical efficacy.

Mesenchymal stem cells peripheral vein infusion has also emerged as a possible therapeutic approach for end-stage liver disease. The infusions of MSCs were effective with beneficial outcomes on liver synthetic functions and hepatic fibrosis. With better safety profile, they have also shown improved albumin, prothrombin, and alanine concentrations.

I. Limitations of Stem Cell Therapy

Remarkable investigations by various biologists, using transplantation techniques for inducing pluripotent cells (iPSCs) in neurodegenerative disease models, have aided in optimizing the current treatment protocols. Despite these advances and technical exploration, the approach of NSPCs or iPSCs transplantation may not be practically fruitful to cure neurodegenerative disorders due to inadequate knowledge of disease mechanisms and limitations in the treatment of brain diseases. Furthermore, transplantation of neuronal cells in the early stage of disorder can additionally complicate the therapy and result in greater risk than benefit.

The aberrant neurogenesis has been reported in response to the disturbed CNS environment. Aging can significantly reduce the neurogenic capacity of neural stem or progenitor cells, leading to a much lower yield of sufficient neurons for replacing degenerated neurons or integrating into CNS.

Strategies to Overcome the Limitations of Using Stem Cells Safely and Effectively

To address these limited applications of transplanting NSPCs exogenously, one constructive approach would be the stimulation of endogenous neural stem cells, residing in the subventricular zone and subgranular zone of dentate gyrus. Alternatively, augmenting the neurogenesis in non-neurogenic niches can also be valuable such as

the continuous addition of new neurons in the spinal cord. The other useful endogenous stimulation advances are discussed as follows.

1. Stimulation of Ependymal Cells in The Spinal Cord

Ependymal cells are specialized ciliary cells of brain which arise from radial glia. NSCs can also be recruited from central canal surrounded by ependymal zone, serving as primary pool of stem cell. Further, the in vitro stem cell proliferation of ependymal cells is reported in comparison to astrocytes. The ependymal niche of spinal cord has shown rapid proliferation of ependymal cells after spinal cord injury. Although, more research is required to identify the exact role of ependymal zone of spinal cord in regard of proliferation and differentiation in to oligodendrocytes and astrocytes after injury. These findings can conclude that the subpopulation of ependymal cells of spinal cord can hold the underlying neural stem cells properties.

2. Neuronal Stem Cells Stimulation Through Endogenous Factors

The molecular signalling pathways which are involved in NSC proliferation and differentiation can be a biomarker of endogenous neuronal stimulation. Numerous factors are identified for modulating neurogenesis. Stimulation and migration of the neural stem cells at site of injury in response to neuronal deficit or trauma suggests that there are some endogenous factors which can affect proliferation and migration of precursor cells at the injury site. Moreover, intracerebral delivery of neurotrophic factors and stromal-derived factor -1 has influenced expansion and migration of intravenously administered NSCs into the brain.

These advances reveal that a well-synchronised anti-inflammatory response is also essential for tissue restoration processes. This should preferably be accomplished in presence of beneficial factors affecting proliferation, migration, and integration or replacement of neuronal cells. Conversely, a continuous and intensified response may initiate more exacerbated neuroinflammatory cascade, which may

accelerate the neurodegenerative signalling and result in worsening of disease. Considering this fact, the approach to augment endogenous neurogenic potential of NSPCs may achieve the therapeutic goals by recruiting and differentiating appropriate cell lineages.

3. Targeting Immature Neuroblasts

Adult-born NSPCs that are not damaged in early days of cell division retain unique morphology before undergoing physiological maturation in to granular neuron. There are new approaches to amplify endogenous neurogenic capacity that could target immature adult neuroblasts under critical period of maturation. Immature neuroblasts holds membrane resistance properties, reduce glutamatergic activity and distinct firing. These properties permit development of a maturation stage in which immature neuroblasts can be stimulated through several biological and environmental factors. The hyper excitable state of immature granular cells are important to concern in the perspective of neurodegeneration. The endogenous neurogenesis, stimulated by immature neurons, can also alter the behavioural patterns.

4. Enhancement of Neuronal Stem Cells Through Type 2 Amplifying Cells

There are certain specialized cells in hippocampus which are called as type 2 amplifying cells. These cells have shown rapid proliferation and yield the pool of many progenitor cells, presenting these cells as potential target for endogenous stimulation strategies. Neuronal proliferation can be activated through physiological as well as pharmacological pathways.

5. Endogenous Stimulation Through Neurogenic Compounds

Another captivating approach in modulation of endogenous neurogenesis is through screening and use of new molecules termed as neurogenic compounds. The development of certain synthetic compounds or natural molecules in recent research trends has shown promising results in stem cell-based therapies. These

molecules or compounds have advanced the therapeutic options for neurodegenerative disorders.

The neuroactive compounds have shown promising results in neurodegenerative animal models. Furthermore, neuroactive molecules have been explored as modulators of neurogenesis, which can also be implicated in stem cell based regenerative therapy. For example, isoxazole 9 (Isx-9) has been found to enhance adult hippocampal progenitor cell proliferation and differentiation into mature neuroblast without perturbing the number of neural stem cell. This compound also has role in enhancing spatial memory in mice. These small molecules have been identified to improve cognition by influencing the neuronal plasticity at various junctures of differentiation and maturity. Isx-9 also has neurogenic potential as it can induce neuronal genes to re-express and generate differentiated astrocytes *in vitro*. Therapies with such compounds can be an alternative strategy for recruitment of progenitor cells that may functionally be differentiated into neuronal lineage.

6. Targeting Adult-Born Neurons

One more approach is to target the different stages of adult-born neurons, for example by increasing synaptic connectivity, integrity into network, or maturation period. The phase of adult borne neurogenesis is also affected by physiological and pharmacological agents.

Endogenous neurogenesis for neurodegenerative treatment can also be achieved by initiating the migration of dividing and mature cells to desired region of brain. Various investigations have identified that NSPCs originating from, for example, SGZ and SVZ neurogenic niches are commonly reorganized to maintain their regular migratory tracks in the neuropathological state.

7. Applications of Endogenous Neurogenesis

It is possible that advance methodologies affecting endogenous neurogenesis will enable researchers to direct the migration of cells. For example, the lost neurons in Alzheimer's disease can be replaced

by dentate gyrus NPCs in cortical region. The spiny neuronal cells degenerated in Huntington's disease (HD) can be substituted from neurons of SVZ region into the striatum. Moreover, the exogenous transplantation of NSPCs in Parkinson's model of rat has shown to induce release of some trophic factors like stromal cell-derived factor-1α (SDF-1α) in local NSPCs, substantially promotes migration of endogenous neural progenitors to the grafted area.

However, the effectual migration of NSPCs to injured or deficit areas is needed to be discovered, for the said purpose of extensive exploration of NSPCs. In addition, the extracellular pathways and transcriptional regulation of migrating cells should also be investigated.

J. Biomarkers for Neuronal Progenitor Stem Cells

Development of stem cells into neurons and astrocytes require working of certain protein biomarkers which are expressed at various stages of differentiation processes. These include nestin, glial fibrillary acidic protein (GFAP), and bromodeoxyuridine (BrdU). However, it is unknown at which stage the progeny is limited to the neuronal fate, either in the subgranular zone of the hippocampus or subventricular zone. Moreover, the number of cells incorporating into the existent neural network is also unclear.

1. Nestin

Nestin is a protein belonging to the sixth class of intermediate filament proteins. The expression of nestin occurs in adult neural stem cells (NSCs) and immature progenitor cells. However, it disappears as cell differentiation occurs. This protein has also been recognized as a marker of neural stem cells in both adult brain and embryo. Nestin is involved in self-renewal and proper survival of NSCs. Most nestin-positive cells, in the early stages of embryonic development, work as progenitor stem cells that can proliferate and differentiate in certain brain regions. As the cell division and differentiation of these cells terminate, the expression of nestin is also downregulated.

2. GFAP

Glial fibrillary acidic protein (GFAP) is a known marker for astrocytes (the specialized glial cells). It is an intermediate filament that is responsible for maintaining the mechanical strength of the astrocytes. During neurogenesis, the cells possessing astrocytic properties can function as an origin for new neurons. GFAP-positive progenitor cells are able to produce distinct neuronal cell types during the process of neurogenesis. Studies have shown that radial astrocytes also function as DG neuronal stem cells (NSC).

3. BrdU

Bromodeoxyuridine (BrdU) is a synthetic structural analogue of thymidine nucleoside. It has been used as an efficient source to label cells involved in the s-phase of the cell cycle, in both perinatal and adult proliferating cells. BrdU labelling is a prevalent technique used for studying neurogenesis. BrdU targets the proliferating cells at distinct stages of neurogenesis. Although these techniques are considered as valuable tool for monitoring adult neurogenesis, they have limited application in the analysis of stem cells. When exposed to proteins like BrdU, the expression of stem cell markers is reduced or they exhibit transient labelling.

K. Transcriptional Regulators for Neuronal Development: From Stem Cells to Neurons

Neuronal cell differentiation is regulated by one of the super families of transcriptional factors called basic Helix-Loop-Helix (bHLH). These neuronal factors are responsible for adequate production of glial and neuronal cells. The conversion of somatic stem cells or pluripotent stem cells into neuronal cells also requires bHLH genes functioning. The aberrations and mutations in bHLH factors are related to development of various cancers and neurological disorders. Two most important bHLH genes are NeuroD and Neurogenin.

1. NeuroD

The family of transcription factors that possess a fundamental role in tissue development and maintenance is the bHLH superfamily. The neural lineage bHLH factors, a subgroup belonging to this family, have a great importance in the development of the central nervous system (CNS). The NeuroD is the subset of neural lineage bHLH factors that are involved in neuronal development and progression. As neuronal differentiation is a complex process, the NeuroD gene also serves as a marker for differentiation of adult hippocampal neurogenesis. It is also classified as an indicator of adult cells in the sub-granular zone and the inner granular layer. In the adult brain, NeuroD is also responsible for neuronal cell proliferation. Moreover, the polysialylated neural cell adhesion molecules (PSA-NCAM) are also positive for NeuroD simultaneously, therefore the NeuroD expression can also be detected in these molecules.

2. Neurogenin

Neurogenin is another transcription factor belonging to the bHLH family and it plays a fundamental role in neurogenesis. This proneural gene can initiate a neurogenic program, both in vitro and in vivo, in distinct progenitor cells. Various studies have reported that these factors are involved in cell-type-specific neurogenesis. The importance of neurogenins can also be seen through gene mutations. Neurogenin 1 (Ngn1) or Neurogenin 2 (Ngn2) single or double mutant mice have shown a loss of spinal cord neurons, as well as spinal and cranial sensory ganglia. Apart from inducing neurogenesis, Neurogenin 1 inhibits NSCs differentiation into astrocytes by restraining CBP-Smad1 complex away from genes that are responsible for astrocyte differentiation.

3. Signaling Mechanisms Involved in Differentiation Processes of Neuronal Stem Cells

Beside transcriptional activation, the stimulation of signalling pathways, such as epidermal growth factor (EGF), fibroblast growth

factor (FGF), notch and bone morphogenetic proteins (BMP) have been well recognized for their role in neural progenitor, astrocytes, and neural stem cell proliferation, differentiation and fate decision. Several pathways that stimulate neural differentiation can effectively generate different types of neurons using in vitro methods. These cell types include dopaminergic neurons, cholinergic neurons, spinal motor neuron, and oligodendrocytes.

The in vitro differentiation protocols of producing neurons has great importance, such as functional spinal neurons are produced from NSCs through induction of Sonic Hedgehog signalling and retinoic acid treatment. One of the clinical approaches for Parkinson's disease (PD) therapy is to generate dopaminergic neurons from NSCs after exposure with fibroblast growth factor 8 (FGF8) and stimulation of Sonic Hedgehog activity. The inhibition of transforming growth factor beta (TGF beta) and BMP signalling also induce differentiation of NSCs towards dopaminergic neurons. Following differentiation, the subtype specification of dopaminergic neurons is influenced by various transcription factors, including Nurr1, Lmx1a, Pitx3 and FoxA.

Identification of cellular features in developmental brain can introduce effective differentiation approaches of desired and appropriate neural identity, such as obtaining neuronal midbrain dopaminergic cell groups from induced pluripotent stem cells (iPSCs) or mesenchymal stem cells (MSCs) in in vitro methods. Additionally, differentiation can be improved using co-culture of neural progenitor stem cells with bone marrow stromal cells (BMSCs) or neonatal cortical astrocytes.

L. Stem Cell-Based Therapies for Neuronal Degenerative Disorders

Every organ and tissue of the body has a replacement and repair system for organizing new cells and replacing the older and dead cells. Regeneration of neuronal cells is the most crucial phase for restoring

neuronal functions. This is because of the fact that CNS has weaker aptitudes for developing proper cell repair and cell replacement.

Various therapeutic approaches have been designed for neuronal cells repair therapy. These include neuroprotective drug therapies for enhancing neurogenesis, neural stem cells transplantation and targeting inflammatory pathways for repairing neuronal distortion.

1. Neuroprotective Drug Therapy for Enhancing Neurogenesis

Neuroprotective drug approaches are in investigational stages for revitalizing brain cells. Certain examples include the use of neurotrophic agents, free radical scavangers, metal ion chelator, neuronal gene modulators, and neuronal apoptosis inhibitors.

The potential stimulations of neural stem cells endogenously, for regenerating new neurons in adult CNS, is less invasive technique than cell transplantation. Such an advancement in neuronal cell research has been successful for treatment of complex CNS diseases, for example Alzheimer's disease. Use of mitotic trophic factors has been associated with amplified neurogenic response. Also, Olanzapine, which is used for treatment of schizophrenia, nitric oxide (NO), and 5-phosphodiesterase inhibitors have been screened ex vivo for potential ability for inducing neurogenesis.

2. Neural Stem Cells Transplantation

Neural stem cells transplantation is considered as the most advantageous for reconstructing neuronal cells connections. This requires an appropriate selection of neuronal cells with specificity, possessing proliferative potential, and should be phenotypically plastic. Successful exogenous transplantation of NSCs has upregulated regulatory processes like angiogenesis and neurogenesis after stroke. SVZ-derived neural stem cells have been used for intracerebral transplantation in experimental models of various brain disorders including Parkinson's disease, Huntington's disease, and multiple

sclerosis. Clinical trials have shown successful therapeutic advantages of spinal cord injection of NSCs in treatment for amyotrophic lateral sclerosis (ALS).

3. Neuronal Stem Cells Modification by Anti-Inflammatory Agents

Inflammation has a deleterious impact on neurogenesis, as it affects generation of new neural cells endogenously from brain's cells or by exogenous transplantation of neural cells. Neural cell repair therapy could be modified by use of anti-inflammatory drugs. Neural cell proliferation has been associated by moderating inflammatory pathways. Therefore, optimizing the potential neural inflammatory markers can increase differentiation of neurons and promote recreation of new cells in lesioned areas. In this context, potential treatment with natural or synthetic drugs, possessing anti neural-inflammatory property, should be used. Specifically, this treatment strategy should be specified towards the activation of microglial cells in cumulative neural stem cell proliferation and differentiation into neuronal cells.

Here are some examples of medical treatments utilizing stem cells.

a. Neuronal Stem Cells and Their Relation o Alzheimer's Disease Management

Alzheimer's disease (AD), also known for years as short-term memory loss, is being identified for weaker cognitive functions. The underlying pathology is based on declined cholinergic neurons. This ultimately progresses towards distorted behavioral patterns of learning and emotions. Available drug therapies for treatment of Alzheimer's disease are associated with serious adverse effects that leads to incomplete cure.

Neural stem cells have demonstrated a better portrait of advanced therapeutic approach providing effective behavioral responses. Transplantation with these cells has developed boosted neuronal networks, replacing degenerated neurons. This provides neuronal regeneration backup for AD where neuronal connection is incessantly

disturbed. Transplanted cells have capability to differentiate into cells in damaged area for nurturing cells giving prompt recovery.

Neuronal stem cells are found in hippocampus and sub-ventricular zone in adults. NSCs are genetically redesigned after being differentiated from brain tissues or embryonic stem cells. Development of zebrafish model of AD by Bhattarai et al. (2017) is one of the most advantageous applications of NSCs transplantation. The amyloid toxicity, induced by human Aβ42, enhanced inflammatory markers causing synaptic loss, leading to cell death and lack of behavioral responses. Interleukin-4 mediated NSCs transplantation produced significant outcomes for regenerating neuronal cells.

The population of cholinergic neurons are mostly affected in Alzheimer's disease (AD) and resulted in cognitive decline. Basal forebrain cholinergic neurons have been generated by application of two methods which include transplantation of neuronal stem cells with transcription factors Gbx1 and Lhx8 and controlled differentiation of NSCs to cholinergic neurons by using diffusible ligand Bone Morphogenetic Protein 9 (BMP9). The obtained cholinergic neurons also successfully engrafted into hippocampal slices and they expressed functional properties of cholinergic neurons in electrophysiological experiments. These differentiated forebrain cholinergic neurons can be an effective therapeutic alternative for AD treatment.

Neuronal stem cells have also been transplanted in 3xTg (triple transgenic) AD model of mice. The transplanted NSCs differentiated into glial cells, enhancing synaptic density. Improved behavioral responses are the key results in novel object recognition and Morris-water-maze tests while Tau and Aβ protein levels remain unchanged.

b. Parkinson's Disease Treatment with Neuronal Stem Cells

The most common neurodegenerative diseases include Parkinson's disease (PD), a prevailing disorder which has been increased to 4% in aged people over 80. It is diagnosed with presence of aggregates of α-synuclein. Genetic mutations in genes such as parkin (PRKN) and alpha-synuclein (SNCA) are associated with progression of PD.

Basic treatment of PD includes restoration of dopamine levels through monoamine oxidase-B inhibitors and L-Dopa. Deep brain stimulation (DBS) is also in current strategies for PD treatment. All these treatment protocols have failed in reversing neuronal degeneration. Therefore, a compliant treatment protocol should be developed for recuing motor and non-motor symptoms of PD.

Innervation of undifferentiated stem cells in brain areas with decreased dopaminergic neurons are having inclination for producing better effects in in vivo PD models. Effective transplantation therapy with neuronal cells depends on brains recognition process of donor cells. This is also involved in reducing dyskinetic side effects associated with PD in clinical trials. Effective protocols must be programmed for reducing chances of immune rejection.

Transplantation treatment of PD primates along with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have produced significant effects of cellular survival and also moderate behavioral patterns. The neural cells are not capable of being pre-differentiated into dopamine containing cells. However, investigations are still in process for transplanting dopaminergic neuronal stem cells in PD models. Neural stem cells may provide a repaired system within the brain through creating anti-inflammatory and neurogenic factors. Neural stem cells have the functional capability to be transformed into dopamine neurons but their quantity is insufficient to produce significant effects. Neural stem cells are usually differentiated into astrocytes. These astrocytes released glial cell line-derived neurotrophic factor (GDNF), which is responsible for neuronal homeostasis.

c. Functional Responses of Neural Stem Cells in Ischemia

Recent in vitro NPSCs transplantation have produced successful outcomes in ischemia-induced rat model. Oligodendrocytes have been originated from subventricular zone (SVZ) cells of neonatal rats. Findings of in vivo experiments also supports the positive effects of neural stem cells in stroke. An Increase in neuronal protein biomarker BrDU is also associated after neuronal stem cells transplantation

in hypoxia-induced neuronal cells. Neuroblast and mature cells proliferation also increases after neuronal stem cells treatment after ischemia.

d. Neural Stem Cells as Effective Treatment for Gliomas

The administration of human glioma (U251) cell lines with NSCs increases the animal's survival rate in nude mice model. The neuronal cells treatment can decrease extracellular-regulated kinase (ERK1/2), tumor suppressor gene (p53), and phosphorylation of protein kinase B (AKT) genetic markers. Following administration of NSCs, there is also a substantial upregulation in apoptotic markers known as caspase-3. This suggests that NSCs have potential antiapoptotic activity. The coculturing of NSCs conditioned medium with U87 glioma stem-like cells has shown decreased viability of glioma cells. Endogenous stimulation of stem cells in the subventricular zone also targets glioma cells and decreases.

Neural stem cells after genetic modulations can stimulate endogenous secretion of anti-cancer compounds near tumor zone. These endogenous antitumor compounds are also called as immunomodulators, include interleukin-4 (IL-4) and interleukin-12(IL-12). IL-4 is extensively involved in recruiting precursor T-cells. This is advantageous to kill cancer cells through increased immune response. IL-4 is also regraded as tumor combating cytokine, which improves survival rate of neurons. IL-4 can also be transmitted through retroviral transfer, which in turn can improves survival rate. IL-12, a potentiator of T-cells, enhances differentiation of T cells into CD4+ and CD8+ T-cells. IL-12 also induces natural killer cells activation. Both immunomodulatory approaches of interleukin production by NSCs have successful implications in enhancing survival rate and decreasing tumor burden.

e. Neural Stem Cells Induction in Spinal Cord Injury

In spinal cord injury (SCI), various factors affect the regeneration of neurons. These factors include cell loss, neutrophins deficiency, and glial scar. Therapeutic strategies of using neural stem cells are

more effective in recovering cellular loss after SCI. Neural stem cells are considered as potent candidate for SCI therapy for improving neuronal functional distortions.

Nogo66 receptor (NgR) vaccine is a nucleic acid vaccine, which targets Nogo66 receptors found in central nervous system. Combination of Nogo66 receptor vaccine with neuronal stem cells transplantation increases recovery phase of SCI in preclinical testings. NgR+ NSCs vaccine could promote better functional recovery than when NgR vaccine or NSCs are used alone. This vaccine also prevents motor neuronal entry in the injured tissues of spinal cord. Also, this therapy enhances neuronal cells differentiation into oligodendrocytes and neurons.

M. Effects of Neurotrophic Factors Secreting Mesenchymal Cells in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder in which there is a progressive motor neurons degeneration. This eventually affects muscles of whole body. Muscular paralysis affects movement, speech, and ultimately leads to respiratory failure and death.

An average of 4–7 per 100,000 people is affected by ALS worldwide, with survival of 3–4 years. Rilizole and Edaravone are two medications for ALS treatment, approved by the US Food and Drug Administration. These drugs are moderately treating ALS by slowing the functional decline of each organ system affected by ALS.

Clinical trials are in advancements for using neurotrophic factors, released by mesenchymal stem cells, in treatment of muscular degeneration of ALS. A multi-step approach is in progress in current clinical trials. This is initiated by removing patient's own bone marrow and then extracting undifferentiated MSCs from a bone marrow cell.

The MSCs are then cultured further in ex vivo cell culture techniques and allowed to differentiate. The differentiated MSCs are

now releasing essential neurotrophic factors, required for phases of neuronal cells development, growth, proliferation and survival. These cells are then transplanted back in the patient.

The neurotropic factors involve vascular endothelial growth factor (VEGF) for angiogenesis and nourishment of neuronal cells, glial-derived neurotrophic factor (GDNF) for promoting neuronal survival, hepatocyte growth factor for promoting angiogenesis, and morphogenesis in various organs and brain-derived neurotrophic factor (BDNF) for neuronal plasticity. Neuronal plasticity is one of many important neuronal functions to change cellular structural and functional responses accordingly to certain stimuli or injuries. The functional importance of neurotrophic factors in modifying neuronal plasticity and survival makes them useful in treating progressive degeneration in ALS.

The differentiated transplanted MSCs have achieved favourable results in Phase II clinical trials for 48 ALS patients in United States in 2017. The transplanted cells are found to be well tolerated and safe. The cells have also improved ALS functional rating responder score (ALSFRS-R). Additionally, inflammatory markers have been downregulated alternatively with increase in neurotrophic factors, improving cerebrospinal fluid biomarker profiles. However, Phase III clinical trials in 2019 did not produce the required responses to meet primary endpoints.

N. Clinical and Biological Applications of Stem Cells in Neurological Disorders

It is important to take into consideration that all the applications of stem cells are either in the animal modelling stage or in the initial phases of clinical trials. Some of these are elaborated as follows.

1) Treatment with mesencephalic tissue in Parkinson's disease

   A project namely TRANSEURO in the United Kingdom has been investigating the advantages of transplanating fetal ventral mesencephalic tissue derived allogeneic dopaminergic neuroblasts

in to PD patients. It was useful in targeting areas which regulates normal neuronal function within the brain.

2) Multiple sclerosis therapy with mesenchymal stem cells

   The therapeutic effects of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) have been evaluated in patients with multiplesclerosis. Symptoms of the hUC-MSC-treated patients were improved compared to patients in the control group.

3) Neural stem cell therapy approach for amyotrophic lateral sclerosis

   A cell therapy approach has been developed for the treatment of later-phase ALS patients. This clinical trial has used fetal human neural stem cells (hNSCs) into the anterior horns of the spinal cord to test for the safety of both cells.

4) Treatment of spinal cord injury with human embryonic stem cells

   Human embryonic stem cells have been utilized in the treatment of spinal cord injury. All patients have shown significant power and movement of limbs, improvement in their control and sensation of bowel and bladder and sitting balance.

5) Bone marrow-derived cells in treatment of brain injury

   Transplantation with bone marrow-derived cells is a good alternative and valid strategy to treat a focal brain injury.

6) GABAergic interneurons in treatment of epilepsy

   Transplantation of GABAergic interneurons (INs) differentiated from embryonic stem cells can provide long-term functional benefits in animal models of epilepsy and other neurological disorders.

7) Autism spectrum disorder treatment with pluripotent stem cells

   Three-dimensional neural cultures (organoids) derived from induced pluripotent stem cells (iPSCs) have been investigated

neurodevelopmental alterations in individuals with severe idiopathic autism spectrum disorder (ASD).

O. Evidences for Safety and Effectiveness of Stem Cell-Based Therapy for Neurological Disorders

Promising outcomes of human umbilical cord mesenchymal stem cells (hUC-MSCs) have been found in the treatment of ischemic neurological disease. The safety profile and therapeutic effects of hUC-MSCs have been successfully evaluated in clinical trials. The intravenous administration of hUC-MSCs in ischemic patients has markedly improved neurological outcomes such as emotional reaction, extrapyramidal function, and cognition ability.

One of the most serious neurological disorders are intracerebral haemorrhage. Treatment with conventional strategies have been associated with greater risks. MSCs have been found safe and efficacious for treatment of intracerebral haemorrhage. MSC therapy has been associated with neuro-restoration and clinical improvement. Patients treated with this stem cell-based approach have not been associated with any serious adverse effects including de novo tumor development.

Treatment of progressive neurological disorders has been associated with limited safety and efficacy issues. Complicated diseases such as multiple sclerosis is of greater concern as its treatment is highly linked with conventional drugs toxicities. Adipose-mesenchymal derived stem cells (AdMSCs) show a better therapeutic option with minimal invasive procedures. There are improved safety outcomes in treating patients with multiple sclerosis and measures of treatment have shown recovering effects with AdMSCs infusions. PDA-001, human placenta-derived cells have been emerged as well tolerated and efficacious in treating progressive multiple sclerosis patients. There were no signs of paradoxical worsening of lesions with doses of PDA-001.

Cordstem-ST, an IV transplantation of umbilical cord-derived mesenchymal stem cells, has been found to be safe and has greater therapeutic potential in the treatment of acute cerebral infarction. Patients treated with Cordstem-ST experienced no serious adverse effects.

Differentiation of mesenchymal stromal cells into neuron-like cells has also attenuated recovery in cerebral oedema and acute stroke patients. The intravenous infusions of differentiated stem cells have accelerated recovery and improvement with no clinical adverse effects in these patients.

The regenerative therapy, also known as stem cell therapy, is now a part of the treatment regimen of various neuronal diseases. The basic concept behind this advanced therapy is improving the repair response of dysfunctional neuronal cells by transplanting stem cells. This is a revolutionary therapy approach for treating PD, AD, ALS, etc. All these diseases are characterized by the loss of specific neuronal cells and deposition of insoluble and unfolded proteins. Cognitive impairment, motor neuronal dysfunction and paralysis are serious manifestations of these diseases.

The critical pathology of these diseases and involvement of multiple molecular signalling proteins makes treatment more difficult. Also, serious adverse effects associated with conventional therapy makes the patient to leave therapies prior to the completion of treatment. Therefore, there is still a need for efficacious treatment with lower consequences of adverse events. Stem cell therapy has emerged as a safe and beneficial strategy in treating neurodegenerative diseases. It involves isolation of specific neuronal subtypes and reviving a neural network in replacement of the damaged and lost neurons in the disease. Although more research work is still required especially preclinical and clinical studies.

The recent clinical trials have set the stage to continue progress in stem cell-based therapy. Moreover, technological developments using hydrogels and nanoparticles are being in evaluation processes to make stem cell-based treatments more effective. In the future, regenerative

therapies involving stem cell-based treatments for neuronal diseases are expected to be used successfully in clinical settings, making an impossible cure to a better and more effective remedy.

P. Conclusion

Neurodegenerative diseases have disturbing adverse effects profile with conventional pharmacological therapies. To date, therapy with stem cells is probably the most efficacious and preferred treatment option for patients suffering from neurodegenerative diseases. From in vitro studies to animal model, now stem cells have been extensively evaluated in clinical settings in the treatment of various neurological diseases, such as PD, AD, etc. The cost, manpower requirement, and post-transplant monitoring are some of the concerns that are still under investigation for making this therapy more effective and advantageous.

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Chapter 8

The Potential of Cd34+ Hematopoietic Stem Cells to Increase Fibroblast and Collagen Skin in Ultraviolet B-Exposed Skin

Mochamad Syaifudin, Wimpie Pangkahila, Ida Sri Iswari, Basuki Supartono, Mochamad Wildan

A. Introduction

Aging is a process characterized by the decline and death of cells, which occurs with advancing age. The skin, which accounts for approximately 16% of the human body weight, is frequently exposed to sunlight, toxic substances, air pollution, and heavy metals daily (Parrado et al., 2019). The aging process affects all organs, including the human skin. Skin aging can be attributed to extrinsic factors, such as exposure to ultraviolet (UV) light, cigarette smoke, and air pollution, as well as intrinsic factors, such as genetics, race, and hormones (Krutmann et al., 2021).

Several studies demonstrate that the aging process causes a decline and depletion in the number of Langerhans cells in the

M. Syaifudin, W. Pangkahila, I. S. Iswari, I. G. K. N. Arijana, B. Supartono, M. Wildan

Clinic Regenerative Medicine MMC Lamongan, e-mail: msyaifudin1608@gmail.com

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epidermis. These cells are known as immunogen-effector cells in the skin and their reduction results in decreased resistance after exposure to the environment (Said et al., 2015). In the epidermal layer, there is a narrowing of the dermo-epidermal junction. Additionally, there is atrophy of the dermal layer, a reduction in fibroblasts, mast cells, and blood vessels, as well as abnormal nerve endings. Other changes include hair loss of pigment, abnormal nail beds, a reduced number of glands in cell size, reduced melanocyte cells, and a decrease in Langerhans cells (Niculet et al., 2020).

Ultraviolet (UV) light is considered one of the most important factors in premature skin aging, often referred to as photoaging (Amaro-Ortiz et al., 2014). Photoaging occurs when the skin is chronically and repeatedly exposed to UV rays over some times. UV light is one of the spectra from sunlight that reaches the earth, in addition to visible light and infrared light (Guan et al., 2021) evidence has also shown their efficacy in the prevention of photoaging, dyspigmentation, DNA damage, and photocarcinogenesis. In the USA, most broad-spectrum sunscreens provide protection against ultraviolet B (UVB.) Based on wavelength, UV rays can be further divided into ultraviolet A (UVA), ultraviolet B (UVB), and ultraviolet C (UVC) rays. Chronic exposure to UVA and UVB rays plays a significant role in photoaging and photocarcinogenesis (Bosch et al., 2015).

Exposure to UVB rays affects skin tissue and produces free radicals, which cause damage at the cellular level and ultimately lead to the death of collagen fiber cells and fibroblast cells (Yin et al., 2019). Collagen is a polypeptide that adopts a triple helix form, with each chain composed of glycine-X-Y linkages. The chains can be damaged by certain enzymes, resulting in the release of glycine groups. Currently, dermal collagen staining is identified based on the amount of glycine. Furthermore, glycine staining can be expressed using the Sirius Red dye (Chen et al., 2019).

A promising method is the utilization of stem cells. Stem cells are cells that can form and structure body tissues. They are early-life cells that can develop into other cells and form various tissues in the

body (multipotent). Furthermore, when stem cells are transplanted into the body, they will form body tissues in that specific location. The characteristics of stem cells are undifferentiated, self-renewal, and the ability to differentiate into more than one cell type (multipotent/pluripotent) (Romito & Cobellis, 2015).

Hematopoietic stem cells are progenitor cells that form blood cells. The sources of these cells are bone marrow and blood. Hematopoietic stem cells can be isolated directly from peripheral blood or through mobilization techniques. These stem cells possess pluripotent and plastic properties, allowing them to differentiate into non-hematopoietic cells (Ogawa et al., 2015). Research on the subcutaneous administration of human peripheral blood CD34+ stem cells to the skin of male Wistar rats exposed to UVB, while observing the number of fibroblast and collagen cells, has never been conducted. This article will discuss the potential of CD34+ hematopoietic stem cells to increase fibroblast and collagen skin in ultraviolet B exposed skin.

B. Skin

Skin is the largest organ in the human body. The appearance of the skin provides information about the individual, such as their overall health, ethnicity or race, lifestyle, and age. The quality of the appearance of the skin is determined by skin color, texture, and shape. As the largest organ in the human body, the skin has a surface area of 1.5–2 m^2 and accounts for about 15% of the total body weight of an adult (Meléndez-Martínez et al., 2019).

Skin is made up of three layers, from outermost to innermost: epidermis, dermis, and hypodermis (subcutaneous tissue). The epidermis, or skin's outermost layer, serves as a waterproof barrier and helps skin tone. The epidermis consists of five layers: stratum corneum, stratum lucidum, stratum spinosum, stratum granulosum, and stratum basale (Yousef et al., 2020). The epidermis is a dynamic structure, of which 95% is composed of differentiated keratinocytes. Other cells in the epidermis include melanocytes, Langerhans cells, and Merkel cells. Melanocytes are melanin-producing cells, which

are the pigment of the skin. Langerhans cells have an immunological function, and Merkel cells play a role in sensory perception (ter Horst et al., 2018).

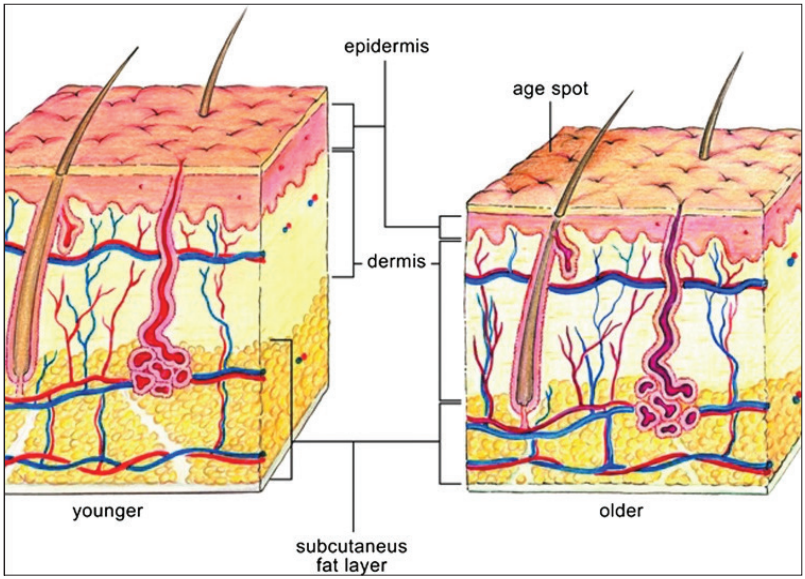
The dermis is divided into two layers: the papillary dermis on the surface and the reticular dermis beneath. The papillary dermis contains collagen, elastin, fibrous, and ground substance (mucopolysaccharides, hyaluronic acid, chondroitin sulfate) and is rich in microcirculation. The reticular dermis contains coarser bundles of collagen with scattered elastin fibers (Brown & Krishnamurthy, 2022).

Aging is an irreversible and natural phenomenon that occurs as a person ages. It is characterized by a gradual decline in the function of various organs and systems in the body. As a result of this reduction in function, many indications and symptoms of aging develop, which are divided into two parts, namely:

1) physical symptoms such as muscle mass, increased fat, wrinkled skin, decreased memory, impaired sexual function, decreased work ability, and bone problems (Amarya et al., 2018); and
2) psychological symptoms include decreased vitality, difficulty sleeping, anxiety, irritability, and feeling worthless (Kang & Kim, 2022).

Anti-aging medicine is defined as a branch of medicine that uses the latest scientific knowledge and medical technology to early detection, prevention, treatment, and reversal of age-related dysfunctions, disorders, and diseases. The goal of anti-aging medicine is to prolong life in a healthy state (Ok, 2022).

The structure of old and younger skin is indeed structurally different (Figure 8.1). In general, the epidermis was thicker in young compared to old skin. Younger epidermis has smoother epidermis. In older skin, the epidermis becomes thinner and flatter, and cell turnover slows down (Mine et al., 2008). The total thickness of the skin in old age decreases due to the loss of collagen and elastin in the dermis; collagen fibers become thicker and irregular than younger skin, reducing the elasticity of the skin. Collagen, the protein molecules made up of amino acids, is the main constituent of the dermis and is



Source: Farage et al. (2013)

Figure 8.1 Differences between Younger and Older Skin in Skin Structure

made of fibroblast collagen fibers. Young skin has a thick dermis rich in collagen and elastin fibers. As we age, collagen fibers become sparser, more damaged, and less able to support the skin (Karim et al., 2021).

The hypodermis, the deepest skin layer, is the subcutaneous fat layer on the skin. Fat, blood vessels and nerves are the main structural components of the hypodermis. It has many important functions, including storing energy, connecting the dermal layer of the skin to muscles and bones, insulating the body, and protecting against damage. Even though the hypodermis is located in the deepest layer of skin, aging also occurs in this layer, causing loss of fat on the hands and face, as well as increasing fat in the area between the hips and ribs (Liang et al., 2023).

Chronological aging is a natural process of physiological change and is influenced by genetic and hormonal factors. Chronological aging is characterized by xerosis, sagging, wrinkles, sluggishness, and

Table 8.1 Histological Manifestations of Chronological Skin Aging

Epidermis	Dermis	Other Tissues
Flattening of dermo-epidermal junction	Atrophy (decrease in dermis volume)	Hair depigmentation
Change in thickness	Changes in skin supporting tissue	Hair loss
Varying cell shape and sixe	Decreased fibroblast	Conversion of terminal hair to vellus hair
Atypical cell nuclei	Decreased mast cell	Nail plates abnormal
Decreased melanocyte	Decreased blood vessel	Abnormal glands
Decreased Langerhans cells	Shortening capillary looAbnormal nerve vessels	

the appearance of seborrheic keratosis and cherry angiomas (Karim et al., 2021). In the epidermal layer (Table 8.1), the most consistent structural changes in aged skin include flattening of the dermo-epidermal junction (Lynch et al., 2022), variation in thickness, changes in the size and shape of cells, occasional atypical nuclei, a decrease in the number of melanocytes, and a reduction in the number of Langerhans cells (Papaccio et al., 2022).

Dermis thickness decreases with age. In the dermis layer occurs atrophy, fibroblasts decrease, mast cells decrease, blood vessels decrease, capillary loops shorten, and nerve endings become abnormal as the result of aging. Other changes include hair loss of pigmentation, hair loss, terminal hair becoming fine hair, abnormal nail bed, and decreased glandular number (Farage et al., 2013).

Relatively few changes occur in the thickness of the epidermis, the shape of keratinocytes, and the cohesion of corneocytes, and there is a significant loss of melanocytes and Langerhans cells. The major skin changes in chronological skin aging are seen at the dermo-epidermal junction, which shows flattening of the rete ridges, which causes a reduction in contact between the epidermis and dermis, leading to a reduction in nutrient and metabolite exchange between these two compartments (Rittié & Fisher, 2015).

Skin aging is a complex biological process that is a consequence of both intrinsic and extrinsic factors. Intrinsic aging, also known as

chronological aging, results in changes in all layers of the skin (Karim et al., 2021). The epidermis undergoes a slowdown in regeneration. In young skin, epidermal turnover takes 28 days, but in old age, it takes 40–60 days. This slowdown results in thinning of the epidermis, making the skin appear translucent. The slowdown in epidermal regeneration also disrupts the skin's defense and repair functions (Kim & Leung, 2012).

Keratinocytes accumulate on the surface of the skin, making it appear rough and scaly. Histology of old skin shows thinning of the dermal-epidermal junction, which increases skin fragility and reduces nutrient transfer to the epidermis and dermis (Karim et al., 2021).

The population of melanocytes in the epidermis decreases and the existing melanocytes experience a decrease in activity. Old skin experiences dyschromic changes such as pigmented spots, freckles, and lentigines. Old skin is also more susceptible to sunburn because the skin is thinner and has fewer melanocytes (Karim et al., 2021). Skin aging also affects Langerhans cells. The number of Langerhans cells decreases by up to 50%, resulting in a decrease in skin immunity and an increased risk of skin cancer (Chambers & Vukmanovic-Stejic, 2020).

The dermis appears hypocellular, with fewer fibroblasts and mast cells and loss of dermal volume. Electron microscopy studies have shown that collagen fibers become loose and there is a moderate increase and thickening of elastin fibers with resorption of most sub-epidermal fibers. In addition, there is a decrease in the number of dermal blood vessels, a shortening of capillary loops, and a decrease in the density of Pacinian corpuscles and Meissner's corpuscles, which are skin-end organs responsible for the perception of pressure and light touch. Loss of sensory and autonomic innervation involves the epidermis or dermis (Russell-Goldman & Murphy, 2020).

C. UV Rays and Photoaging

Ultraviolet (UV) radiation is a type of nonionizing radiation that can be found at the lower end of the electromagnetic spectrum, between

X-rays and visible light. UV is an invisible electromagnetic radiation with a wavelength range of 100–400 nm. UV can be divided into four wave bands: vacuum UV, UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm) (Williamson & Neale, 2022).

UV radiation is classified as a "complete carcinogen" since it is a mutagen as well as a non-specific damaging agent, as well as a tumor starter and promoter. UV is the most important modifiable risk factor for skin cancer and many other environmental-influenced skin illnesses when it is abundant. UV, on the other hand, enhances human health by mediating natural vitamin D and endorphin synthesis in the skin; thus, UV has complicated and mixed impacts on human health (Amaro-Ortiz et al., 2014).

The three categories of UV radiation markers are as follows (de Jager et al., 2017).

1) UVA (320–400 nm)

   The most common UV radiation encountered since it passes through air ozone with little alteration. When UVA is overexposed, it causes pigment darkening (tanning) followed by sunburn. Although UVA is required for Vitamin D generation in humans, excessive exposure can cause epidermal hardening, immune system suppression, and cataract formation. UVA is often utilized in cosmetics, the production of sunbeds, or tanning booths.

2) UVB (290–320 nm)

   UVB is a crucial contributor to photochemical DNA damage. UVB is also required for the formation of Vitamin D in humans. However, excessive exposure may be damaging to the human body. These negative effects include sunburn, cataracts, and the start of the carcinogenic process in the skin.

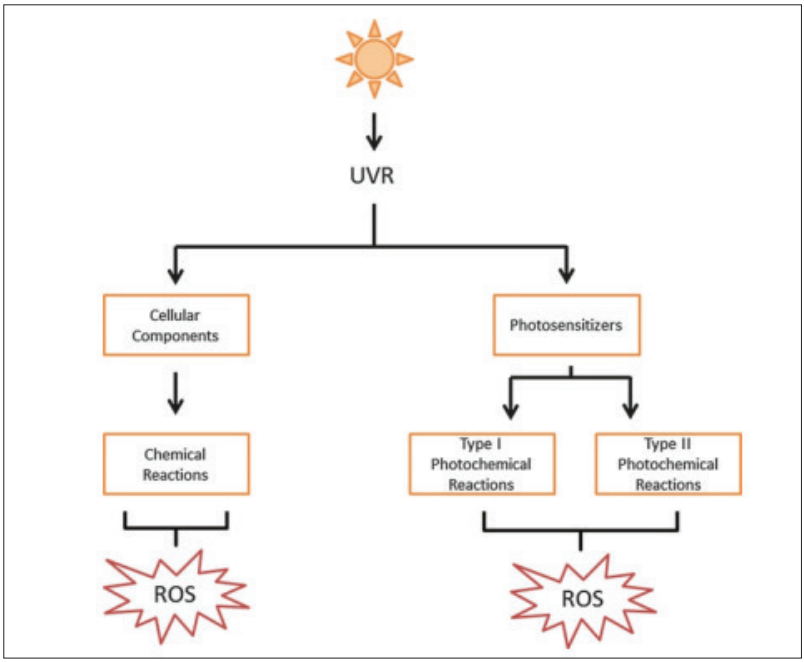
3) UVC (220–290 nm)

   UVC is almost entirely absorbed by ozone in the atmosphere and has little effect on human health. UVC is emitted by germicidal lamps to kill germs. Exposure to UVC by humans may result in

ocular burns and snow blindness. Because UVC is absorbed by the dead outer layer of the dermis, exposure can produce acute pain that subsides in a few days.

UV radiation triggers the formation of reactive oxygen species (ROS), which can damage DNA and inhibit the activity of tyrosine phosphatase. UV can also reduce retinoic acid receptor (RA) and trigger an increase in nuclear factor-kappaB (NF-kappaB), with a final effect of reducing collagen production, and collagen breakdown, due to the activity of matrix metalloproteinases (MMPs) (Gromkowska-Kępka et al., 2021).

UV radiation can mediate damage to cellular components in two ways (Figure 8. 2). The first mechanism involves the direct absorption of incident rays by the cell and its components. This results in the production of an excited state of the components and subsequent



Source: Jager et al. (2017)

Figure 8.2 Mechanism of UV radiation mediates cellular damage.

chemical reactions. The second mechanism is photosensitization. Incident rays are absorbed by endogenous or exogenous photosensitizers such as bilirubin. As a result, the sensitizers are excited to their triple states. The excited photosensitizers work in two ways. Type I photochemical reactions involve electron transport and the process of hydrogen abstraction to create free radicals. Type II photochemical reactions require the transfer of energy with O_2 to produce reactive state singlet oxygen (1 O_2) (de Jager et al., 2017).

Photoaging refers to the skin changes that are caused by chronic sun exposure on top of the layers of chronological skin aging. Photoaging is produced from the cumulative damage of UV radiation that causes severe skin disorders (Tanveer et al., 2023). The UVC part of the spectrum is not present in sunlight on earth, except at high latitudes, because the UVC part is absorbed by the ozone layer of the atmosphere through the absorption of UVA and UVB rays by cellular chromophores such as urocanic acid, riboflavin and melanin precursors, which work as photosensitizers that play a major role in the production of reactive oxygen species (ROS) and free radicals (WHO, 2017).

Long-term exposure to UVA radiation can induce the same changes as those induced by UVB, including dermal hyperplasia, thickening of the stratum corneum, thinning of Langerhans cells, dermal inflammation, and accumulation of lysozymes on dermal fibers. Clinically, photoaged skin shows characteristics of roughness, fine and coarse wrinkles, uneven hyperpigmentation that can be in the form of lentigines or spots (freckles), weakness, swelling, and telangiectasia (Maeda, 2018).

D. The Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are the definitive architects of hematopoiesis, which functions as a continuous producer of blood cells throughout the life of an organism (Pinho & Frenette, 2019). To keep the immune system and hemostasis functioning normally throughout the life cycle, HSCs continuously produce blood, a process

known as hematopoiesis. Anatomically, hemostasis mostly takes place in the bone marrow of the skull, pelvis, sternum, and vertebral column (Boes & Durham, 2017).

Each HSC is programmed to produce various blood cell components efficiently, thereby enabling red blood cells to transport oxygen, megakaryocytes, and platelet derivatives to interact with injured vascular and immune system cells to protect against microbial attack (Ng & Alexander, 2017). The first appearance of HSCs in the embryonic stage of hematopoiesis was identified in the aorta-gonad-mesonephros area, which then shifted to the fetal liver and continued to the bone marrow over time (Julien et al., 2016).

The hematopoiesis, which serves as a constant maker of blood cells throughout an organism's life, is unquestionably designed by the HSC. Red blood cells that carry oxygen, megakaryocytes, and platelet derivatives can interact because each HSC is engineered to produce different blood cell components effectively (Chapman & Zhang, 2018).

Each HSC is engineered to effectively create different blood cell components, allowing oxygen-carrying red blood cells, megakaryocytes, and platelet derivatives to collaborate with damaged vascular and immune system cells to fend against microbial onslaught (Ng & Alexander, 2017). HSC first appeared in the aortogonado-mesonephros region during the embryonic stage of hematopoiesis. Over time, they moved to the fetal liver and then on to the bone marrow. Since HSCs were originally discovered, there has been a great deal of research into stem cell-specific markers (Chapman & Zhang, 2018). This is predicated on the fundamental tenet that every cell possesses distinctive markers, including blood cells, referred to as clusters of differentiation (CD). CD45-positive hematopoietic cell markers predominate (Yadav et al., 2020). HSC research has focused on examining the differentiation of HSC into progenitor cells in vitro colony assays and HSC/progenitor transplantation experiments in myeloblastic experimental mice (Yadav et al., 2020).

Because mature blood cells have a short lifespan, they must constantly be replenished HSCs, a limited subset of cells with the

capacity for self-renewal and differentiation, carry out this function. HSCs cannot form non-hematopoietic cell groupings like MSCs, which makes it possible for them to differentiate under MSCs (Chen & Ju, 2019).

HSCs are adult stem cells from the bone marrow that show the markers CD34+, CD133+, and Thy1+, but not CD38- or CD33-. They come from the hematopoietic system. Aside from self-renewal, HSCs are capable of actively differentiating by developing all blood components (multipotent). HSCs can also go into the cell cycle phase known as G0, which is known as dormancy and is devoid of cell division activity. However, G0 phase cells are still required, especially in cases of tissue injury (Rix et al., 2022).

1. Origins of HSC

Since HSCs mostly comes from the bone marrow, it is typically challenging to locate HSCs in peripheral blood. Therefore, activation with specific cytokines is required to cause HSC migration to peripheral blood arteries. Granulocyte colony-stimulating factor (G-CSF) or cytotoxic drugs (myelosuppressive bone marrow suppressants) are specifically used to stimulate and produce HSCs. Cytotoxic substances can disrupt the communication between hematopoietic cells and bone marrow stromal cells, causing a significant number of progenitor cells and HSCs to be released into the bloodstream (Xie et al., 2021).

2. Plasticity of Hematopoietic Stem Cell (HSC)

There is a growing body of evidence that HSCs are plastic and that, at least under some circumstances, they can participate in the generation of tissues other than those of the blood system. A few studies have shown that HSCs can give rise to liver cells. Those findings have scientists speculating about the biological response of HSCs to disease or tissue damage and about the early differentiation of the embryonic tissues into discrete layers. It was unexpected that a component of blood could crossover a developmental separation to form a tissue

type that ordinarily has a completely different embryonic origin (Ogawa et al., 2015).

The findings noted above and other reports of cardiac and muscle tissue formation after bone marrow transplantation in mice and of the development of neuron-like cells from bone marrow have raised expectations that HSCs will eventually be shown to be able to give rise to multiple cell types from all three germ layers. One study has demonstrated that a single HSC transplanted into an irradiated mouse generated not only blood components (from the mesoderm later of the embryo) but also epithelial cells in the lungs, gut, (endoderm layer), and skin (ectoderm layer). If HSCs are truly multipotent, their potential for life-saving regenerative therapies may be considerably expanded in the future (Ogawa et al., 2015).

3. The Possible Risk of HSCs

Allogeneic HSCT is used to treat hemoglobinopathies; after conditioned to overcome the immunological barrier, allogeneic stem cells are used as vectors to correct the basic genetic defect by replanting genes that are essential for normal hematopoiesis. Although the benefits of HSCs are widely used in cell therapy, transplanted HSCs can have fatal side effects in bone marrow transplantation in thalassemia patients for indications in high-risk category patients. Allogeneic stem cell transplant from a matched sibling donor is an option to treat certain types of thalassemia and has shown 15-year survival rates reaching near 80%. However, recent retrospective data showed similar overall survival compared to conventional treatments with multiple blood transfusions (Khaddour et al., 2023).

When the allogeneic graft starts to proliferate in the recipient, an immunological graft versus-host reaction graft-versus-host disease (GVHD) may occur. GVHD can affect one or more organs to varying degrees, with the most frequently affected being the skin, gastrointestinal tract, and liver. GVHD is a serious complication of bone marrow transplantation and can be fatal. Therefore, the prophylactic administration of cyclosporine (an immunosuppressive

drug) is an important part of the pretransplant and post-transplant treatment (Lucarelli et al., 2012)

4. HSC Marker

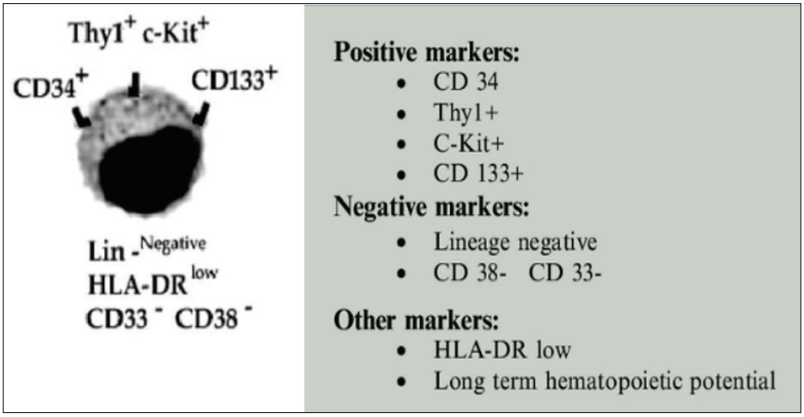
Numerous markers found in HSC serve as intrinsic and signal integration molecules in the activation of transduction pathways. However, CD34+/CD38- can be used as a substitute for the standard HSC markers CD34+/CD133+/Thy1+/CD38-/CD33- (Figure 8.3). Because it is more feasible, using markers is more common (Sudo et al., 2013).

Systematically, HSC markers are divided into two.

1) HSC conventional or traditional markers

   The three categories of traditional HSC markers are as follows (Rix et al., 2022).

   a) CD34+ HSC express the sialomucin transmembrane protein CD34+, which serves as an adhesion molecule. Lymph node endothelial cells that T cell selectin ligands bind to when they enter the lymph nodes also express CD34+ protein.



Source: Rix et al. (2022)

Figure 8.3 Markers of HSCs

b) Localizing cells is the function of the glycoprotein CD133+, sometimes referred to as prominin 1, which is expressed by HSC, progenitor, and endothelial cells.

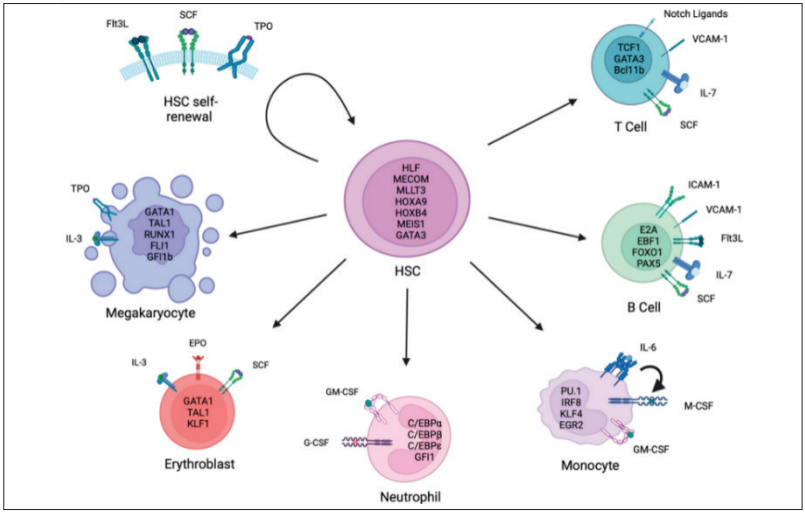
c) Thy1+ Thy1+, also referred to as CD90+, is a glycoprotein that is expressed by HSC, thymocytes (T cell precursors), MSC, NK, neurons, and endothelium and serves as a cell-to-cell communication molecule and matrix contact. The illustration below explains the HSC conventional marker.

The HSCs do not express CD38 or CD33 markers but do express CD34, CD133, and Thy1 markers. Except for self-renewal, HSCs can actively differentiate to generate all blood components.

2) HSC complex markers

Systematically, HSC complex markers are divided into four types (Figure 8.4), namely (Bozhilov et al., 2023):

a) CD34+/CD38-/c-Mpl+, these markers function as cellular physical markers;



Source: Bozhilov et al. (2023)

Figure 8.4 HSC Molecular Complexity Markers

b) Thrombopoietin (TPO), this marker functions as a self-renewal activity;
c) stem cell factor (SCF), marker functions as a proliferative and differentiation activity;
d) transforming growth-factor-betas (TGF-β); this marker functions as a dormant cell cycle.

HSCs express the standard marker CD34+/CD38-/c-Mpl+, the self-renewal marker TPO, the proliferation and differentiation marker SCF, and the dormant cell cycle marker TGF-β. SCF and TPO are two HSC molecular markers that are important. These two marker proteins have cytokine regulator roles. SCF specifically affects hematopoietic progenitor cell promotion and differentiation, whereas TPO affects self-renewal (Mann et al., 2022).

On the other hand, because TPO cytokines and their receptors, including c-Mpl, are involved in the early stages of HSC hematopoiesis, cells that express the markers CD34+/CD38-/c-Mpl+ have significantly higher HSC engraftment activity (Li et al., 2022). By encouraging HSC adherence to bone marrow osteoblasts and maintaining long-term repopulating activity, signals from angiopoietin-1 via Tie2 control HSC dormancy (He et al., 2014).

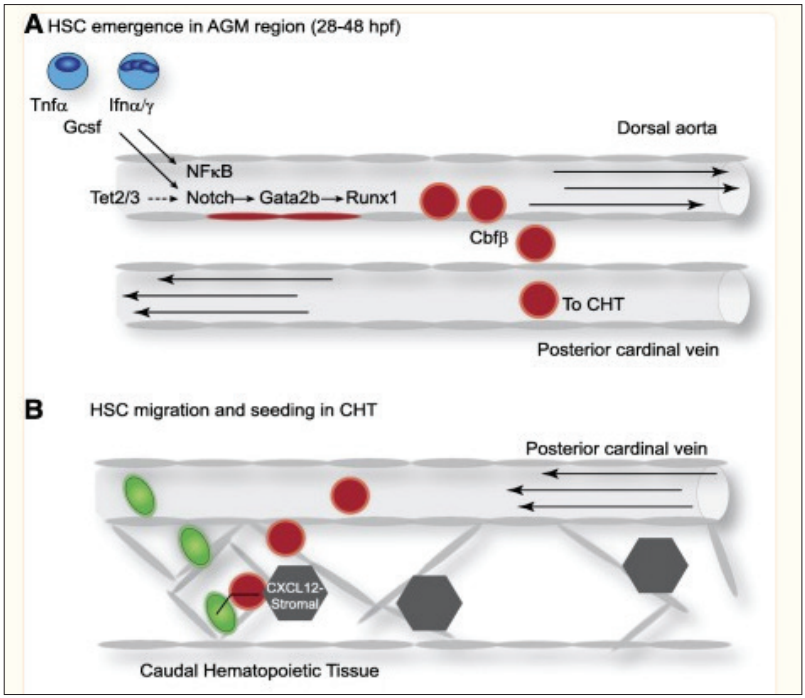
5. HSC Development

The integration of several intrinsic factors and signal transduction pathways is necessary for the emergence and specification of HSCs, from early mesodermal precursors to the development of HSCs in the bone marrow. Animal experiments have revealed several regulatory pathways for the emergence of HSCs, including the vascular endothelial growth factor (VEGF) pathway, which can promote cell differentiation and migration, the sonic hedgehog (SHH) pathway, and the bone morphogenetic protein (BMP) pathway, which control arterial wall polarization (HSC specification). Notch lines for HSC shape and specification, as well (Thambyrajah & Bigas, 2022).

Development of HSCs occurs in a region termed the aortagonad-mesonephros (AGM). Within this region, HSCs specifically arise from specialized hemogenic endothelium found in the ventral wall of the dorsal aorta (DA) in a process termed the endothelial-to-hematopoietic transition. The dorsal section of the aorta (DA), which is formed by the vascular cord, which is originally formed by HSC precursors that migrate to the middle region of the embryo from the posterior lateral plate of mesoderm (PLM). HSCs grow from specialized hemogenic endothelial cells after the development of DA; these cells then leave the aorta and enter the bloodstream, where they seed in a niche region for additional development (de la Garza et al., 2017).

The process begins with the release of pro-inflammatory signal molecules Tumor Necrosis Factor Alpha (TNF-α) and Interferon Alpha/Gamma (IFN-α/γ) by myeloid effector cells which can promote the emergence of HSCs via the NF-κB and Notch signaling pathways. The other side of the Tet2/3 protein also regulates Notch signaling. All these conditions trigger the expression of Gata2-b and runx1 in hemogenic endothelial cells. The Cbf-β molecule is needed to encourage extravasation of emerging HSCs from within the dorsal aorta (DA) so that nascent HSCs (newborn HSCs) appear. Nascent HSCs then seed into the caudal hematopoietic tissue (CHT) to induce endothelial remodeling to form a micro-niche consisting of HSCs surrounded by endothelial cells adjacent to stromal cells that express CXCL12 (Figure 8.5) (de la Garza et al., 2017).

Myeloid effector cells emit the pro-inflammatory signaling chemicals TNF- and IFN-α/γ, which can encourage the formation of HSCs via the NF-B and Notch signaling pathways. This is the first step in the process. Contrarily, Tet2/3 protein also controls Notch signaling. The expression of Gata2-b and Runx1 in hemogenic endothelial cells is induced by each of these circumstances. For nascent HSC (newborn HSC) to manifest, the extravasation of HSC emerging from the dorsal aorta (DA) must be stimulated by the Cbf-



Source: de la Garza et al. (2017)

Figure 8.5 HSC Molecular Development

molecule. To cause endothelial remodeling and create a micro-niche with HSCs surrounded by endothelial cells next to stromal cells that produce CXCL12, nascent HSCs were then sown into CHT sections. The image below explains how HSCs are formed molecularly (de la Garza et al., 2017).

The process of HSC formation begins with the release of TNF-α and IFNα/γ activating the NF-κB and Notch signaling pathways. Together with the Tet 2/3 protein it triggers the expression of Gata2-b and runx1 in hemogenic endothelial cells and together with the Cbf-β molecule promotes the emergence of HSCs as nascent HSCs from within DA. Nascent HSC is then seeded into the CHT section forming a micro-niche consisting of HSC endothelial cells and stromal cells

expressing CXCL12, so that HSCs reside in the bone marrow (de la Garza et al., 2017)

Regulation and coordination of HSC transcription factors namely Gata2, Scl, Runx1, Lmo2, and C-myb. The coordination of HSC transcription factors with epigenetic factors is important in determining the fate of HSCs. Gata2 plays an important role in hematopoiesis, especially downstream of Notch signaling during HSC specification. Gata2-a also plays a role in vascular, conversely, Gata2-b is needed in the formation of HSC (de la Garza et al., 2017).

6. HSC Characterization Based On CFU Test

The definition of HSC was based on the capacity of donor HSCs to rebuild (reconstruct) the recipient's bone marrow blood hematopoietic system after radiation ablation had previously caused damage to it. This demonstrates the evolution of the idea that HSCs are bone marrow cells capable of producing the full blood system (Eaves, 2015).

The lymphatic organs of the recipient can be used to directly identify different donor-derived (clonogenic) cell colonies. In particular, the progenitor cells—cells in charge of hematopoietic recovery—allow characterization by examining the recipient lymph colony forming unit (CFU). To preserve stem cell properties, HSC generates progenitor cells that are capable of self-renewal and numerous hematological or multipotent offspring (Kronstein-Wiedemann & Tonn, 2019)

An in vitro test called the CFU test (CFU assay) is used to evaluate hematopoietic progenitor cells, particularly multipotent progenitor cells (MPP) and progenitor cells with a restricted lineage, such as erythroid, granulocytic, and monocyte cells. Although the majority of the CFU found in bone marrow and blood have limited potential in vivo, primitive HSCs and progenitors can colonize under specific culture conditions (Li et al., 2015)

CFU testing is performed by planting low-density single cells in methyl cellulose-based semisolid medium, such as MethoCultTM, together with a specific combination of cytokines. As a result, discrete

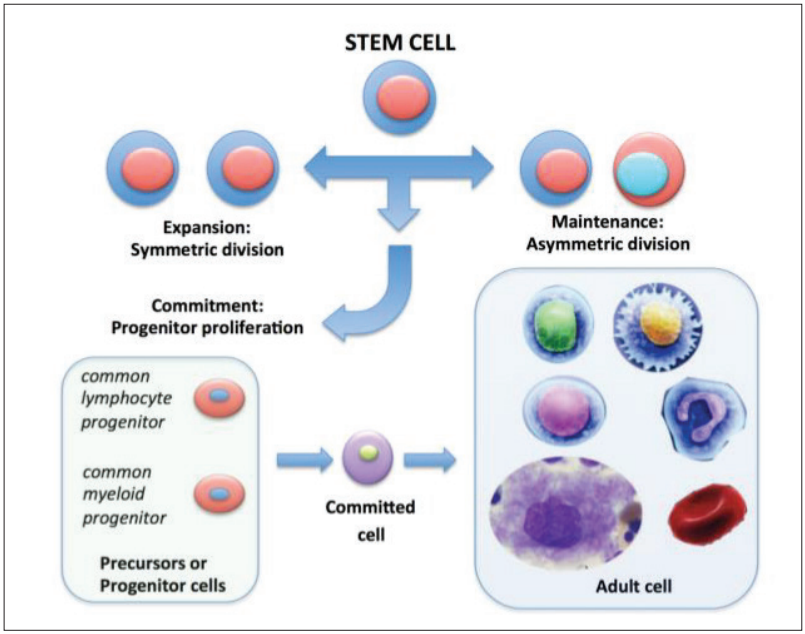
and separate colonies are created as HSCs multiply and develop into certain progenitor cells. Using morphological and phenotypic criteria, colonies originating from several progenitor cell types were categorized and counted according to the quantity and kind of mature cells produced. When long-term transplant studies are both costly and unfeasible, CFU studies are helpful for HSC (Li et al., 2015).

E. The Physiological Dynamics of Hematopoiesis

Proliferative behavior is a characteristic of in vivo HSC activity. In Basic Molecular Stem Cells, HSCs divide more slowly than progenitor cells. G0 state or quiescence status are two ways to measure the slow rate of HSC cleavage as they progress through the cell cycle. The G0 state is connected to the condition of cells that have stopped dividing (gone dormant) but are still capable of reversing their actions. This state can be distinguished from senescence, which is the condition of cells that are permanently kept in the G1 phase (Cheung & Rando, 2013).

Two modes of cell division are referred to as asymmetric cell division and symmetric cell division (Figure 8.6). The asymmetric division of a stem cell characterized by only produces one differentiated cell and one stem cell, or two distinctly differentiated daughter cells for self-renewal and homeostatic control of the stem cell pool. Therefore, every SC produces copies of itself plus differentiated cells. The second mode, symmetric division of a stem cell characterized only produces two identical stem cells or differentiated daughters (Caocci et al., 2017; Majumda & Tao Liu, 2020).

Intestinal cells and hair follicles are two examples of tissues with high rates of cell turnover that provide evidence for the function of stem cells, including HSCs. Stem cells with a high rate of proliferation are present in this tissue. This shows that the HSCs have an active compartment that can guide hematopoiesis towards a stable state and a dormant compartment that serves as a reserve for preserving the stem cells' capacity for long-term self-renewal (Rompolas & Greco, 2014).



Source: Caocci et al. (2017)

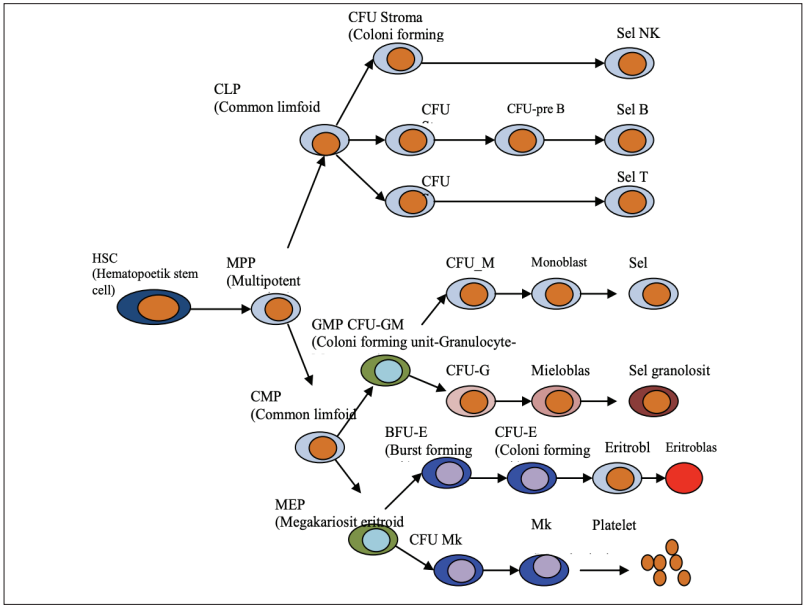
Figure 8.6 HSC in Bone Marrow

However, HSCs in their latent form can still react to stress. HSC proliferative activity differs from asymmetric cleavage activity, which calls for a correlation between HSC activation and differentiation. When tissue injury occurs, HSC cleavage takes the form of self-renewal activity to replenish HSC that were lost owing to differentiation (Pinho & Frenette, 2019).

Injury signaling molecules are released by damaged tissue, suggesting a significant need for tissue repair and regeneration. To sustain the hematopoiesis system and restore active compartment HSCs that had previously divided asymmetrically to satisfy the needs of injured tissue regeneration, the injury signal causes active dormant compartment senescence HSCs to divide symmetrically (Nugraha & Putra, 2018).

F. Maturation of HSC Development

HSCs continue to multiply and develop into adult cell lines. HSC derivatives go through various changes during this differentiation process depending on the stages of maturation (Figure 8.7), starting



Notes: LT-HSC : long term-hematopoietic stem cell;
ST-HSC : short-term hematopoietic stem cell;
MPP : multipotential progenitor;
CLP : common lymphoid progenitor;
CMP : common myeloid progenitor;
CFU-GEMM : colony-forming unit-granulocyte/erythrocyte/macrophage/megakaryocyte;
BFU-E : burst-forming unit-erythroid;
CFU-E : colony-forming unit-erythroid;
CFU-Mk : colony-forming unit-megakaryocyte;
CFU-GM : colony-forming unit-granulocyte/Macrophage;
CFU-G : colony-forming unit-granulocyte;
CFU-M : colony-forming unit macrophage.

Source: Wognum and Szilvassy (2015)

Figure 8.7 HSC Hierarchy

with becoming multi-potential progenitors (MPP), then progenitors that have been committed to certain lineages (lineage-committed progenitors), and so on, until they finally reach maturation into specific cells, such as monocytes or monocytes, lymphocytes, and so forth. An MPP commits in bone marrow to become either common myeloid progenitor (CMP) or common lymphoid progenitor (CLP). The CMP and CLP give rise to mature blood cells in peripheral blood, such as granulocytes, red blood cells (RBC), platelets, monocytes, T cells, B cells, and natural killer (NK) cells (Tober et al., 2018).

G. HSC Therapy in Skin Aging

Stem cells (SCs) have changed the old paradigm of anti-aging and gained increased attention as a new therapeutic technique in the advancement of biotechnology (Table 8.2). Stem cell (SC) therapies

Table 8.2 Clinical Trials Applications of Stem Cell for Facial Skin Aging and Photoaging

SC Preparation	Outcomes	Time	Results	Reference
Secretome adipose-derived stem cells (AD-SC)	1. The epidermal and dermal thickness 2. Collagen density	6 weeks	The epidermal and dermal thickness ↑ The expression of TIMP-1 and dermal collagen density ↑	Putri et al. (2022)
Exosomes derived from human umbilical cord blood mesenchymal stem cells	Collagen I dan Elastin synthetis	4 weeks	Increased expressions of Collagen I and elastin ↑ after 3 days	Kim et al. (2017)
Stromal vascular fraction (SVF)	Quality of skin: spots, pores, UV spots, brown spots, and red areas	6 months	Improved spots, pores, UV spots, brown spots, and red areas	Kuo et al. (2021)

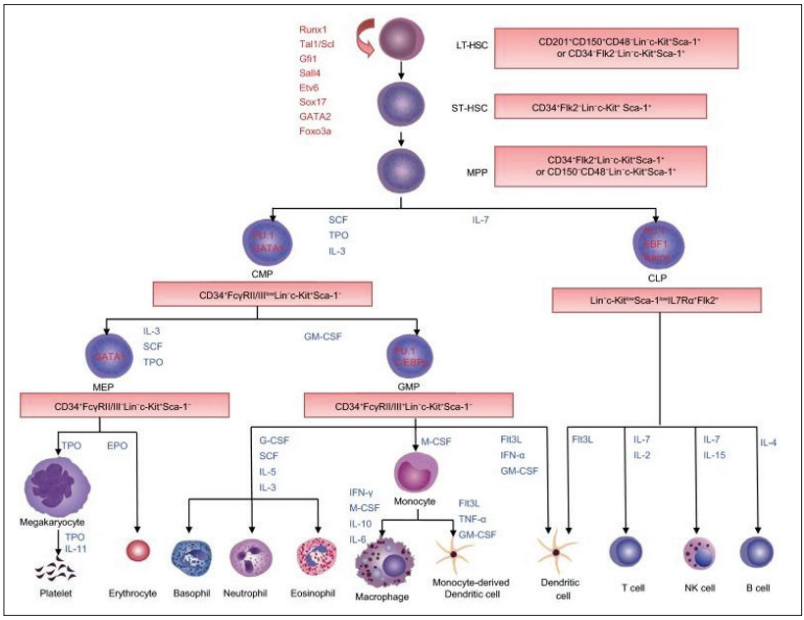
SC Preparation	Outcomes	Time	Results	Reference
Adipose-derived stem cells (AD-SCs)	Collagen synthesis	4 weeks	Significant wrinkle reduction and hingher collagen density	Jeong et al. (2015)
Adipose-derived mesenchymal stem cells (AD-MSCs)	1. Presences of oxylatan and elaunin 2. Total fibrilin and tropoelastin	6 weeks	Increased and ordered presence of oxytalan and elaunin fibers in zone 1 Total fibrillin and tropoelastin increased after treatment	Charles-De-Sá et al. (2020)

have broad application prospects in the field of regenerative medicine due to the inherent biological characteristics of SCs, such as their plasticity, self-renewal, and multidirectional differentiation potential. Thus, SCs could delay or even reverse aging (Chang et al., 2022).

H. Molecular of HSC Differentiation

Numerous cytokines, chemokines, receptors, and intracellular signaling molecules have an impact on the mechanisms governing HSC self-renewal and differentiation. Growth factors and cytokines, such as CSF and IL, that activate intracellular signaling pathways control the differentiation of HSCs. Traditionally, HSC differentiates into the oligopotent progenitor cell lineages of lymphoid and myeloid-erythroid, which are both confined progenitor cell lineages (Lee & Hong, 2020).

HSC lineage development model of the HSC lineage (Figure 8.8). Common myeloid progenitor cells and lymphoid progenitor cells are produced by the differentiation of HSC. T cells, B cells, NK cells, and dendritic cells are produced by common lymphoid progenitor cells, whereas granulocyte-macrophage progenitor cells and megakaryocyte



Source: Cheng et al. (2020)

Figure 8.8 HSC Lineage

erythroid progenitor cells are produced by common myeloid progenitor cells. Myeloblasts and monocytes are produced by granulocyte-macrophage progenitor cells, whereas macrophages are produced by monocytes. Erythrocytes and platelets are produced by collections of megakaryocyte erythroid progenitor cells (Cheng et al., 2020)

I. CD34+

CD34+ is a surface glycophosphoprotein expressed on early hematopoietic stem and progenitor cells, microvascular endothelial fibroblasts, and embryos. Bone marrow (BM) CD34+ cells represent only 1.5% of bone marrow mononuclear cells, but contain precursors of all lymphocyte lineages, as evidenced by the finding that CD34+ cells purified from marrow can restore hematopoietic recovery in primates, humans, or mice undergoing infusion. autologous bone

marrow after keloid treatment. CD34+ hematopoietic cells obtained from marrow or blood are used clinically in transplantation and gene therapy studies, including ongoing efforts to expand hematopoietic progenitor cells ex vivo (Hassanpour et al., 2023).

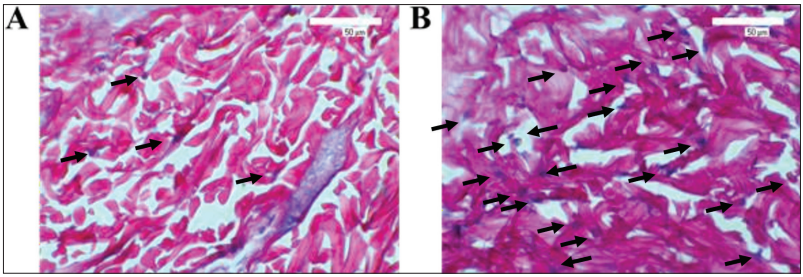
Despite the importance of CD34+ as a marker of early hematopoietic progenitor cells in experimental and clinical hematopoiesis, the function of CD34 remains unclear. Due to its potential role in fundamental processes such as hematopoietic progenitor cell development and inflammation, research into the regulation and function of CD34+ is ongoing in several laboratories (Sidney et al., 2014).

Recent experiments on CD34 function suggest that CD34 expressed on endothelial cells may play a role in leukocyte adhesion and "homing" during inflammation. It is hypothesized that CD34 plays a role in the stable localization of progenitor cells in the BM, CD34 may also play a role, involved in the maintenance of the hematopoietic phenotype of Sted's ancestors (Hughes et al., 2020).

1. Effect of CD34+ Hematopoietic Stem Cell to Increase Fibroblast and Collagen Skin in Ultraviolet B-Exposed Skin

CD34+ cells make up roughly 1%–2% of all bone marrow cells. CD34+ cells are typically collected through the process of leukapheresis, whereby blood is processed in a way that concentrates white blood cells and removes many of the red blood cells. Once a concentrated sample containing hematopoietic stem cells is acquired, researchers can apply additional separation methods to extract the desired cell population from the leukoplakia. Even though leukoplakias are specifically designed for further cell separation, harsh isolation methods can result in cell damage that affects viability and cell function. Gentle isolation is especially critical when separating rare or fragile cell populations, such as CD34+ cells, to acquire a sufficient number of cells that retain their normal function (Romito & Cobellis, 2015).

The control group with UVB exposure, there were fewer blue-nucleated fibroblasts overall, but in the CD34+ group with UVB



Notes: A: UVB plus a control group;
B: CD34+ group plus UVB. Increased blue cell nuclei in fibroblast cells were seen. Fibroblasts were marked by white arrows (100× magnification).

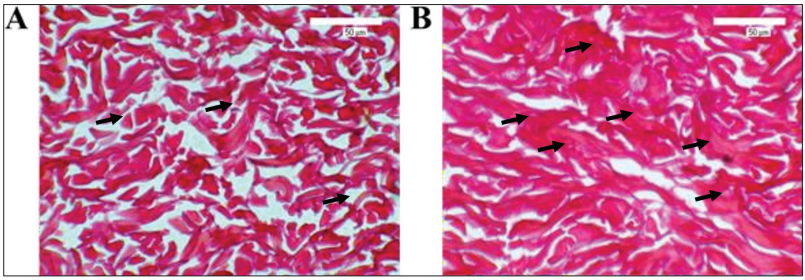
Source: Syaifudin (2015)

Figure 8.9 Wistar Rat Dermal Tissue Fibroblast Cell Count with HE Staining

exposure, there were more blue cell nuclei in fibroblasts (Figure 8.9 A). Based on the results, the treatment group's fibroblast cell count increased by 68.39%, and its collagen content increased by 18.66% when compared to the control group (Figure 8.9B). It is because stem cells may create and organize bodily tissues. Stem cells are young, multipotent cells that can differentiate into other types of cells and create new bodily tissues. When implanted into the body, stem cells will develop into body tissue at that precise site. Stem cells have the qualities of being undifferentiated, having the capacity for self-renewal, and having the capacity to differentiate into several cell types (Bacakova et al., 2018).

In group with UVB plus as a control group (Figure 8.10A), damage to the structure and content of collagen is evident in the thin-appearing red collagen fibers. Incomplete collagen fibers are shown by white arrows. In group with UVB and CD34+ subset (Figure 8.10B), collagen that has red collagen strands seems to be bigger and broader. Intact collagen fibers are indicated by arrows.

Hematopoietic stem cells, especially CD34+ stem cells, are one such option. Progenitor cells called hematopoietic stem cells produce blood cells. Blood and bone marrow are the origins of these cells. Direct isolation of hematopoietic stem cells from peripheral blood



Notes: A: UVB plus a control group;
B: CD34+ group plus UVB (100× magnification).

Source: Syaifudin (2015)

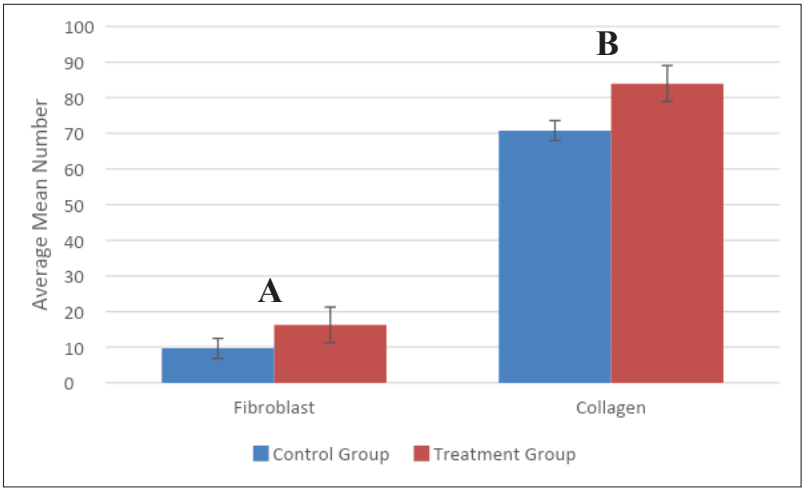
Figure 8.10 Collagen content in the dermis of male Wistar rats stained with Sirius Red.

is possible, as well as mobilization methods. These stem cells can develop into non-hematopoietic cells because they have pluripotent and plastic features (Supartono et al., 2018).

The differences in the mean number of fibroblasts and collagen between groups after administration of CD34+ stem cell suspension was measured (Figure 8.11). The histogram is presented as mean ± standard deviation. Asterisk symbol shows significant difference in modulators at difference treatment between control group and treatment group ($P < 0.05$).

When donor peripheral blood is isolated directly, no medications are used in the procedure. After being cultivated and identified by cytometric examination, the separated cells are given a medium for growth and application. Peripheral blood can be used to collect hematopoietic stem cells directly, or they can be mobilized. By isolating donor cells without the use of medication, direct isolation is carried out (Supartono et al., 2019).

Hematopoietic stem cells that express the CD34 cell marker are known as CD34+ cells. The most accurate indicator of hematopoietic stem cells is thought to be CD34. Pluripotent blood stem cells, unipotent myeloid cells, vascular endothelium, brain membrane



Notes: A. Fibroblast; B. Collagen (*p<0.05 showed significance compared to the control group).

Source: Syaifudin (2015)

Figure 8.11 Differences in the Mean Number of Fibroblasts and Collagen between Groups after Administration of CD34+ Stem Cell Suspension

components, and human skin follicular cells all express the CD34 antigen. Studying the plasticity—the capacity of hematopoietic stem cells to differentiate into non-hematopoietic cells—of CD34+ cells is made possible by their features. According to this study, delivering CD34+ hematopoietic stem cells to the skin's dermis may enhance the number of fibroblast and collagen cells there. This has therapeutic importance since it causes the skin to grow firmer and develop a smooth and robust texture (Sidney et al., 2014).

Male Wistar rats exposed to UVB radiation had more fibroblast cells in their skin after receiving subcutaneous injections of human peripheral blood CD34+ stem cells. Additionally, it caused the skin of male Wistar rats exposed to UVB radiation to produce more collagen. By highlighting the potential use of CD34+ hematopoietic stem cells in boosting skin fibroblast cells and collagen, this research aids in the regeneration of face skin (Zorina et al., 2023).

Hyaluronic acid products and laser technology have traditionally been utilized to promote the formation of fibroblasts and collagen. In contrast to typical cosmetic techniques, utilizing hematopoietic stem cells gives the benefit of sustained skin restoration. As only standard injectable syringes were utilized, it is crucial to emphasize that this study had restrictions regarding observation duration, dosage, and depth of subcutaneous injection. Therefore, these elements may have an impact on the fibroblast cells' considerable rise as compared to collagen. It is advised that more studies be conducted to increase the observation period and make use of injection equipment to precisely gauge CD34+ hematopoietic stem cell dose and depth. Clinical studies are required to verify the results of this study in people (Brohem et al., 2013).

J. Conclusion

Based on the discussion in this chapter, the following four conclusions can be drawn.

1) Anti-aging medicine is a branch of study to the early detection, prevention, treatment, and reversal of age-related dysfunctions, disorders, and diseases.
2) UV radiation can mediate damage to cellular components and photoaging that is produced from the cumulative damage of UV radiation can cause severe skin.
3) CD34 is considered the most reliable marker for hematopoietic stem cells. The characteristics of CD34+ cells provide an opportunity for studying their plasticity, which refers to the ability of hematopoietic stem cells to transform into non-hematopoietic cells.
4) Administration of human peripheral blood CD34+ stem cells subcutaneously increased the number of fibroblasts and collagen in the skin of male Wistar rats exposed to ultraviolet B (UVB) radiation.

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Chapter 9

Induced Pluripotent Stem Cells (iPSCs) and Neurological Diseases

Ahmad Faried
Yulius Hermanto

A. Introduction

A cell is the smallest unit of an organism. The cell was first discovered by Robert Hooke in 1665. All cells in the human body come from a single-cell zygote, and although there are about 220 types of cells, the mechanisms of their work are synergistic with each other. Some of them work independently, like blood cells, while others form tissues, like synapses from the brain to the ends of the body. Stem cells play an important role in this developmental process. Stem cells are the term for groups of undifferentiated cells (Ohnuki & Takahashi, 2015).

The ability to self-replicate and differentiate into different cells with specific functions are two main characteristics of stem cells. Stem cell hierarchies are based on their differentiation potential and

A. Faried, Y. Hermanto
Universitas Padjadjaran, e-mail: ahmad.faried@unpad.ac.id

Faried, A., & Hermanto, Y. (2025). Induced pluripotent stem cells (iPSCs) and neurological diseases. In B. Supartono & A. Noviantari (Eds.), *Discovering the miracle of stem cells* (223–248). BRIN Publishing. DOI: 10.55981/brin.1128.c1303, E-ISBN: 978-602-6303-50-9

classified into totipotent stem cells, pluripotent stem cells, multipotent stem cells, and unipotent stem cells. The highest hierarchy is totipotent stem cells, which means that cells have the ability to produce a whole organism. In the development of the human body, this is only present until the morula phase. The lower the ability to differentiate, for example a unipotent stem cell, can produce only one type of cell (Hochedlinger & Plath, 2009).

The Waddington epigenetic landscape explain process of stem cell differentiation as a mole seed rolling down from a hill to one of several valleys, where the potential for stem cell development decreases. Stem cells do not have the ability to choose a different type of route when passing through their crossroads (biffurcatio point) (Counce, 1958).

In certain situations, nuclear reprogramming occurs when differentiated cells can return to their previous condition. For example, embryonic stem cells (ESCs) are made from B lymphocytes or neurons using somatic cell nuclear transfer (SCNTs), giving a group of transcription factors that transform mature cells into pluripotences (producing induced pluripotent stem cells; iPSCs). Induced pluripotent stem (iPSCs) are a type of pluripotent stem cell derived from adult somatic cells that have been genetically reprogrammed to an embryonic stem (ES) cell-like state through the forced expression of genes and factors important for maintaining the defining properties of ES cells (González et al., 2011; Takahashi et al., 2007).

Induced pluripotent stem cells (iPSCs), which remarkably resemble ESCs, developed as the two scientific streams. Although the reprogramming procedure used to create iPSCs is still a mystery, the products show promise in several fields, including drug discovery, pathological research, toxicology, the assessment of side effects from drugs, and regenerative medicine (Wang et al., 2020). Soon after the conversion of human somatic cells into iPSCs, the modelling of neurodevelopmental and neurodegenerative diseases offers new insights into the biology of diseases and the potential of new therapies.

This article will discuss induced pluripotent stem cells (iPSCs) from development to utilization, applications in neurology and

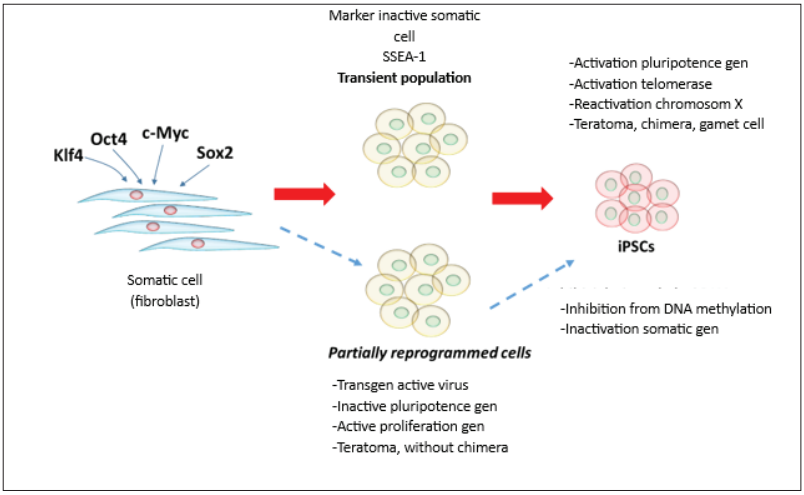
neuropsychiatry, potential advantages, and challenges that will be faced in the future. iPSCs are considered a valuable resource for regenerative medicine because they can be generated from any healthy person or patient.

Development of Pluripotent Cells

The first method of converting mature cells into pluripotent cells is known as nuclear transfer. Experiments conducted on amphibians, mammals, and humans showed that the genome of each adult cell, after undergoing terminal differentiation, can still produce live cloning. This suggests that the boundaries of development on the genome cannot be removed (Kumar et al., 2015).

Using a combination of four retrovirally transcripting factors from 24 candidate genes, Sox-2, Klf-4, Oct-4, and c-Myc, Kazutoshi Takahashi and Shinya Yamanaka managed to transform adult human cells (fibroblasts) into iPSCs5 (Takahashi et al., 2014). Initially, iPSCs were isolated by drug selection against the expression of the ESC marker (Fbx15), which was specific but not essential to ESCs, so that the first generation of iPScs was similar but not identical with ESCs (Halevy & Urbach, 2014). The transcription and epigenetic patterns of the first generation of iPSC indicate partial reprogramming conditions from fibroblasts to ESCs; these cells cannot produce chimera when injected into the blastocyst or do not contribute to the formation of gametocytes, indicating the status of mass reprogramming (Hochedlinger & Jaenisch, 2015).

In-depth studies replace iPSC selection with promoters of genes important for pluripotence, such as Oct-4 or Nanog (Olariu et al., 2016). These genes are thought to be more selective in cells that have undergone complete reprogramming. While Fbx15 is activated in virus-infected cells, so many cells that only undergo partial reprogramming are selected (Figure 9.1)(Okita et al., 2007). On a molecular level, the next generation of iPSCs showed patterns of transcription, DNA demethylation, and histone methylation (histone trimetylation H3 lysin 4 (K4) and lysin 27 (K27)) similar to the ESC pattern (Hanna et al., 2008).



Notes: This figure shows direct reprogramming of somatic cells into pluripotence cells, while partial reprogrammation produces iPSCs that require DNA demethylation agents and somatic gene inactivation.

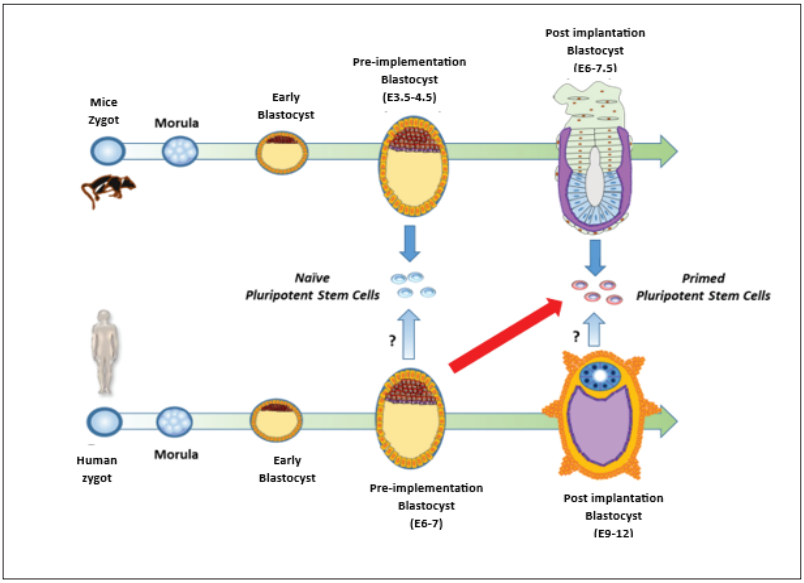
Source: Okita et al. (2007)

Figure 9.1 Direct Reprogramming of Somatic Cells into iPSCs

iPSCs have been successfully made from a wide range of cells on scatter since their invention by Kazutoshi Takahashi and Shinya Yamanaka. The technology can also be used on a variety of species, such as humans, monkeys, naked mole rats, and others (Aoi et al., 2008). This suggests that with the correct combination of transcription factors, somatic cells can be converted into pluripotent cells.

B. Relevance of Pluripotent Cells for Human Embryological Development

Conventional human pluripotency cell cultures cannot maintain cells in pre-implantation status, as most ESCs and iPSCs have characteristics similar to epiblast-derived stem cells (EpiSCs). Human pluripotent cells are identical to post-implant epiblasts, according to the transcription examination of primate embryos at the various



Notes: This figure shows the status of human pluripotence and scit. The characteristics of the human ESC and the scabies are very different, although they both originate from pre-implanted blastocysts. Human ESC is molecularly and functionally similar to post-implant epiblast tissue or comparable to epiSC mice.

Source: Smith (2017)

Figure 9.2 The Status of Human Pluripotence and Scit

phases of embryonic development (Boroviak & Nichols, 2017). Furthermore, there are differences in gene and epigenetic expression between pluripotential cells and pre-implantable epiblastic cells in humans. Therefore, experts agree that cells produced from the cell mass in the human body are more similar to primed epiblast stem cells (EpiSc humans; naive ESCs. (Figure 9.2) (Nichols & Smith, 2012; Smith, 2017).

The difference in species characteristics in pluripotency status is an interesting topic in the study of naive human pluripotent cells, which shows differences in the process of human embryonic development. Success in determining naive pluripotency status in

human cells is crucial for the study of embryonic development and human pluripotence and is expected to provide further benefits in clinical applications such as stable cell expansion and efficiency.

Understanding the evolution of the human body is the biggest challenge in biology. This is caused by the desire to learn about life and the pragmatic need to find a cure for a variety of human diseases. With their ability to proliferate and differentiate, human pluripotent cells are highly promising as a subject of human biological research. The long process has resulted in many discoveries and knowledge about human pluripotent cells. This allows research into the disease to understand the cause of the disease and find its cure. With current technological advances, human cells can be produced in large quantities with high quality. This allows cell therapy to replace damaged body cells (Bai, 2020).

C. Development of the Central Nervous System

Often, there is no cure for diseases or brain abnormalities. In addition, difficulties in obtaining human brain tissue for research and unrepresentative measurement models hinder progress in the science and therapy of neurological diseases. The development of human pluripotent cell technology, especially iPSCs, has excellent prospects for neurological disease research. The ability of iPSCs to produce certain patient cells or tissues connects clinical and animal studies. To make certain cells or tissues in the patient, a good understanding of the development of the brain and nerve tissue is required (Xie & Tang, 2016).

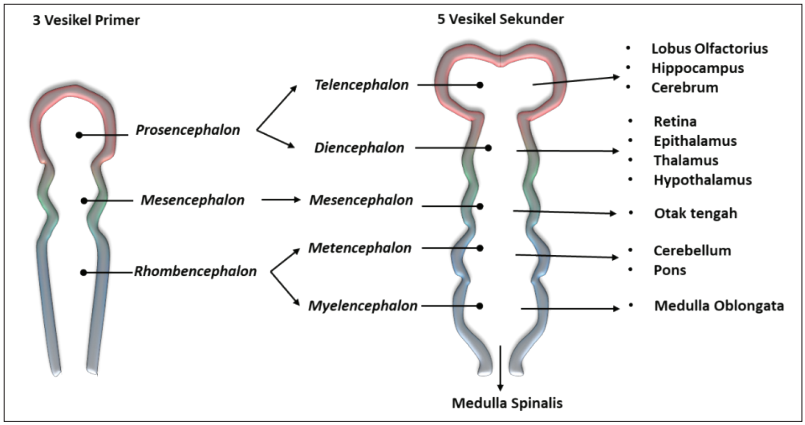
The embryonic phase in humans begins with conception and continues until the eighth week of gestation (GW), when each layer of the embryo forms a large number of specific organ tissues (organogenesis). The main structures of the brain and central nervous system have grown at the end of the embryonal phase. These include segmentation of neural tubes, gastrulations, and primary and secondary neurulations (Kostović & Jovanov-Milošević, 2006).

During the development of the human embryo, gastrulation is the beginning of the birth of the central nervous system. The reorganization of embryonic disc structures (embryonic bilaminary networks) into layered structural tissues is a sign of gastrula, an important process that prepares the embryo for organogenesis, the formation of more complex tissues (Kostović & Jovanov-Milošević, 2006).

The formation of neuralis tubes, which will form the brain and medulla spinalis, is known as neurulation. This process in humans begins at the 3rd GW, which is shown by the formation and fusion of the neuralistic pathway in the middle line of the embryo. The notochord pushes the ectodermal cells on it to form neurulation plates, which starts the neuralization process. The ectodermal layer naturally tends to be pro-neural rather than epidermal because signals from primitive nodes stop the formation of the epidermis. The apico-basal thickening process of the ectodermal layer, known as placode, is caused by the suppression of bone morphogenetic protein (BMP) and signals from the WNT pathway. At the ends of the skull, the placode nerve tends to be wider than in the caudal (Kostović & Jovanov-Milošević, 2006).

At first, the human neural tube had a straight tube structure. However, before the closure of the posterior neuropore, the anterior portion of the tube underwent significant changes. Three primary vesicles are composed of neural tubes in this area: the anterior cerebral vesicle (prosencephalon), the mid-brain vesicules (mesencephalone), and the posterior cerebral vesicles (rhombencephalon). When the posterior neural tube is closed, the optical vesicle, which is a secondary protrusion, appears from the lateral side of each of the frontal brain vesicles (Figure 9.3) (Elshazzly et al., 2023).

The neuralistic body has a polarization in its dorsal-ventral focus. For example, the regio dorsal medulla spinalis is where spinal neurons receive input from sensory neurons, and the regio ventral is where the spinal motor neurons are located. Many interneurons in the mid-medulla spinalis process information between neurons. Signals from



Notes: The first structure of the human brain three primary cerebral vesicles will develop, producing five secondary vesicles that help in the derivation of adult brain tissue.

Source: Moore (1993)

Figure 9.3 The First Structure of the Human Brain Three Primary Cerebral Vesicles

the environment affect this polarization. The epidermal membrane and roof plates affect the dorsal regio, while the notochord and floor plates impact the ventral regio (Elshazzly et al., 2023).

D. Neural and Human Pluripotent Cells

Neurons and glia form complex but orderly human brain tissue. Many types of cells in the mice (1) and human (2) brains were found through single cell profiling research (Molnar & Gair, 2015). However, the underlying mechanisms and processes of this cellular diversity are still not fully understood, especially in humans. Embryonal stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are interesting models for the study of the specifications of human neural subtypes (Tao & Zhang, 2016).

In the early stages, most neurological diseases tend to attack certain neural subtypes. The cerebrocentral dopaminergic neurons, mainly those that regulate motor function (A9-dopamine neurons), are damaged in Parkinson's disease. On the other hand, in patients

with Huntington's disease, the spiny medium neuron (GABAergic) in the striatum has primary nerve damage. Otherwise, with many decay systems, oligodendrosites are usually subjected to degenerative processes. Why only certain types of neurons or glia are sensitive or resistant to damage remains a mystery or question mark. The ability to direct human pluripotent cells to a specific neural subtype is expected to reveal the susceptibility of neuronal subtypes and the mechanisms responsible for neurological diseases (Xiao et al., 2016).

This organized differentiation naturally occurs gradually and usually takes three to four times longer than measuring, resulting in expensive operating costs and the risk of microbial contamination. By using a combination of external transcription factors to change cell phenotypes, the concept of direct reprogramming offers a technical solution to this problem. This concept developed after the invention of iPSCs and stems from the overexpression of MyoD in fibroblasts that produce muscle cells (Nogami et al., 2018).

The combination of ISL1, LHX3, and NGN2 with the Sendai virus managed to convert iPSCs into HB9+ cells, which are immature motor neurons, accounting for more than 90% of the total cells in just 14 days. A practical illustration of obtaining a particular neuron. Interestingly, these three combinations are not enough to convert fibroblast cells into motor spinal neurons; additional transcription factors such as ASCL1, BRN2, NEUROD1, and MYT1L are needed to assist the conversion process. This suggests that iPSCs and fibroblasts have different epigenetic resistances. However, research has shown that, compared to iPSCs, direct conversions from fibroblasts do not undergo complicated processes of chromatin remodelling, which makes them better suited to the patient's genetic background (Akter & Ding, 2022).

E. Modeling of Neurological Disorders Using IPSc-Derived Neural Cells

iPSC-based disease modelling has become increasingly popular for the study of neurological diseases due to its advantages. Reprogrammed iPSCs of human somatic cells originate from humans, thus avoiding

concerns about species differences associated with using animal models.

Most importantly, reprogrammed iPSCs of the patient's somatic cells retain their original genomic characteristics, such as gene mutations and chromosomal abnormalities. These genomic characteristics can be retained after differentiation, so they can be used to study the effects of certain genomic defects on cellular function. This is especially beneficial for drug development. Liu et al. showed the advantages of using an iPSC-based system for drug development by suggesting that treating neurons derived from iPSC with potential drugs for Alzheimer's disease could better reflect biomarker changes in real patients (Liu et al., 2014). With recent advances in genomic editing technologies, notably TALEN and CRISPR/Cas9 iPSCs can be manipulated from single nucleotide changes from one gene of interest to the removal of specific fragments on chromosomes related to disease (De Masi et al., 2020). The flexibility of genomic editing in iPSCs allows us to compare molecular and cellular phenotypes on the same genetic background, leading to more relevant conclusions about the mechanisms of disease (Gaj et al., 2016).

Using NSC-derived iPSC, a variety of neurological diseases have been studied, including monogenic and complex nerve disorders. Using small molecules including CHIR99021, SB431542, dorsomorphin, and compound E, Liu et al. produce NSCs from iPSCs derived from Parkinson's disease patients, which carry LRRK2 mutations (Liu et al., 2012).

The study found a new phenotype in iPSC patients derived from NSCs, which indicated nuclear architectural defects associated with LRRK2 mutations and increased proteasomal stress. However, since cells derived from iPSC have been shown to have characteristics similar to fetal cells, the role of NSCs in aging diseases needs to be carefully evaluated in iPSC-based modelling systems. Aging-inducing compounds, for example progerin, MG132, and concanamycin A, have been identified to facilitate the aging of nerve cells derived from

iPSCs, which may benefit the modelling of age-related neurological diseases (Weykopf et al., 2019).

Neurons, as the basic work unit in the brain, are affected by most neurological diseases. Neurons derived from iPSC have attracted great interest in the modelling of neurodegenerative diseases. To model the disease using iPSCs, the first challenge is to produce neuronal subtypes that are relevant to the disease. The diversity of neuronal subtypes is determined by complex genetic and environmental factors. During embryogenesis, the morphogenesis of the neuroectoderm is determined by a combination of morphogens along two axes: the rostro-dorsal axis by WNT, fibroblast growth factors (FGF), and retinoic acid (RA); and the dorso-ventral axis by WNT, BMP, and Sonic hedgehog protein (SHH). With this knowledge from evolutionary biology, the researchers have used morphogens and growth factors to produce specific neurons in the subtype and region of iPSC (Li et al., 2018).

Astrocytes are the most numerous cell type in the brain but are largely overlooked compared to their extensive focus on neurons to date. With increasing knowledge of astrocyte biology, its role in neurological diseases is now increasing. Similar to neurons, astrocytes are modelled by morphogenic gradients along the rostro-dorsal and dorsal-ventral axes and show heterogeneity in terms of subtype and regionality (Chiareli et al., 2021).

Neurological disease models using astrocytes derived from iPSC can be traced back to 2012. Jouperri et al. observed emptiness in Huntington astrocyte-iPSC-derived patients as well as peripheral lymphocyte-derived patients, suggesting a new phenomenon of Huntington's disease. Astrocytes derived from ALS iPSC patients carrying transactive DNA protein-binding reactions (TDP-43) mutations showed cell autonomic defects, including TDP-43 proteinopathies and cell death, but no adverse effects on motor neurons were observed (Juopperi et al., 2012).

F. Modelling Neuropsychiatric Disorders with Human Pluripotent Cell Technology

The scientific world still faces a huge challenge in understanding the biological foundations of nervous system diseases. This is mainly due to the complexity of the human brain, which consists of many types of cells with a variety of complex functions and relationships. In addition, the symptoms and severity of neuropsychiatric disorders vary from one person to another due to genetic, environmental, psychosocial, and developmental history factors affected. As a result, neurological disorders remain difficult to treat and have a negative impact on the health of individuals and communities.

Recent biotechnological developments can accelerate mechanistic research on nervous system diseases and drive new therapies. First, the genomic revolution allowed to discover specific gene variants and copy numbers that potentially cause neurological abnormalities. Second, rapid, accurate, and easy-to-do genetic modification through genetic engineering techniques with CRISPR-Cas9 enables the study of genetic function in human cells, squirrels, and primates (Knott & Doudna, 2018).

The discovery of iPSCs allowed the transformation of a patient's somatic cells into pluripotency cells that could distinguish different cell types. Cellular reprogramming can now be used to obtain neurons and other brain cells from patients with different genetic abnormalities. Furthermore, advances in bioengineering, known as 3D culture or organoid, show very small brain structures within a culture cup that have a design similar to the biological structures found in vivo.

The latest biotechnology development of new therapies can be assisted by these advanced technologies, which can speed up mechanical research on nervous system diseases. iPSC technology has a major advantage in exploring unusual genetic diseases by using patient genetic data; this increases specificity and approaches research to clinical applications. The most promising diseases to study are those identified by genetic mutations.

In most cases, there is a causal relationship that can be observed in the neural derivatives of the patient's iPSCs. Neural derivatives of monolayed-cultured (2D) iPSCs have been used to investigate various mechanisms responsible for neuropsychiatric disorders. For example, there is a decrease in the number of glutamatergic synapses in iPSC monolayer neurons in patients with Rett syndrome. The administration of IGF-1, a peptide often used in clinical trials of neurodevelopmental disorders, can alter this phenotype. In addition, the neurons of patients with Timothy syndrome (mutations in the Ca2+ channel type-L) showed changes in dendritic retraction and production of neurotransmitters (Centeno et al., 2018).

Furthermore, research on psychiatric disorders brought on by ancestors, but not caused by the disease is very beneficial with iPSC technologies. In one study, the glutamatergic neuron culture of schizophrenia patients showed a decrease in connectivity and number of neurons; anti-psychotic drugs could restore some of the molecular and cellular phenotypes of patients' neurons. There are mitochondrial abnormalities and neuron hyperexcitability in the hippocampus of patients with bipolar disorder, according to studies conducted on their hypocampal neurons. Lithium can recover this neuronal hyperexcitability, but it only occurs in patients' neurons with bipolar symptoms that indicate a response to lithium. This suggests that iPSC technology can be used to predict drug therapeutic responses (Osete et al., 2023).

In addition to successfully recapitulating synaptic and neurodevelopmental disorders, neural derivatives of iPSC patients can also be used to investigate neurodegenerative diseases or proteinopathies. These diseases are usually more characterized by neurotoxicity due to oxidative stress and proteasome disorders than synapses. Nguyen et al. (2011), for example, found that the gene expression of dopaminergic neurons associated with oxidative stress and -synuclein proteins increased in Parkinson's patients with the LRRK2 mutation, or leucine-rich repeat kinase-2. As a result, compared to control neurons, these mutant neurons are more

prone to cell death due to exposure to H2O2, MG-132 (proteasome inhibitors), and 6-hydroxydopamine (neurotoxic) (Weykopf et al., 2019; Xiao et al., 2016). The findings about the susceptibility to stress in patients' neuron derivatives provide a new understanding of the mechanisms of disease and form the basis for drug screening. Soon after the conversion of human somatic cells into iPSCs, the modelling of neurodevelopmental and neurodegenerative diseases (see Table 9.1 for a summary of neurological disease modelling with iPSC) offers new insights into the biology of diseases and the potential of new therapies.

Neural culture with a single-layer system has produced a lot of results, but the system does not have features like the human brain, so it cannot be used to model some disease phenomena. Intercellular and ligand-receptor interactions lead to neurobiological development and synaptic connectivity. When neurons are cultured monolayer, this signalling dynamics is unclear. The development of in vitro models

Table 9.1 Summary of Neurological Disease Modelling with iPSC

Disease	Gen	Neurological Symptom	Phenotypes in Neural Derivatives	Testing Therapy	Ref.
Adrenoleu-kodystrophy	*ABCD1*	Demyelination and loss function system nerve center and edge in a manner progressive	Increasing *very long chain fatty acids* (VLCFA) in oligodendrocytes	Lovastin. 4-phenylbu-tyrate degrades VLCFA levels	Jang et al. (2011)
Alzheimer`s disease	*PS1*, *PS2*, *APPs*, Sporadic	Disturbance cognition and memory as well as progressive disorientation	Increasing secretion of amyloid β (Aβ), *phospho-tau* (Thrc231) and *glycogen synthase kinase-3β* (aGSK-3β) in neurons	γ-secretase inhibitors decrease Aβ secretion β-secretase inhibitors decrease phosphotau (Thrc231) and aGSK-3β levels The combination of Bromocrip-tine, Cromolyn and Topimarate lowering Aβ _	Yagi et al. (2011)

Disease	Gen	Neurological Symptom	Phenotypes in Neural Derivatives	Testing Therapy	Ref.
Amyotrophic lateral sclerosis (ALS)	*SOD1*,*VAP*, *TDP43*	degeneration and loss *upper* and *lower motor neurons* in a manner progressive	VAPB: decline VAPB levels in motor neurons. TDP43: motor neuron mutant own enhancement dissolved and *detergent-resistant* TDP-43 protein levels, decreased neuron survival and increased vulnerability to antagonist PI3K pathway	*Anacardic acid* lower mutant TDP43 protein levels Bosutinib promotes autophagy and decreases amount *protein misfolding* Ropinirole prevents death cells, abnormal protein aggregation, and production molecule oxygen-radicals	Kondo et al. (2017)
Huntington's disease	CAG repetitions in the Huntingtin gene (HTT)	Chorea and progressive dementia be marked with loss *medium spiny neurons* striatal and *cortical neurons*	NSCs-HD shows stress susceptibility to decline BDNF levels, increased death cells, and disorders bioenergetic mitochondria Formation inclusion protein aggregates after administration of *proteasome* inhibitors (MG132) Disturbance *corticogenesis* Vacuolization of astrocytes increasing activity lysosomes in iPSCs	Correct genetics *Pyruvate* increase rate ATP and activity bioenergetic mitochondria	Mehta et al. (2018)
Familial dysautonomia (FD)	*IKBKAP*	Degeneration of sensory and autonomic neurons	Decline gene expression related to neurogenesis and neuronal differentiation Disturbance migration *neural crest*	Kinetin repair phenotype	Lee et al. (2009)
Parkinson's disease (PD)	*LRRK2*, *PINK1*, *SNCA* and *parkin*	Degeneration of dopaminergic neurons substantia nigra	PINK1: Interference function mitochondria in dopaminergic neurons LRRK2 and SNCA: sensitivity against oxidative stress	Inh2 reduces degeneration of dopaminergic neurons with LRRK2 mutation	Weykopf et al. (2019)

Disease	Gen	Neurological Symptom	Phenotypes in Neural Derivatives	Testing Therapy	Ref.
Syndrome Rett (RTT)	*MeCP2 CDKL5* _	Disturbance function motor, regression skill, hypotonia, seizures. *Atypical Rett syndromes*: disabilities intellectual, epilepsy, and autism.	MeCP2: neuronal maturation deficit, decreased amount synapse and *dendritic spines*, larger soma size small, increasing LINE1 retrotransposition CDKL5: aberrant dendritic spines	IGF-1 repairs phenotype *in vitro*	Ricciardi et al. (2012)
Schizophrenia	Multi-factorial	Hallucination, delusions, disturbances talk. Abnormality neurotrans-mitter, decrease arborization dendritic, and disorder myelination	Lost neuronal connectivity, Decline amount neurites, PSD95, and receptors glutamate Abnormality migration and myelination oligodendrocytes	Loxapine, *valproic AC* ID repair phenotype *in vitro*	Windrem et al. (2017)
Timothy's syndrome	*CACNA1C*	Syndrome *Long-QT*, deficit neurological characteristics autistic	Decline gene expression in layers cortex lower and callosal projection neurons Increased production of norepinephrine and dopamine abnormality in retraction dendritic	Roscovitine restore phenotype *in vitro*	Paşca et al. (2011)

that can measure the structural and functional complexity of the human brain is motivated by the limitations of monolayer culture. Human pluripotenic cells (iPSCs and ESCs) can produce human organoid brains that have structural characteristics similar to fetal brains by using the intrinsic ability of cellular aggregation (Lancaster & Knoblich, 2014).

All efforts to create experimental models for neuropsychiatric disorders will only succeed if the models can describe the processes that occur in the human brain. The monolayer (2D) culture system has advantages mainly in scalability and homogeneity, which facilitate the screening of genetic and pharmacological systems that have

many tests. However, brain organoid culture is more related to the characteristics of cellular interactions and the structural organization of the human brain. Basically, the choice of a cultural system depends on the purpose of the investigation and the design of the experiment. Current biotechnological advances have expanded our understanding of a variety of neuropsychiatric conditions. Therapeutic innovations are also expected to improve the quality of life of patients.

G. Prospects of iPSCs-Based Therapy in Neurological Diseases

The brain and medulla spinalis have been the subject of cell therapy studies since the emergence of stem cell biology. Due to the numerous diseases associated with ineffective cell replacement therapy, the central nervous system is attracted to cell substitution therapy. Nevertheless, because the brain is an organ that has many structural classifications and complex intercellular interactions, cell therapy cannot always help the brain regenerate structurally after damage.

This problem is becoming increasingly complex due to the limitations of the repair capacity of the adult human brain. Although neural stem cells exist in the human brain, their role in structurally repairing brain tissue remains a mystery. According to early stem cell biology, it is expected that donor cells (also known as NSCs or pluripotenic cells) may vary depending on the situation after the transplant. However, further research found that spontaneous differences in donor cells may not produce the expected cell type. At present, it is known that when the neuronal or glial subtypes suffer degeneration replacement should be done with the same cell subtype or their forefathers in order to achieve proper structural repair. Luckily, many nervous system diseases only attack certain types of cells. This means neuronal or glial cell therapy can be used for many neurological diseases (Li et al., 2018).

In this regard, glial progenitor cells, which can produce astrocytes and oligodendrosites (similar to oligodendrocyte progenitor cells, or

OPCs), have been thoroughly studied as a potential drug to repair myelin in the spinal cord and the human fetal brain. These cells can be extracted from human pluripotent cells (both ESCs and iPSCs). In trial animals, transplanted OPCs tend to spread and migrate along the neural focus, becoming myelinating oligodendrosites of the dismielinated locus. Human perinatal OPC transplantation into a hypomyelinated shiverer scab, which usually dies at the age of twenty weeks, prolongs the scab life locus. stores myelinated and neurological phenotypes. This is a demonstration of proof of concept for remyelination therapy with OPC on various white skin disorders.

The very common post-transplant OPC characteristics allow the treatment of dysmyelination diseases caused by enzyme deficiency or disorders of lysosome storage such as mucopolysaccharidoses, Krabbe's disease, and metachromatic leukodystrophy. In the future, autologous cell therapy is highly likely to be used in patients with leukodystrophy abnormalities. Hopefully, the transplanted cells could prove effective in remyelinating and correcting the patient's metabolic abnormalities. This can be achieved through the integration of genetic engineering technology to repair mutations and iPSC technology.

In the 1980s, a Swedish scientist, A. Bjorklund, had an idea of treating patients with Parkinson's disease by transplantation; they tried to replace the dopaminergic neurons in the substantia nigra of an aborted fetus with the middle brain tissue. This discovery promoted the development of cell therapy for Parkinson's disease. Technically speaking, about 2–3 fetuses are needed for one patient, which makes its implementation difficult due to donor issues and ethical issues. However, with advances in human pluripotency cell technology, dopaminergic neurons can be produced efficiently from ESCs or iPSCs in large quantities (Björklund & Lindvall, 2017).

Although its clinical trials are promising, there are still some problems with unresolved cell transplants. If fetal midbrain tissue is implanted into the striatum of a Parkinson's patient, the results are inconsistent, ineffective, and produce many side effects (graft-induced dyskinesia). In addition, the nigral dopaminergic cells are located at

a heterotopic location in the putamen or striatum. Donor cells do not have normal afferent connectivity and are exposed to different neural circuits. Furthermore, it is not known whether intrastriatal dopaminergic neuron transplantation can improve the normal basal function of the ganglia in Parkinson's patients. Additionally, some studies show that the -synuclein and Lewy body aggregates are transmitted from the recipient cell to the donor cell, so that the donor neurons are involved in the disease process (Björklund & Lindvall, 2017).

At least, there was caution in the use of these strategies in clinics due to confusion about the spread of the disease from the recipient to the donor, the need for immunosuppression, and the ideal dosage. Overall, the benefits and risks of this approach should be compared to the availability of effective pharmacotherapy for Parkinson's disease and deep brain stimulation to reduce the severity of the disease. Whether dopaminergic cell transplants are beneficial and whether the cost is comparable to the benefits or risks is still debated. Patients who were refractory to current medical choices and did not experience cognitive impairment were a small group.

H. Problems in Cell Therapy Applications

To ensure that cell therapy is safe and effective, a variety of issues need to be studied or considered before being applied clinically. Some problems with cell replacement therapy with stem cells are as follows (Dodson & Levine, 2015; Mousaei Ghasroldasht et al., 2022). Somatic cell reprogramming is not a perfect process. There are imperfections in somatic cell reprogramming, epigenetic marker retention, and new mutations that can cause tumors.

However, this does not indicate that the ESC is better. There is evidence that long-term culture processes increase the likelihood of genetic erosion or genetic mutation in human pluripotent cells (ESCs and iPSCs). To ensure the safety of cell derivatives before clinical use, a thorough examination of iPSCs and ESCs is required.

If the test design is inappropriate, many potentially effective agents fail in clinical trials. For optimal and objective results, clinical trial designs should understand pathogenesis, disease course, and disease heterogeneity.

One should not expect too much from the therapeutic ability of cells to cure diseases because animals cannot recapture all the pathological conditions that exist in human diseases. To ensure that human pluripotent cell derivatives are safe, examinations of the potential for tumor genesis, immune reactions, heterotopic differentiation, and microbial transmission should be carried out before implementing cell therapy in clinical conditions.

I. Conclusion

Induced pluripotent stem cells (iPSCs) have opened new avenues for stem cell research and unique opportunities in the pharmaceutical industry and clinical practice. Reprogramming technology has also made it possible to study cell fate decision mechanisms and model human diseases. This has greatly increased the chances of discovering new drugs. iPSCs show promise in some fields, including drug discovery, pathological research, toxicology, the assessment of side effects from drugs, and regenerative medicine. Soon, after the conversion of human somatic cells into iPSCs, the modelling of neurodevelopmental and neurodegenerative diseases will offer new insights into the biology of diseases and the potential of new therapies. However, like many other fields, reprogramming technology has several challenges, such as clinical trial design, risk, and benefit considerations.

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Chapter 10

The Art of Ethical Dimensions in Stem Cell Research

Dito Anurogo

A. Significance of Stem Cell Research

Stem cell research is a groundbreaking field with vast potential to revolutionize medicine and biotechnology through regenerative therapies and disease treatments (Mao et al., 2022). Stem cell research, promising for medicine and regeneration, faces ethical dilemmas, mainly concerning embryonic cells. Stem cell advancements, like CRISPR-Cas9 gene editing, offer transformative prospects for personalized medicine (Wang et al., 2022). Stem cells hold immense transformative potential in regenerative medicine, disease modeling, and drug development, offering hope for treating various debilitating conditions.

D. Anurogo

Universitas Muhammadiyah Makassar, e-mail: dito.anurogo@med.unismuh.ac.id

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Responsible research practices in stem cell studies require collaboration among policymakers, researchers, bioethicists, and the public to balance progress with ethics, ensuring benefits and safeguarding stakeholder well-being (Neil et al., 2022). Ethical issues in stem cell researches, from the moral status of embryos to stem cell sourcing, demand careful attention. Upholding informed consent, ethical reviews, and standards are essential to safeguard the rights and well-being of both human and animal participants. (Lovell-Badge, et al., 2021). As stem cell therapies advance to clinical trials and commercialization, strong legal frameworks are essential to guarantee patient safety, oversee research, and avoid exploitation (Moradi et al., 2019). Ultimately, the vast benefits of stem cell research must be balanced with its ethical considerations to align science with societal values.

B. Ethical Issues in Stem Cell Research

Ethics in scientific research is a fundamental aspect that governs the conduct of scientists and researchers in their pursuit of knowledge and advancements (Sato & Suzuki, 2022). At its core, ethics in scientific research involves adherence to moral principles, values, and standards that ensure the responsible and respectful treatment of all individuals involved, including research participants, colleagues, and the broader community. Ethical considerations in stem cell research are particularly critical due to the complex nature of these cells and their potential implications for human health (Assen et al., 2021).

Central to the concept of ethics in stem cell research is the principle of respect for human dignity and autonomy. This principle necessitates obtaining informed consent from research participants, ensuring they are fully aware of the risks, benefits, and potential outcomes of the study. Researchers must also protect the privacy and confidentiality of participants, respecting their rights to autonomy and self-determination throughout the research process (Harris et al., 2022).

Another crucial ethical consideration in stem cell research relates to the source of stem cells. The use of embryonic stem cells (ESCs) has been a subject of significant debate due to the destruction of embryos in the process of obtaining these cells. This raises complex questions regarding the moral status of embryos and the balance between scientific advancement and respect for human life. As a result, researchers have sought alternative sources of stem cells, such as induced pluripotent stem cells (iPSCs), which can be generated from adult somatic cells without the ethical concerns associated with embryonic sources (Singh et al., 2015).

Moreover, researchers must uphold principles of scientific integrity and honesty, ensuring transparent reporting of research findings. Negative results and potential limitations of the research should be openly communicated, preventing publication bias and fostering a more comprehensive understanding of stem cell science (Valdés & Lecaros, 2023).

Ethical issues in stem cell research also extend to the potential for commercialization and patenting of stem cell technologies. The pursuit of financial gain can sometimes conflict with considerations of equitable access to therapies and the public good. Striking a balance between incentivizing innovation and ensuring affordability and accessibility of stem cell treatments is essential to navigate these ethical challenges (Alahmad et al., 2020).

Stem cells hold immense promise for regenerative medicine, but the ethical considerations surrounding their sources have been a focal point of debate in the scientific community and beyond (Volarevic et al., 2018). Among the various types of stem cells, embryonic stem cells (ESCs) derived from early-stage embryos have been at the heart of controversy due to ethical implications related to the moral status of embryos and the potential for harm. Understanding the arguments for and against the use of embryonic stem cells is crucial for a comprehensive analysis of the ethical challenges in stem cell research (Al-Agele, 2023).

1. Harnessing Embryonic Stem Cells: Unveiling their Potential

Embryonic stem cells offer a fascinating discussion due to their unique features, especially pluripotency. This trait allows these cells to transform into various cell types, forming the complex human body. Such potential brings optimism to regenerative medicine and disease research, paving the way for new medical breakthroughs (Żakowska-Henzler et al., 2023).

Pluripotency in embryonic stem cells holds transformative promise for regenerative therapies. Supporters see a future where regrowing damaged tissues, like heart muscle after an attack or nerve cells in degenerative diseases, is possible, challenging traditional medical boundaries (Cho et al., 2022).

Moreover, embryonic stem cells are valued not only for their adaptability but also for their ability to self-renew indefinitely. This trait provides a consistent supply for research, meeting the growing demand in scientific studies and ensuring continuous exploration (Chen et al., 2022; Varzideh et al., 2023).

Embryonic stem cell advocates navigate beyond science to ethics, addressing questions about life's beginnings and research morality. Nonetheless, they believe the research's potential could transform healthcare and offer healing to many (Hauskeller et al., 2019).

Embryonic stem cells represent a complex potential in science, with their pluripotency offering vast possibilities and their self-renewal fueling continuous knowledge. Their significance triggers both scientific and ethical discussions. Navigating this, a balanced approach to ethics, research, and hope is essential for progress (Blasimme & Sugarman, 2023).

2. Arguments for the Use of Embryonic Stem Cells

Advocates for embryonic stem cells highlight their pluripotency, which lets them transform into any human cell type (Fléchon, 2022). Embryonic stem cells' ability to differentiate offers potential

for regenerative therapies and disease modeling. Their prolonged self-renewal also makes them a sustainable research resource (Chen et al., 2022).

3. Arguments Against the Use of Embryonic Stem Cells

A main ethical concern with embryonic stem cells is the embryo's destruction during extraction. Critics believe embryos, with their potential for human development, warrant protection, equating the process to taking human life and raising ethical issues about their use in research (Koplin, 2023).

The ethical implications of obtaining and using embryonic stem cells extend beyond their origin. Their potential clinical applications introduce additional concerns, such as the risk of teratoma formation—the development of tumors comprising various cell types—following transplantation. Researchers must carefully weigh the potential benefits of embryonic stem cell-based therapies against these risks and address safety concerns responsibly (Volarevic et al., 2018).

As stem cell research advances, the ethical discussions about their source and use highlight the need for a careful approach (Abubakar et al., 2023). Balancing science with ethics and exploring alternatives like iPSCs and adult stem cells is crucial for advancing stem cell research while maintaining societal values and standards (Moradi et al., 2019).

C. Informed Consent and Human Subjects in Stem Cell Studies

Informed consent is fundamental to ethical research, ensuring participants fully understand a study's aims, risks, benefits, and their rights. In stem cell research, with its potential experimental treatments, securing informed consent is vital to protect participants' autonomy and well-being (Riordan et al., 2022).

Stem cell studies, from lab research to clinical trials with humans, require researchers to offer clear, comprehensible information

to potential participants or their legal representatives (Yui et al., 2022). Informed consent should promote open dialogue, ensuring participants' voluntary, and informed choices. Consent should be continuous, with the option to withdraw at any time without consequences (Padilla et al., 2022).

Particular attention is required when obtaining informed consent for stem cell clinical trials, as these studies involve novel therapies with unknown risks and potential benefits. Participants must be thoroughly informed about the investigational nature of the treatment and the uncertainties surrounding its outcomes (van Rijssel et al., 2022). Researchers must uphold the principles of beneficence and non-maleficence, ensuring that the potential benefits outweigh the risks for research participants (Hosseini et al., 2022).

In the context of stem cell research, the role of human subjects and human donors is pivotal. Both aspects require rigorous ethical considerations, particularly concerning informed consent, the fate of the cells collected, storage and potential use of these cells, and compensation to donors, including considerations around commercialization and potential royalties (Assen et al., 2021).

1. Human Subjects in Stem Cell Research

Human subjects participating in stem cell research, especially in clinical trials, need to be fully informed about the investigational nature of the study. This includes a clear understanding of the potential risks and benefits, the experimental status of the treatments, and the fact that outcomes are uncertain. Continuous informed consent is crucial, and participants must have the option to withdraw at any time without any repercussions. This aligns with ethical principles, ensuring the autonomy and safety of participants (Yamanaka, 2020; Zarzeczny, 2019).

2. Human Donors in Stem Cell Research

In cases where stem cells are sourced from human donors, informed consent becomes even more complex. Donors must understand the

purpose of the donation, the process involved in cell collection, and the future use of these cells (Guo et al., 2021).

a. Fate of the Donated Cells

Based on the purposes, the fate of the donated cells will fall into two categories.

1) Research purposes: Initially, cells might be collected for specific research purposes. This could involve studying cell behavior, testing new treatments, or understanding disease mechanisms (Charitos et al., 2021).
2) Post-research fate: After the research is completed, the cells might be either destroyed, stored for future research, or used for other research projects. The specific fate of the cells should be transparently communicated to the donor during the consent process (Orzechowski et al., 2021).

b. Storage and Use of Donated Cells

Donated cells may be stored in biobanks. The duration of storage can vary depending on the research requirements and the viability of the cells. These cells could be used for (Zalaf et al., 2020):

1) future research initiatives;
2) development of treatments for other individuals, subject to ethical approvals and regulatory guidelines;
3) educational or training purposes in some cases.

c. Compensation to Donors

The issue of compensation for donors is complex. Typically, donors are not paid for their donations as it could influence their decision to donate and raise ethical concerns about commodification of body parts. However, donors may be compensated for associated costs like travel or time off work (Zalaf et al., 2020).

3. Commercialization and Royalties

If stem cells are commercialized, the ethical and legal frameworks become even more intricate. Generally, donors do not receive royalties from products developed from their cells. The principle here is that once donated, the cells become a part of a common resource for scientific advancement. However, this is a topic of ongoing ethical debate, particularly in cases where the commercial use of donated cells leads to significant profit (Padovano et al., 2022).

Ultimately, both human subjects and donors in stem cell research are protected by stringent ethical guidelines, emphasizing informed consent, the careful handling and use of donated cells, and clear communication about all aspects of the research and its potential implications. The principles of autonomy, beneficence, non-maleficence, and justice, guide these practices, ensuring ethical conduct in this rapidly evolving field (Lovell-Badge, et al., 2021).

D. Considerations for Conducting Research on Adult and Somatic Stem Cells

Amidst the ethical debates surrounding embryonic stem cells, adult and somatic stem cells offer a promising alternative for research and therapeutic applications. Adult stem cells are found in various tissues throughout the body and play a crucial role in tissue repair and regeneration. Their inherent ability to self-renew and differentiate into specialized cell types has sparked interest in exploring their therapeutic potential. However, conducting research on adult and somatic stem cells raises unique ethical considerations that must be thoughtfully addressed (Hoang et al., 2022).

One ethical consideration involves the collection of adult stem cells, which can be obtained from donors through minimally invasive procedures. Ensuring that the collection process poses minimal risk to the donor while adhering to the principles of informed consent and privacy is essential (Escoto et al., 2023). Researchers must also consider issues of equity and justice, as access to stem cell therapies

should not be limited to a privileged few but should be accessible to a diverse range of patients, regardless of socioeconomic status or geographical location (Farajkhoda, 2017).

In addition to the sourcing of adult stem cells, ethical questions arise concerning the use of somatic stem cells for research purposes. Somatic stem cells are responsible for maintaining and renewing specific tissues in the body, and their exploration offers insights into tissue-specific diseases and regenerative medicine (Ji et al., 2023). Ethical considerations revolve around potential risks associated with manipulating and modifying somatic stem cells, as well as ensuring their safe application in clinical trials and therapies (de Jongh et al., 2022).

E. Ethical Dilemmas in Stem Cell Clinical Trials and Experimental Therapies

The translation of stem cell research into clinical trials and experimental therapies presents ethical dilemmas. Rigorous ethical standards must be upheld to protect research participants and ensure the validity of findings. Ethical concerns involve patient selection, informed consent, and transparent reporting of outcomes (Drolet et al., 2023). Offering unregulated stem cell treatments outside clinical trials raises safety and exploitation concerns, necessitating comprehensive guidelines and regulations. Responsible and ethical practices are crucial to fulfill the promise of stem cell research while upholding human rights and scientific integrity (Peng et al., 2020).

F. Ethical Guidelines and Best Practices

Ethical guidelines serve as critical foundations for ensuring the responsible conduct of stem cell research. Leading organizations, including the International Society for Stem Cell Research (ISSCR), the National Institutes of Health (NIH), and the World Health Organization (WHO), have developed comprehensive frameworks to address the ethical complexities of stem cell research and its

applications. These guidelines cover essential aspects such as informed consent, protection of research participants, transparency in scientific reporting, and the appropriate use of stem cell sources. By providing a shared ethical framework, these guidelines facilitate collaboration among researchers, promote transparency, and instill public confidence in stem cell science (Assen et al., 2021).

As stem cell research continues to advance, it is essential to develop a robust framework for ethically responsible practices. This framework must embrace ethical considerations at every stage of the research process, from laboratory investigations to clinical applications. Researchers and institutions should prioritize the principles of beneficence, non-maleficence, autonomy, and justice in their work, ensuring that the potential benefits of stem cell research are maximized while minimizing any potential harm or risk to patients and society (Kidha, 2020).

Central to this framework is the establishment of transparent and accountable mechanisms for oversight. Ethical review boards and institutional committees play a vital role in evaluating proposed research studies, scrutinizing ethical aspects, and ensuring that research adheres to the established guidelines and regulations. Engaging with stakeholders, including patients, the public, and ethicists, can further enrich the ethical considerations of stem cell research, facilitating diverse perspectives and avoiding undue concentration of decision-making power (Assen et al., 2022).

The pursuit of scientific progress and ethical considerations in stem cell research is an intricate balancing act. On one hand, the urgency to address critical health challenges propels researchers to explore novel avenues and potential therapeutic applications. On the other hand, responsible scientific inquiry must be guided by robust evidence, adherence to ethical guidelines, and patient safety (Weinbaum et al., 2019).

Researchers should conduct stem cell research with humility, dedication to thorough study design, and peer review. Transparently sharing all results, good or bad, supports scientific integrity. Reviewing

the ethics of new technologies, like gene editing, ensures alignment with societal values and priorities (Joseph et al., 2022).

Ethical training and education are indispensable for researchers and professionals engaged in stem cell research. As new technologies and discoveries unfold, staying abreast of ethical guidelines and best practices is essential for informed decision-making. Researchers should receive comprehensive training that addresses the nuances of informed consent, patient privacy, the responsible use of stem cell sources, and the potential implications of their work on patients, communities, and future generations (Assen et al., 2022).

Promoting ethical reflection and open discussions in research institutions is crucial. Interdisciplinary collaborations and dialogues among researchers, ethicists, and policymakers ensure ethics remain central to stem cell research (Torres-Padilla et al., 2020).

G. Ethical Guidelines and Best Practices in Indonesia

Indonesia, a diverse archipelago, blends cultural spirituality, religious jurisprudence, and Western bioethics. Medical professionals in Indonesia must balance international practices with these unique cultural perspectives (Hefner, 2021). In Indonesian bioethics, personal autonomy and informed consent are paramount. While aligning with international standards, Indonesia emphasizes respecting cultural and spiritual beliefs in the decision-making process for medical or research activities (Subandi et al., 2023). Indonesian ethics prioritize collective well-being. While individual rights are essential, they might be limited during public health crises. Any such measures should be proportionate, transparent, and temporary to prevent potential authoritarianism (Casey & Vermeule, 2022). In Indonesia, biomedical research follows stringent ethical rules. Human cloning is banned, as are genetic modifications inheritable by future generations, reflecting global caution and Indonesia's respect for human life (Florea, 2023; Staunton et al., 2019). In Indonesia, end-of-life care is influenced by diverse religious beliefs. Physicians must understand these cultural

nuances, especially regarding topics like passive euthanasia, ensuring alignment with patients' spiritual values and family wishes (Kuhn, 2022). In Indonesia, the challenge for policymakers is translating ethical norms into laws. Due to the evolving nature of medicine and ethics, regular reviews involving bioethicists, religious leaders, and the public are essential (Ten Have & Neves, 2021). In Indonesia, ethical guidelines merge international standards with local cultures and beliefs. For healthcare and research success, it's vital to blend global insights with an understanding of the unique Indonesian context (Charitos et al., 2021; Fauziah & Mukhlis, 2019).

In the Indonesian context, the practical application of ethical guidelines in healthcare and biomedical research often reflects a unique blend of international standards and local cultural and religious values. This duality presents both challenges and opportunities in implementing ethical practices (Mathur et al., 2019).

1. Practical Realities in Indonesia

a. Balancing Autonomy and Collective Well-being

Indonesian healthcare professionals often navigate a fine line between respecting individual autonomy and prioritizing collective well-being. This balance becomes particularly evident during public health emergencies, where individual rights might be tempered for the greater good. The response to the COVID-19 pandemic is a case in point, where the government had to implement measures that balanced individual freedoms with public health necessities (Newnham & Kirkham, 2019; Søvold et al., 2021).

b. Cultural Sensitivity in Medical Decision-Making:

In a culturally diverse nation like Indonesia, medical decisions are heavily influenced by the patient's cultural and religious background. This sensitivity is especially apparent in end-of-life care, where healthcare providers must align their practices with the patient's religious beliefs and family preferences, which can vary significantly across the archipelago (Pentaris & Christodoulou, 2021).

c. Bioethics in Research

In biomedical research, Indonesian researchers adhere to stringent ethical guidelines that prohibit practices like human cloning and inheritable genetic modifications. These regulations reflect a global cautious approach and are in line with Indonesia's respect for the sanctity of human life. The challenge lies in ensuring these guidelines are consistently applied and understood across various research institutions, which can be diverse in their resources and expertise (Earp et al., 2020).

d. Regulatory Framework

Indonesia's approach to bioethics in medicine and research is to harmonize international norms with local beliefs and practices. However, translating these ethical norms into enforceable laws is an ongoing challenge. The dynamic nature of medical science requires that these laws and guidelines be regularly reviewed and updated, involving a wide range of stakeholders including bioethicists, religious leaders, and the public (Pimenta et al., 2021).

e. Education and Training

To effectively implement these ethical guidelines, there is a need for continuous education and training for medical professionals and researchers. This training should not only cover the technical aspects of bioethics but also emphasize the importance of cultural competence in dealing with patients from diverse backgrounds (Ignatowicz et al., 2022).

d. Public Awareness and Engagement

Public understanding and engagement in bioethical issues are crucial for the success of healthcare policies in Indonesia. This involves educating the public about their rights and the ethical considerations in medical treatments and research, thus fostering a more informed and participative society (Fletcher, 2023).

Ultimately, the reality of applying ethical guidelines in Indonesia's healthcare and research sectors involves a complex interplay of respecting international standards while also honoring the nation's diverse cultural and religious ethos. Continuous education, regulatory vigilance, and public engagement are key to navigating these challenges effectively (Resosudarmo, 2022).

H. Future Directions and Ethical Deliberations

As stem cell research advances, new technologies continue to emerge, each with their unique ethical implications. One such technology is organoid development, which involves growing three-dimensional mini organs in vitro, providing a platform for studying human organ development and disease. While organoids hold great promise for disease modeling and drug testing, concerns arise over the potential for these structures to acquire unforeseen functions or even consciousness, raising questions about the ethical status of organoids and the boundaries of human-like entities (Kendal, 2022).

Additionally, while these technologies offer potential benefits in personalized medicine and regenerative therapies, ethical considerations must address issues of consent, equity, and the responsible use of powerful gene editing tools (Li et al., 2023). As these emerging technologies push the boundaries of what is scientifically possible, the ethical dimensions of responsible innovation must be carefully deliberated (Stahl et al., 2021).

The development of regenerative medicine and tissue engineering holds tremendous potential for addressing critical health challenges, such as organ failure and tissue degeneration. By harnessing the regenerative capabilities of stem cells, researchers aim to create replacement tissues and organs tailored to individual patients, minimizing the risk of rejection and enabling more effective treatments (Dzobo et al., 2018).

However, ethical considerations accompany these advancements. As researchers seek to create complex human tissues or organs using stem cells and scaffold materials, questions arise about the moral status

of these engineered tissues. Determining when these structures attain the status of a living organism or individual requires thoughtful ethical deliberation (Chen & Liu, 2016). The potential for commodification and commercialization of human body parts further complicates the ethical landscape, calling for robust safeguards to uphold the dignity of the human body and protect patients from undue exploitation (de Kanter et al., 2023; Lau, 2023).

I. Conclusion

Stem cell research offers vast medical potential, but poses complex ethical challenges, from embryonic stem cell use to informed consent and equitable access. The legal landscape features intricate regulations and patents affecting research and affordability. Ensuring a responsible approach requires collaboration among researchers, ethicists, patients, and the public, supported by ethical boards and global partnerships. As the field advances, continuous dialogue, ethical awareness, and responsible innovation are vital to align scientific progress with societal values and well-being.

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Chapter 11

Stem Cells Are a New Hope, a New Horizon for Humanity and the Future of Human Beings: Representing Indonesia to the World

Basuki Supartono

A. Introduction

Numerous ailments—such as infections, traumas, degenerative conditions, malignancies, and congenital anomalies—have the potential to assail the human body, jeopardizing its survival. These illnesses can impact diverse systems, organs, tissues, and cells, culminating in structural impairments and functional disturbances. The integumentary, musculoskeletal, nervous, cardiovascular, endocrine, and other bodily systems may all be implicated. Failure to effectively manage these maladies can result in disability and mortality. Consequently, they engender myriad challenges and anguish for

B. Supartono

UPN Veteran Jakarta, e-mail: basuki@upnvj.ac.id

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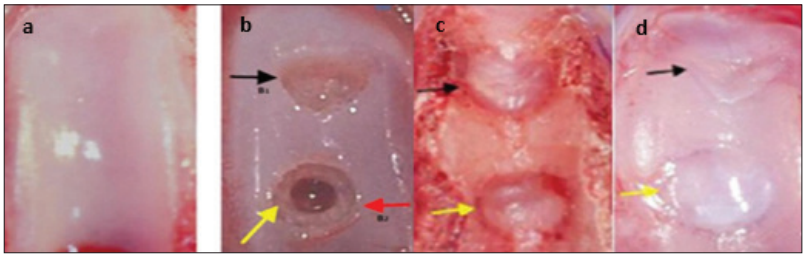
humanity, imposing burdens upon individuals, families, and societal and economic frameworks. Humanity endeavors to mitigate ailments via a multitude of strategies. However, not all interventions yield positive outcomes, with a fraction resulting in failure. Both medical and surgical therapies sometimes fail. Failure may occur because these therapies are unable to repair structures and restore tissue function to their original state (Supartono, 2018a).

Such failures can occur due to the nature and condition of the tissues themselves. Certain body tissues lack the capability for regeneration (healing). If these tissues experience structural disintegration and tissue dysfunction, they cannot heal because they are incapable of undergoing the healing process. An example of this is the cartilage tissue in knee joints. This tissue is avascular, meaning it lacks blood vessels, which results in the cartilage's inability to heal. When this tissue is diseased, injured, or aged, it cannot recover. In the event of knee joint cartilage damage, the regenerative response culminates in the formation of suboptimal, fibrous tissue. The quality of this tissue is not as good as the original tissue (Figure 11.1). To address this condition, the damaged tissue must be removed and replaced with artificial tissue made of metal and plastic materials. This procedure is known as joint replacement surgery. While offering therapeutic advantages, this surgical intervention is associated with



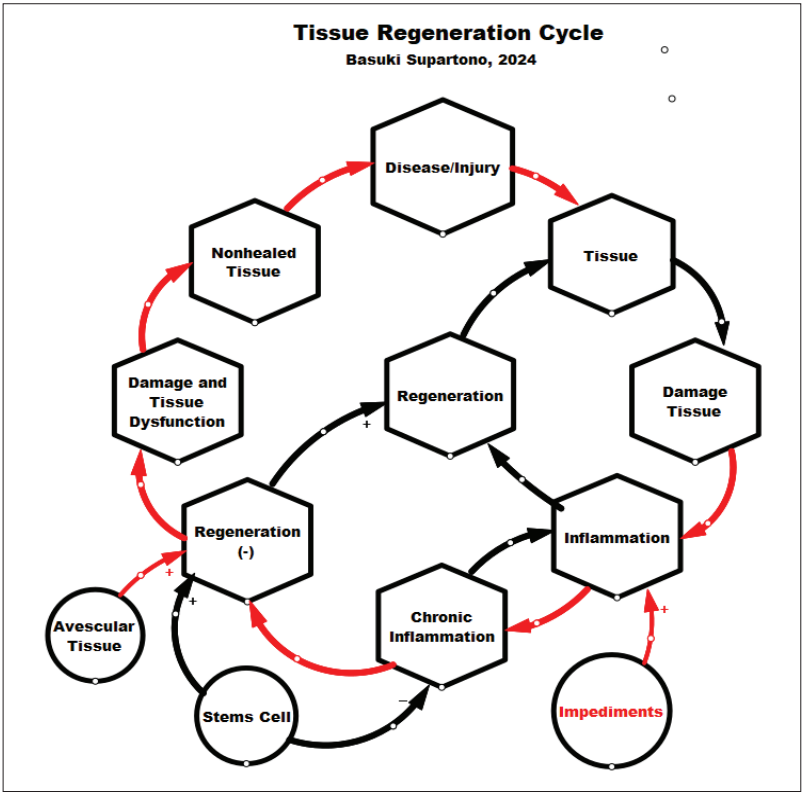
Notes: Knee joint cartilage of animals model: (A) normal cartilage, (B) cartilage defects, (C) 1 month after defects, (D) 2 months defects.

Source: Supartono et al. (2018)

Figure 11.1 Natural regeneration of cartilage defects in animal models results in fibrous tissue formation.

significant health risks and cannot fully re-establish the joint's original functional capacity (Supartono, 2018a).

Another factor contributing to healing failure arises from the impediments encountered by normal tissues during the regeneration process (Figure 11.2).



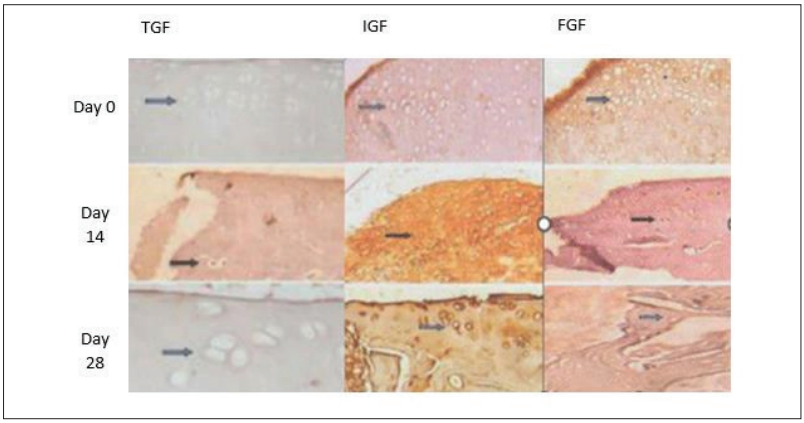
Notes: The normal tissue regeneration cycle consists of disease/injury, tissue damage, inflammation, and regeneration. Regeneration does not occur in avascular tissues and under conditions of chronic inflammation due to impediments caused by specific diseases. Stem cells play a role in modulating inflammation and act as agents of regeneration.

Source: Supartono (2023)

Figure 11.2 Tissue Regeneration Cycle

The healing cycle—encompassing tissue damage, inflammation, and regeneration—is disrupted, arresting at the inflammation stage. All normal tissues subjected to pathology, trauma, or senescence undergo damage, eliciting a counteractive inflammatory response from the tissue to mitigate the disturbance. This inflammatory phase is pivotal in establishing the requisite conditions for the onset of the regeneration phase

Our research shows that tissues affected by damage will respond with inflammation, release growth factors (GFs), and undergo tissue regeneration. These GFs are temporary and spatial. They appear after the first week, following the end of the inflammatory process, and persist for two weeks to stimulate cell formation. After two weeks, the GFs disappear to allow the restoration of the extracellular matrix and the formation of new, high-quality tissue (Figure 11.3) (Supartono, 2016b).



Notes: Tissue impacted by injury releases growth factors (GF) such as TGF, IGF, and FGF. These growth factors are temporary and act in a spatially specific manner. GFs emerge after the first week, following the conclusion of the inflammatory process, and persist for two weeks to stimulate cell formation. After two weeks, the GFs dissipate, allowing the restoration of the extracellular matrix and facilitating the formation of high-quality new tissue.

Source: Supartono (2023)

Figure 11.3 Tissue Regeneration Cycle

Nevertheless, under specific circumstances, the inflammatory response inadequately resolves the injury, persisting over an extended period and transitioning into chronic inflammation. A prototypical example is observed in wounds associated with diabetes, where prolonged hyperglycemia over years fosters an environment conducive to chronic inflammation, thereby inhibiting regenerative capabilities. Moreover, this chronic inflammatory state impedes the cellular and tissue regeneration processes, rendering the wound recalcitrant to healing or irreparable.

An additional determinant of tissue healing failure emanates from assorted pathologies, including degenerative conditions, malignancies, and traumatic injuries, all of which may precipitate chronic inflammation. Degenerative diseases and injuries have become a global health issue. Currently, degenerative diseases are among the top ten diseases in Indonesia (Dilogo, 2019). The cost burden of treatment is substantial, as seen in the treatments for diseases such as stroke, heart attack, and breast cancer (French & Emanuele, 2019). The incidence of trauma or injury is on an increasing trend (Supartono, 2014, 2015, 2016a). Injuries in the sports community, especially among athletes, pose a threat to their achievements and future prospects (Supartono, 2017). The aforementioned issues present a multifaceted and considerable challenge to public health, necessitating immediate remedial strategies. This scenario poses a substantial obstacle within the healthcare domain. Traditional medical and surgical interventions are often inadequate in fully addressing disease management. Consequently, there is an imperative need for an innovative methodology, specifically, a biological approach (Dilogo, 2019). A disease healing approach that accommodates the properties, characteristics, behavior of cells, and the regenerative capabilities of each tissue.

Efforts to address these issues have been pursued through a range of research endeavors, encompassing in vitro investigations, animal experimentation, and clinical trials, both globally and within Indonesia. Over the past decade, a novel medical approach has

emerged, termed interventional methodologies. This descriptor is apt as it involves direct intervention at the cellular level. Alternatively known as tissue engineering techniques, this methodology involves the modification of diseased tissues to generate healthy, functional replacements. This approach holds promise in addressing diseases that have historically posed significant treatment challenges. Furthermore, it can be executed without resorting to surgical procedures, thereby reducing costs and mitigating the risks of complications, disabilities, and mortality. The advent of this methodology presents a fresh and hopeful avenue for disease management and the evolution of healthcare (Supartono, 2023).

B. Tissue Engineering Technique

Tissue engineering harnesses the properties of tissues and the capabilities of engineering components. Its implementation involves employing three primary engineering components: cells, signaling molecules, and scaffolds. A scaffold is an artificial environment conducive to cellular growth and maturation, facilitating the maintenance of cellular life cycles, proliferation, differentiation, and extracellular matrix production. Scaffolds can be fashioned from natural, synthetic, or hybrid materials, playing a pivotal role in the efficacy of tissue engineering endeavors. Signaling molecules, also known as growth factors (GFs), are compounds capable of eliciting cellular responses and promoting extracellular matrix synthesis. In contemporary practice, scaffolds and growth factors are readily available in pharmaceutical formulations. Tissue engineering methodologies entail the integration of all three engineering components. However, they can also be implemented using individual components. Monocomponent applications predominantly involve the utilization of cells, specifically responsive cells capable of proliferation and the formation of cellular and tissue matrices.

At first, healthy cells sourced from the target tissue were utilized. These cells are terminally differentiated, signifying that they have assumed specific morphological and functional characteristics. For

instance, mature chondrocytes harvested from cartilaginous tissue are cultured in vitro before being transplanted into damaged tissues to facilitate tissue regeneration. However, this approach encounters numerous challenges and limitations. The process necessitates several sequential stages, including cell isolation, cultivation, and implantation. Cell collection involves surgical interventions that are discomforting, costly, and associated with inherent morbidity risks. Furthermore, the quantity of harvestable cells is restricted, and their quality is contingent upon the patient's age and health status. Moreover, the harvesting process carries the risk of structural damage and diminished cellular functionality, leading to the formation of low-quality regenerative tissue (i.e., scar tissue). Consequently, the utilization of such cells has been largely discontinued. In pursuit of a more promising alternative, researchers, scientists, and clinicians have shifted their focus to stem cells, owing to their capacity to generate high-quality tissue structures and functions. Additionally, the procedure is less invasive and can be performed without resorting to surgical interventions (Supartono, 2018a).

C. The Role of Stem Cells in Tissue Regeneration

In human, illnesses and injuries can inflict harm upon cellular and tissues. To prevent the disruption of physiological functions, the restoration or substitution of this damage is imperative. Stem cells, ubiquitous in human tissues, fulfill this vital function. This provision, bestowed by the Divine in His benevolence, underscores the significance of these cells for human welfare. Tissue-specific stem cells possess both inherent and adaptive qualities, ensuring their capacity to engage in tissue repair endeavors exclusively when necessitated.

Cell division is achieved through symmetric division, producing two identical totipotent daughter cells. Additionally, asymmetric division occurs to produce one identical stem cell and one progenitor cell. Each progenitor cell generates another progenitor cell like itself and another cell that begins the determination process to form a specific cell according to tissue needs. Progenitor cells grow and

develop according to commitment and organize themselves within the tissue architecture. In summary, it can be said that everybody tissue contains stem and progenitor cells with the capability to grow and develop as required by life's demands (Supartono, 2018a).

The Capabilities of Stem Cells and the Plasticity of Tissue Stem Cells

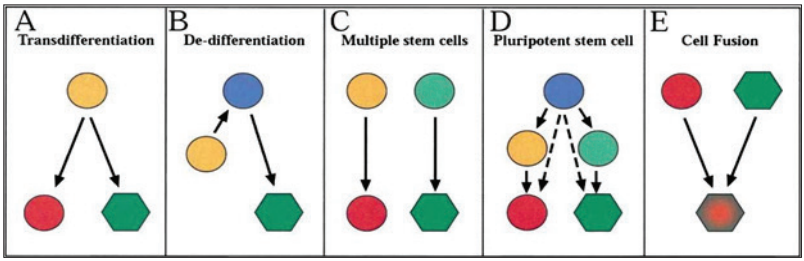
Stem cells exhibit varying degrees of developmental potential based on the exigencies of their tasks and roles. These developmental capacities encompass totipotency, pluripotency, multipotency, oligopotency, and unipotency. Totipotency denotes the unrestricted capacity of stem cells to generate all types of bodily tissue cells, encompassing both embryonal and extra-embryonal tissues, as well as to engender new organisms. Embryonic stem cells and germ layer stem cells exemplify this capability.

Pluripotency denotes the capacity to generate all types of body cells, including germ cells and certain extra-embryonal tissues, albeit without the ability to form a new organism. Multipotency signifies the capacity to generate numerous types of adult cells within the same lineage. Oligopotency denotes the capacity to generate a limited number of adult cell types. Unipotency refers to the capacity to generate a single type of adult cell. These developmental potentials are influenced by various factors, such as the stem cell type, genetic attributes, cell culture conditions, growth factors, microenvironmental cues, and cellular interactions

Furthermore, tissue stem cells demonstrate inherent plasticity, defined as the capacity to differentiate into cell types distinct from their lineage of origin. This characteristic is observable across stem cells from a diverse array of organs and tissues, including but not limited to the liver, pancreas, nervous system, brain, epidermis, bone marrow, muscular tissue, synovial membrane, and hematopoietic system. Within the hepatic environment, oval cells have the potential to differentiate into epithelial cells of the bile ducts. The pancreas harbors multipotent precursor cells, which, while akin to progenitor

cells, exhibit a marginally reduced differentiation potential. These pancreatic precursors are competent in giving rise to both pancreatic endocrine and exocrine cells, as well as neuronal cell types. Similarly, neural stem cells possess the plasticity to differentiate into progenitor cells of the hematopoietic lineage, myocytes, cells of the germ layers, and mesodermal cells. Peripheral blood stem cells have been demonstrated to differentiate into endothelial cells, osteoblasts, and chondrocytes. Notably, Supartono et al's research elucidated that CD34+ hematopoietic stem cells are capable of transdifferentiating into chondrocytes, thereby contributing to the formation of joint cartilage tissue. The plasticity inherent to tissue-specific stem cells heralds a paradigm shift in the approach to the treatment of various diseases (Supartono et al., 2018).

Supartono (2018a) postulated that the plasticity observed in stem cells can be attributed to multiple underlying mechanisms, which include (a) transdifferentiation, (b) dedifferentiation, (c) existence of dual-potency stem cells, (d) pluripotency of certain stem cells, and (e) cell fusion, as illustrated in Figure 11.4. Transdifferentiation refers to the process whereby tissue-specific stem cells undergo a phenotypic



Notes: Mechanisms of stem cell plasticity include (A) transdifferentiation, (B) dedifferentiation, (C) multiple stem cells, (D) pluripotent stem cells, and (E) cell fusion. Tissue stem cells: orange or green spheres; pluripotent cells: blue spheres; orange lineage differentiation cells: red spheres, and green lineage differentiation cells: green hexagons.

Source: Supartono (2018a)

Figure 11.4 Schematic Diagram of the Mechanism of Tissue Stem Cell Plasticity, from Waggers

conversion, resulting in the emergence of cell types divergent from their original lineage. This capacity for transdifferentiation is contingent upon the microenvironment of the resident tissue. For instance, stem cells residing within the bone marrow or circulating in the peripheral blood have the ability to give rise to cell types unrelated to hematopoietic lineage.

Furthermore, both hematopoietic and mesenchymal stem cells within the bone marrow possess the remarkable capacity to migrate to distinct tissue sites, undergoing cellular transformation to adopt the characteristics of the resident tissue cells. Dedifferentiation describes a process whereby tissue-specific stem cells regress to a more primordial or multipotent state, thereby acquiring the capability to differentiate into cell types of a novel lineage. The phenomenon of dual stem cells refers to the capacity of tissue-specific stem cells to simultaneously generate a progenitor cell akin to their original form as well as progenitor cells destined for differentiation into alternate lineages. Pluripotent stem cells embody a transformative mechanism through which tissue-specific stem cells attain pluripotency, facilitating the emergence of dual progenitor cells and, subsequently, divergent lineage pathways. Cell fusion encompasses a process by which two tissue-specific stem cells amalgamate, culminating in the genesis of a distinct cellular entity (Supartono, 2018a).

D. Stem Cells and Their Applications in Tissue Engineering Techniques

Stem cells, inherently undifferentiated, boast the capacity for self-replication and renewal. Through the synthesis of the author's research alongside contributions from other scholars in the field, it has been elucidated that stem cells exhibit three primary functions: modulation of inflammatory responses, facilitation of tissue repair and regeneration, and suppression of microbial proliferation. These intrinsic properties present viable avenues for therapeutic applications. Under certain pathological or traumatic scenarios, an individual's endogenous stem cell reservoir may prove inadequate, attributed

either to a paucity in cell quantity or a decrement in functional quality. In such instances, the clinical scenario may necessitate the exogenous administration of stem cell-derived products to augment or restore the body's reparative and regenerative competencies.

Legislative mandates within Indonesia expressly prohibit the application of embryonic stem cells in clinical settings, permitting instead the utilization of non-embryonic, or alternately termed, tissue-derived stem cells from human sources. Such tissue stem cells are ubiquitously distributed throughout various bodily tissues, encompassing both germinal and somatic cell populations. From a technical perspective, these cells are identifiable within both solid tissues—exemplified by skin, adipose tissue, placenta, muscular tissue, and synovial membranes—and fluidic matrices, including bone marrow aspirate, umbilical cord blood, and peripheral circulatory system. Characterization of tissue stem cells facilitates their classification into several key categories: progenitor or mononuclear cells, mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and induced pluripotent stem cells (iPSCs). These stem cells are harvested from an array of tissues originating from either the patient or a donor (Supartono, 2023).

1. Tissue Stem Cell Production

The production of tissue stem cells within Indonesia holds the potential to diminish reliance on foreign pharmaceutical imports, thereby enhancing national self-sufficiency. The process begins with the isolation of mononuclear progenitor cells, which are then cultured or expansion into various types of stem cells with the desired characteristics (Supartono, 2018a).

2. Techniques and Procedures of Tissue Stem Cell

The production of stem cells encompasses a series of sophisticated steps, including cell isolation, cell characterization, selection, in vitro culture, cell expansion, cryopreservation, cryogenic storage, and subsequent thawing. These processes have been conducted

Notes: (A) manual tissue stem cell processing in the laboratory, (B) a stem cell processing machine.

Source: Supartono (2023)

Figure 11.5 Processing of the Tissue Stem Cells

manually. However, advancements in biotechnology have facilitated their automation. Stem cell processing machines are now available (refer to Figure 11.5). These devices are capable of converting human blood or bone marrow aspiration into assorted stem cell types through an enclosed, sterile system, ensuring the production of clinical-grade products ready for direct patient application. Such machinery offers a cost-effective solution for the large-scale production of diverse stem cell products, catering to broad therapeutic needs. The cells thus processed may be cryopreserved for future use or immediately deployed in therapeutic interventions (Supartono, 2023).

3. Laboratories for Stem Cell Processing

In accordance with prevailing Indonesian regulations, stem cell production is mandated to occur within laboratories specifically designated for stem cell research and processing. There are two types of these laboratories: research laboratories that produce stem cells for research purposes and Laboratories for Stem Cell Processing for Clinical Application (commonly referred to as LPSAK in Indonesian) for producing stem cells for patient treatment.

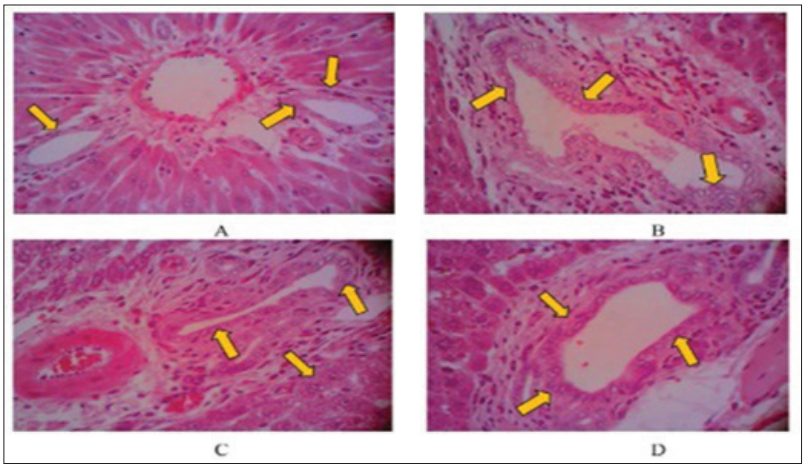
LPSAK must follow the prevailing government regulations and are supervised by the Ministry of Health of the Republic of Indonesia, the Food and Drug Monitoring Agency of the Republic of Indonesia, and the Indonesian Stem Cell Committee. These regulations are outlined in the Minister of Health Regulation of the Republic of Indonesia Number 50 of 2012 regarding the Operation of Stem Cell Processing Laboratories for Clinical Applications (Permenkes No. 50, 2012).

4. Stem Cell Applications

The application of stem cells can be administered through various routes, including topical, intravenous, intra-arterial, intramuscular, and other routes. Once introduced into the host organism, these cells persist, proliferate, and differentiate within the recipient's milieu. Such cells engender the formation of novel cells and tissues, facilitating the repair of tissue degradation and the restoration of tissue functionality to its baseline condition. Consequently, organ and systemic functions are reinstated to their normal states, culminating in the recovey of the patient's health (Supartono, 2018a). The use of stem cells in surgery can save time, reduce complications, shorten the duration of surgery, and provide good outcomes (Dilogo, 2019). However, their implementation must be safe, beneficial, comfortable, efficient, and cost-effective.

5. Safety of Tissue Stem Cells

Stem cell applications have the potential to elicit rejection reactions, thus requiring an evaluation of their safety. Research has shown that the application of tissue stem cells is safe, without causing rejection or toxic reactions. Several studies have documented this. Basuki found that the administration of human hematopoietic stem cells (HSCs) to animal models is safe and does not provoke rejection or toxic reactions (Supartono, 2017). Another study by Supartono showed similar results.



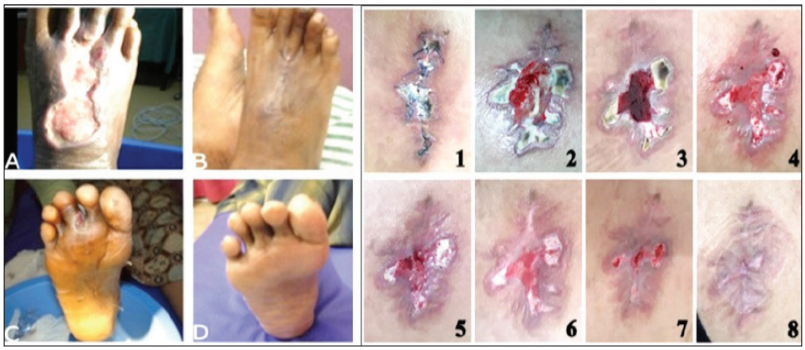
Notes: The number of cells in the bile duct has increased in all of the treated groups compared to the control group, as shown by yellow arrows. The shape of the cells in the treated groups looked more oval than the control group. (A) control, (B) 105 cells, (C) 106 cells, (D) 107 cells.

Source: Supartono et al. (2022)

Figure 11.6 Microscopic Images Of Tissue Regeneration (Oval Cells) in Naive Rats Following Repeated Intravenous Injections of Human PBMC Once a Month for Three Months

The monthly intravenous administration of human progenitor cells (PBMC) to animal models for three months was found to be safe, without any rejection or toxic reactions. The animals remained healthy clinically, in laboratory tests, and histopathologically. Moreover, it was demonstrated that the procedure did not interfere with and still induced tissue regeneration—see Figure 11.6 (Supartono et al., 2022).

Supartono (2018b) reported that the weekly administration of peripheral blood mononuclear stem cells (PBMC) from both patients and donors for two months has been proven safe. There were no



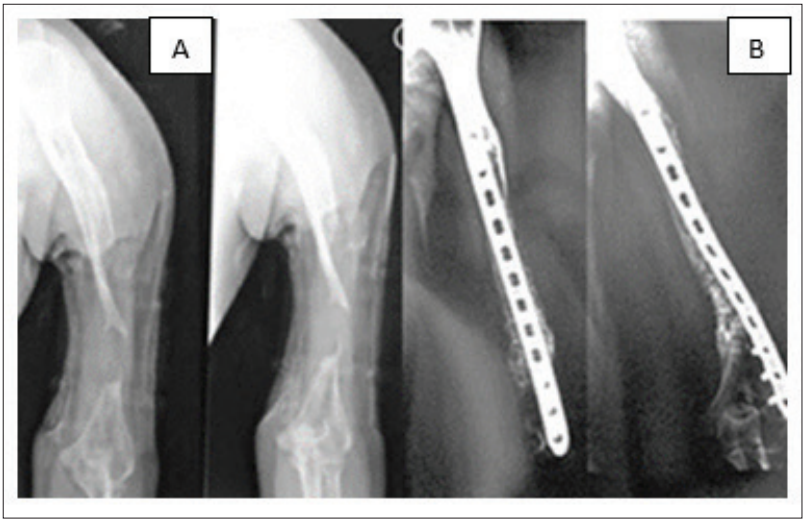
Notes: Figure A, B, C and D describes the weekly administration of peripheral blood mononuclear cells (PBMC) from patients and donors (described by figure 1–8) for two months that has been proven safe. There were no signs of rejection reactions, allergic reactions, infection, toxic reactions, or complications. It aids in the regeneration process or healing of chronic (banal) diabetic wounds that are difficult to heal.

Source: Supartono (2018b)

Figure 11.7 Safety of Administration of Peripheral Blood Mononuclear Cells (PBMC) to Patients with Unhealed Diabetic Wounds

rejection reactions, allergic reactions, signs of infection, toxic reactions, or any adverse complications. Contrary to inhibiting, these interventions facilitated the regenerative process, significantly promoting the healing of chronic, banal diabetic wounds—see Figure 11.7 (Supartono, 2018b).

The application of mesenchymal stem cells (MSCs) and their metabolic products has been proven safe. Ismail reported that the administration of mesenchymal stem cells (MSCs) to patients with bone defects at Cipto Mangunkusumo Hospital in Jakarta was proven to be safe. Furthermore, the application of these stem cells, whether from patients or donors, had a positive impact on the bone regeneration process (Figure 11.8; Dilogo et al., 2019).



Description: The application of mesenchymal stem cells (MSCs) from both patients and donors in patients with bone defects has been proven safe and positively impacts bone regeneration processes. (A) before administration, (B) after administration.

Source: Dilogo et al. (2019)

Figure 11.8 Safety of Administration of Mesenchymal Stem Cells (MSCs) to Patients with Critical Bone Defects

6. Legal Aspects of Stem Cell Services in Indonesia

Indonesia accommodates stem cell-based therapy. The Government of the Republic of Indonesia, through the Health Law No. 17 of 2023, states that stem cells can be used for disease treatment and health recovery (UU No. 17, 2023). Stem cell therapy is primarily directed towards humanitarian objectives, eschewing commercial exploitation. Its application is strictly non-reproductive and contingent upon rigorous validation of its safety and therapeutic efficacy. Moreover, this therapeutic modality is obligated to adhere to established healthcare service protocols, whilst duly acknowledging and integrating societal roles, socio-cultural norms, moral principles, and ethical standards. Details on this matter are regulated in the Minister of Health

Regulation No. 32 of 2018 on the Provision of Stem Cell Services (Permenkes No. 32, 2018).

Stem cell therapy can be administered for degenerative diseases, non-degenerative diseases, and the rejuvenation of cells, tissues, and organs. The source of these stem cells may be autologous or allogeneic, provided voluntarily and without financial remuneration. The types of stem cells that can be administered include mesenchymal stem cells, hematopoietic stem cells, progenitor cells, and secretome.

The secretome is a stem cell product containing growth factors, cytokines, and microvesicular structures, exosomes, and other factors. Stem cells and secretomes must meet quality, safety, efficacy requirements, and have marketing authorization.

Clinical applications of stem cells, whether systemic, regional, local, or topical, are performed by competent medical personnel in hospitals and clinics in accordance with applicable regulations. Clinics are only allowed for local and topical applications. The use of stem cells is carried out in the form of standardized therapy services, the standards of which are set by the Minister of Health of the Republic of Indonesia. Other uses include research-based therapy services organized in hospitals designated by the Minister of Health of the Republic of Indonesia. Hospitals or clinics can set and charge for standardized therapy services or replacement costs for processing in research-based therapy services. Costs incurred from the use of stem cells can be covered by the patient, grants from the central government, local government, research institutions, and donations from the community (Permenkes No. 32, 2018).

7. Religious Aspects of Stem Cells

Indonesia guarantees its citizens the right to worship and practice their religious teachings. In accordance with the constitutional mandate, the state provides protection and assurances regarding the halal status of products utilized by the community through Law No. 33 of 2014 on Halal Product Assurance. The Government of the Republic of Indonesia provides legal certainty in the form of Halal

Product Assurance, evidenced by a Halal Certificate (UU No. 33, 2014). On September 9, 2020, the Indonesian Ulema Council issued a fatwa as a guideline for the government, medical personnel, and the public regarding the use of stem cells. The Indonesian Ulema Council requested the government to strictly supervise stem cell therapy providers and urged medical personnel to always consider the sharia aspects in every medical action. The law on the use of stem cells is twofold: haram (forbidden) or *mubah* (permitted).

The use of human stem cells for any purpose is fundamentally haram if the source of the cells is taken in a manner not in accordance with legal stipulations. Other reasons for being haram include (a) if the extraction of stem cells causes hardship (*masyaqqah*) or harm (*dharar*) to the donor or recipient; (b) if their effectiveness is still in doubt; (c) if used to alter the natural shape of the body to make it more attractive, change identity, or for other purposes that contradict sharia; (d) if stem cells are bought and sold between the cell owner and another party; and (e) for reproductive purposes (to create a new being). Based on the preceding discussion, the administration of human tissue-derived stem cells is permitted for therapeutic treatments, tissue regeneration, and medical research purposes, contingent upon obtaining written consent from the donors (Fatwa Majelis Ulama Indonesia No. 51, 2020).

8. The Use of Stem Cells in Indonesia

As mentioned above, the use of stem cells in Indonesia has been proceeding through research-based therapeutic services. The use of these stem cells is articulated engagingly throughout the chapters of this book. Titled *Discovering the Miracles of Stem Cells*, the book unveils the extraordinary potential of stem cells. Leading stem cell researchers from various research centers in Indonesia report on a range of research findings about the potential and use of tissue stem cells. This includes discussions on the processing of mesenchymal stem cells, induced stem cells, the potential of stem cells for neurological diseases, and the ethical aspects of tissue stem cells. Additionally,

it explains the use of mesenchymal stem cells and their metabolic products for the treatment of orthopaedic diseases, diabetes, skin rejuvenation, and myocardial infarction. Of particular interest is the potential for hematopoietic stem cells to transdifferentiate into skin-forming cells and tissues.

This book is highly engaging as it elucidates the position and contribution of Indonesian stem cell scientists in the scientific journey to discover the potential and benefits of stem cells. It reveals the role of Indonesian scientists in building a golden bridge toward achieving a future of high-quality health life.

9. Bridging the Future

Currently, we stand on the cusp of a new epoch in the fields of medicine and biotechnology, thereby underscoring the importance of contemplating the extensive journey embarked upon in "Discovering the Wonders of Stem Cells". Beginning with innovative techniques in stem cell culture, potential and benefits, to the ethical debates surrounding stem cell research. Each chapter has unveiled various challenging and promising aspects of stem cells.

Jeanne Adiwinata Pawitan elaborated on methodologies for the isolation, culture, and cryopreservation of mesenchymal stem cells (MSCs), highlighting the significance of meticulous sample management and isolation protocols to enhance therapeutic outcomes. Various techniques are utilized to extract MSCs from adipose and umbilical cord tissues, each presenting distinct benefits and considerations. The expansion of MSCs cultures necessitates the adoption of alternative systems, such as hollow fiber systems and hyperflasks, to efficiently accommodate large-scale production requirements. Cryopreservation strategies, inclusive of alternatives to the conventional dimethyl sulfoxide (DMSO)-based solutions, are critical for ensuring the prolonged viability of MSCs without degrading their quality. The application of aseptic procedures is essential to mitigate contamination risks throughout the isolation and culturing phases. The evolution of MSC culture methods, cryopreservation

techniques, and aseptic protocols has significantly contributed to the establishment of effective procedures vital for both research and therapeutic applications. Acquiring proficiency in these techniques is crucial for the successful production of MSCs. Pawitan has introduced cost-effective strategies for the generation of MSCs from bone marrow, adipose, and umbilical cord tissues, which are compatible with current Good Manufacturing Practices (GMP) in laboratory settings.

Ismail Hadisoebroto Dilogo explained thoroughly, the use of stem cells in orthopaedics has surged in the past decade, driven by advances in scientific research. Stem cells offer potential for regenerative medicine in treating bone abnormalities and regenerating tissues like nerves, tendons, ligaments, and cartilage. Mesenchymal stem cells (MSCs) are commonly used in orthopaedic disorders due to their differentiation potential. Clinical studies, particularly in neurology and orthopaedics, have highlighted the prominence of MSC-based therapies. MSCs play a vital role in fracture repair by promoting angiogenesis and bone regeneration, offering promising solutions for non-union fractures.

In treating articular cartilage defects, stem cell-based therapies, including exogenous MSCs and their secretome, show potential in enhancing cartilage repair and slowing or reversing cartilage damage. Research into exosomes derived from MSCs also holds promise in cartilage regeneration by boosting cell proliferation, reducing inflammation, and enhancing cartilage repair. Overall, stem cell-based approaches hold considerable promise for orthopaedic disorders, with ongoing research aimed at optimizing their therapeutic potential and overcoming existing challenges.

Siufui Hendrawan et al. have shown that secretome-based therapy, derived from mesenchymal stem cells (MSCs), exhibits potential in treating diabetic wounds. The secretome provides a cell-free option that possesses lower immunogenicity. Future prospects involve addressing challenges in secretome therapies to expand their clinical applications. Despite facing obstacles, these therapies offer promising alternatives for the treatment of diabetic wounds.

Winawati Eka Putri and Cita Rosita Sigit Prakoeswa have revealed that mesenchymal stem cells (MSCs) have attracted significant attention for their potential in rejuvenating aged skin, attributed to their regenerative capabilities. Research has demonstrated encouraging outcomes of MSC-derived conditioned medium (MSC-CM) in wound healing, photoprotection, and enhancing clinical indicators of skin aging. Various sources of MSC-CM, including bone marrow, umbilical cord, amniotic fluid, adipose tissue, and chorion, have been investigated for their therapeutic impacts on aged skin. Methods of application generally encompass subcutaneous injections or microneedling techniques. Although adverse effects are rare, careful measures should be adopted to prevent complications. Collectively, MSCs and MSC-CM offer promising pathways for the treatment of skin aging, providing potential advantages in tissue regeneration and rejuvenation.

Teguh Santoso et al. have reported the results of their clinical trials on the exploration of stem cell therapy›s efficacy in treating ST-segment elevation myocardial infarction (STEMI) and its aftermath. Stem cell therapy emerges as a promising avenue for replacing or repairing damaged cardiac tissue, with factors like the stem cell source, administration route, dosage, preparation, and timing critically affecting its success. Mesenchymal stem cells derived from the umbilical cord (UC-MSCs) have demonstrated potential in cardiac regeneration owing to their paracrine effects and minimal immunogenicity. Clinical trials employing intracoronary and intravenous administration methods have yielded positive outcomes, including enhancements in left ventricular function and patient quality of life. Despite challenges such as limited sample sizes, stem cell therapy presents a viable strategy for alleviating the detrimental impacts of STEMI and enhancing patient prognosis, underscoring the need for further research to refine its application.

Mochamad Syaifudin et al. have demonstrated the potential for transdifferentiation of CD34+ cells into skin cells and tissues. The characteristics and potential of hematopoietic stem cells, specifically

CD34+ cells, in enhancing fibroblast and collagen production in UV-exposed skin are investigated. The plasticity of HSCs allows them, under certain conditions, to aid in tissue regeneration beyond the blood system, opening avenues for regenerative therapies.

The text delves into several facets of hematopoiesis, including the proliferative behavior of HSCs, modes of cell division, examples of tissues illustrating stem cell functions, and the therapeutic applications of stem cells in combating skin aging. It highlights the crucial role of HSCs in tissue regeneration. Moreover, the text examines the molecular mechanisms behind HSC differentiation and the importance of CD34+ hematopoietic stem cells in skin rejuvenation, particularly their role in boosting fibroblast and collagen production. Mochamad Syaifudin calls for additional research to confirm these results and refine treatment methodologies for clinical use.

Somia Gul et al. theoretically discuss how stem cells therapy presents a promising avenue for addressing neurodegenerative diseases by facilitating neuronal regeneration and correcting abnormalities in neuronal circuitry. Stem cell therapy has the potential to repair hippocampal circuits, improve patterns of cognitive and emotional behavior, and treat neurodegenerative disorders, such as Alzheimer's disease. Despite the associated risk factors and limitations, stem cell therapy has demonstrated promising results in various conditions, including ischemia-induced diseases, gliomas, and spinal cord injuries. Continuous research and technological developments are anticipated to enhance outcomes in the future.

Ahmad Faried and Yulius Hermanto delve theoretically into the depth of induced pluripotent stem cells (iPSCs). The document details the process of nuclear reprogramming to generate iPSCs, emphasizing their potential applications in regenerative medicine. The text proceeds to examine the modeling of neurological disorders using iPSC-derived neural cells. Various neurological diseases, such as Parkinson's disease and Alzheimer's disease, are referenced, alongside the challenges of modeling these conditions with iPSC-derived neural cells. Overall, the document offers an exhaustive review of

the creation, significance, and utility of induced pluripotent stem cells in neurological disease research and treatment. Moreover, the document discusses the prospects of iPSC-based therapies for neurological disorders. Although stem cells therapy shows promise, it faces obstacles, such as challenges in somatic cell reprogramming, genetic alterations, and the necessity for comprehensive preclinical evaluations.

Finally, Dito Anurogo underscores the ethical issues inherent in stem cell research and application. Stem cell research harbors significant potential to transform medicine and biotechnology, providing prospective treatments for various severe conditions through regenerative therapies. Nonetheless, the ethical quandaries associated with the sourcing of stem cells remain a central concern. Paramount to this discussion is the principle of informed consent, particularly in the context of stem cell clinical trials, where the risks and benefits must be transparently communicated to participants. Moreover, adherence to ethical guidelines and best practices is crucial for the responsible conduct of stem cell research. In Indonesia, ethical considerations blend international norms with local cultural and religious values, highlighting the importance of collective well-being while respecting individual autonomy. As stem cell research progresses, the advent of new technologies such as organoid development and gene editing introduces additional ethical challenges. This necessitates continuous ethical reflection to ensure responsible innovation and the protection of human dignity.

At the culmination of the scientific endeavor *Discovering the Miracles of Stem Cells*, it is imperative to deeply explore the essence, implications, and ethical considerations stemming from the groundbreaking discoveries in stem cell research. Stem cell research, as a beacon of light and hope within the medical and biotechnological domains, heralds the potential of stem cells in the treatment and recuperation of diseases. Nevertheless, this path is laden with intricate ethical, technical, and regulatory challenges, thereby necessitating precision and prudence in both its research and application.

E. Conclusion

In summary, stem cell research in Indonesia has advanced swiftly, with its discoveries being applied in the treatment of diverse ailments. The Indonesian government has enacted policies addressing numerous facets of stem cell science, including its production, application, and utilization, from both medical and religious perspectives. These regulations aim to ensure legal certainty, safety, and comfort for patients and stakeholders. However, the application of stem cells remains restricted and is primarily confined to research contexts.

Although the government acknowledges stem cell therapy as a healthcare initiative, it has not officially endorsed it as a standard treatment modality within Indonesia. Consequently, financing for stem cell therapy is currently provided through research grants or community contributions, without governmental support. There is a pressing need for the government to update its policies on stem cell therapy to reflect ongoing scientific progress and the evolving needs of society. Policy enhancement, aimed at fostering health technology use, could be achieved by investing in human resources, budgeting, and infrastructural development within the stem cell domain.

Stem cell therapy ought to be integrated into Indonesia's healthcare framework, enabling patients and the general populace to access stem cell treatments through the national health insurance scheme. It is anticipated that stem cell therapy will, in the foreseeable future, become an integral component of Indonesia's official healthcare services. This integration would ensure that all segments of Indonesian society in need of such treatments can access them safely and affordably. Furthermore, Indonesia could become a destination for global patients seeking stem cell therapy, thereby contributing significantly to the international medical community by elevating life quality and health standards.

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List of Abbreviations

3xTg	: Triple transgenic
6MWD	: Six-Minute Walk Distance
6MWT	: Six-Minute Walking Test
ACL	: Anterior cruciate ligament
AD	: Alzheimer's disease
ADMSCs	: Adipose-derived mesenchymal stem cells
ADSCs	: Adipose-Derived Stem Cells
AF-MSC	: Amniotic fluid-mesenchymal stem cell
AF-MSCs	: Amniotic fluid mesenchymal stem cells
AFSC-CM	: Amniotic fluid stem cells-conditioned medium
AGM	: Aorta-gonad-mesonephros
AKT	: Phosphorylation of protein
AKT	: Protein kinase B
alpha MEM	: Alpha minimum essential medium

ALS	: Amyotrophic lateral sclerosis
ALSFRS-R	: Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised
AMI	: Acute myocardial infarction
AMSC-CM	: Amniotic membrane stem cell-conditioned medium
Ang-1	: Angiopoietin-1
Anti-CMV IgG	: Anti-Cytomegalovirus IgG
ARDS	: Acute respiratory distress syndrome
ASC	: Adipose stem cell
ASC-CM	: Adipose stem cell-conditioned medium
ASCs	: Adipose stem cells
ASD	: Autism spectrum disorder
Bcl-2	: B-Cell lymphoma 2
BDNF	: Brain-derived neurotrophic factor
bFGF	: Basic fibroblast growth factor
bFGF-BP	: Basic fibroblast growth factor-binding protein
BFU-E	: Burst-forming unit-erythroid
bHLH	: Basic Helix-Loop-Helix
BM	: Bone marrow
BM-MSC	: Bone marrow mesenchymal stem cell
BM-MSCs	: Bone marrow-derived mesenchymal stem cells
BMP	: Bone morphogenetic protein
BMP9	: Bone morphogenetic protein 9
BMSC-CM	: Bone marrow stem cell-conditioned medium
BPI	: Brachial plexus injury
BrdU	: Bromodeoxyuridine
BSC	: Biosafety cabinet
CAT	: Catalase

CD	: Cluster of differentiation, as in CD90, CD73, CD105, and CD36
CD34+	: Cluster of differentiation 34 positive
CDSC-CM	: Chorion-derived stem cell conditioned medium cell-derived factor-1α
CFU	: Colony forming unit
CFU-E	: Colony-forming unit-erythroid
CFU-G	: Colony-forming unit-granulocyte
CFU-GEMM	: Colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte
CFU-GM	: Colony-forming unit-granulocyte macrophage
CFU-M	: Colony-forming unit macrophage
CFU-Mk	: Colony-forming unit-megakaryocyte
cGMP	: Current Good Manufacturing Practices
cGTP	: Current Good Tissue Practice
CHT	: Caudal hematopoietic tissue
CINC	: Cytokine-induced neutrophil chemoattractant
CINC-3	: Cytokine-induced neutrophil chemoattractant-3
CLP	: Common lymphoid progenitor
CM	: Conditioned medium
CMP	: Common myeloid progenitor
c-Mpl+	: Cellular myeloproliferative leukemia protein positive
CNS	: Central nervous system
Col	: Collagen
CPD	: Cyclobutane pyrimidine dimer
cPD	: Cumulative population doublings
CRISPR/Cas9	: Clustered Regularly Interspaced Short Palindromic Repeats—CRISPR-associated protein 9

CRISPR-Cas9	: Clustered regularly interspaced short palindromic repeats and CRISPR associated protein 9
DA	: Dorsal aorta
DALYs	: Disability-adjusted life years
DBS	: Deep brain stimulation
DCX	: Doublecortin
DG	: Dentate Gyrus
DM	: Diabetes mellitus
DMEM	: Dulbecco's modified eagle medium
DMSO	: Dimethyl sulfoxide
DNA	: Deoxyribonucleic acid
eASCs	: Expanded allogeneic adipose-derived stem cells
ECM	: Extracellular matrix
EGF	: Epidermal growth factor
EGFR	: Epidermal growth factor receptor
EpiSCs	: Epiblast stem cells
ERK	: Extracellular signal-regulated kinase
ERKs	: Extracellular signal-regulated kinases
ERK1/2	: Extracellular signal-regulated kinases 1 and 2
ES	: Embryonic stem
ESCs	: Embryonic stem cells
ET	: Exercise training
EV	: Extracellular vesicles factor
FBS	: Fetal bovine serum
Fbx15	: F-box protein 15
FGF	: Fibroblast growth factor
FGF8	: Fibroblast growth factor 8
G-CSF	: Granulocyte colony-stimulating factor
GDF	: Growth differentiation factor

GDF-11 : Growth differentiation factor-11
GDNF : Glial cell line-derived neurotrophic factor
GF : Growth factors
GFAP : Glial fibrillary acidic protein
GH : Growth hormone
GM-CSF : Granulocyte macrophage-colony stimulating factor
GMP : Good Manufacturing Practices
GPx : Glutathione peroxidase
GSH : Glutathione
GVHD : Graft-versus-host disease
GW : Gestational week
HA : Hydroxyapatite
HbA1c : Hemoglobin A1c
HBsAg : Hepatitis B surface antigen
HBV : Hepatitis B virus
HCV : Hepatitis C virus
HD : Huntington's disease
HDF : Human dermal fibroblast
HDF-CM : Human dermal fibroblast-conditioned medium
hESCs : Human embryonic stem cells
HF : Heart failure
HGF : Hepatocyte growth factor
hiPSCs : Human induced pluripotent stem cells
HIV : Human immunodeficiency virus
HIV1-2 : Human immunodeficiency virus 1 and 2
HLA : Human leukocyte antigen
HLA-II : Human leukocyte antigen class II
hNSCs : Human neural stem cells

HO-1	: Heme oxygenase-1
HPL	: Human platelet lysate
HSC	: Hematopoietic stem cell
HSCs	: Hematopoietic stem cells
HUC-MSC	: Human umbilical cord-derived mesenchymal stem cell
I/R	: Ischemia-reperfusion
IACUC	: Institutional Animal Care and Use Committees
IC	: Intracoronary
IDF	: International Diabetes Federation
IFATS	: International Federation for Adipose Therapeutics and Science
IFN-α/γ	: Interferon alpha/gamma
IFN-λ	: Interferon λ
IGF	: Insulin-like growth factor
IGF-1	: Insulin-like growth factor 1
IGFBP	: Insulin-like growth factor binding proteins
IGFBP-1	: Insulin-like growth factor binding and IGFBP-2 proteins 1 and 2
IgM	: Immunoglobulin M
IL	: Interleukin
IL-12	: Interleukin-12
IL-18	: Interleukin-18
IL-1b	: Interleukin-1 beta
IL-4	: Interleukin-4
IL-6	: Interleukin-6
INs	: Interneurons
Ips	: Induced Pluripotent Stem Cells
iPSCs	: Induced pluripotent stem cells

ISCT	: International Society for Cellular Therapy
ISSCR	: International Society for Stem Cell esearch
Isx-9	: Isoxazole 9
IV	: Intravenous
IVD	: Intervertebral disc
K4 dan K27	: Lysin 4 dan Lysin 27 (in the context of histone methylation)
kDa	: Kilodalton
KGF	: Keratinocyte growth factor
LPSAK	: Laboratorium Pengolah Sel Punca untuk Aplikasi Klinis/Laboratories for Stem Cell Processing for Clinical Application
LRRK2	: Leucine-rich repeat kinase 2
LT-HSC	: Long-term-hematopoietic stem cell
LV	: Left ventricular
LVEF	: Left ventricular ejection fraction
MC	: Mononuclear cells
MCL-1	: Myeloid cell leukemia 1
M-CSF	: Macrophage colony-stimulating factor
MDA	: Malondialdehyde
MMP	: Matrix metalloproteinase
MMPs	: Matrix mettaloproteinases
MPC	: Mesenchymal precursor cell
MPCs	: Mesenchymal precursor cells
MPP	: Multipotent progenitor
MPTP	: 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine
MRI	: Magnetic Resonance Imaging
MSC	: Mesenchymal Stem Cells or Mesenchymal Stromal Cells

MSC-CM	: Mesenchymal Stem Cell-Conditioned Medium
MSCs	: Mesenchymal stem cells
NADPH	: Nicotinamide adenine dinucleotide phosphate
NE	: Neutrophil elastase
NeuN	: Neuronal nuclei
NF-kappaB	: Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	: Nerve growth factor
Ngn1	: Neurogenin 1
Ngn2	: Neurogenin 2
NgR	: Nogo66 receptor
INI	: National Institutes of Health
NK	: Natural killer
NO	: Nitric oxide
NPCs	: Neuronal precursor cells
NQO1	: NAD(P)H dehydrogenase quinone 1
NSC	: Neural stem cell
NSCs	: Neural stem cells
OB	: Olfactory bulb
OPCs	: Oligodendrocyte progenitor cells
PBMC	: Peripheral blood mononuclear cells
PBS	: Phosphate buffered saline
PCI	: Percutaneous coronary intervention
PD	: Parkinson's disease
PDGF	: Platelet-derived growth factor
PDT	: Population doubling time
PL	: Platelet lysate
PLA	: Processed lipoaspirate
PLM	: Posterior lateral plate of mesoderm

PLM : Posterior lateral mesoderm
P-MSCs : Placenta mesenchymal stem cells
Prdx : Peroxiredoxin
PRKN : Parkin proteins
PRP : Platelet-rich plasma
PSA-NCAM : Polysialylated neural cell adhesion molecule
RA : Retinoic acid
RA : Rheumatoid arthritis
RANTES : Regulated upon activation, normal T cell expressed and presumably secreted
RAR : Retinoic acid receptor
RBC : Red blood cells
ROS : Reactive oxygen species
RTT : Rett syndrome
RWMA : Regional wall motion abnormality
RWMAs : Regional wall motion abnormalities
SC : Stem cell
SCF : Stem cell factor
SCI : Spinal cord injury
SCNTs : Somatic cell nuclear transfers
SCs : Stem cells
SDF : Stromal cell-derived factor
SDF-1α : Stromal cell-derived factor-1 alpha
SGZ : Subgranular zone
SHH : Sonic hedgehog protein
SNCA : Alpha-synuclein
SOD : Superoxide dismutase
STEMI : ST-segment elevation myocardial infarction
ST-HSC : Short-term hematopoietic stem cell

SVF	: Stromal vascular fraction
SVZ	: Subventricular zone
TALEN	: Transcription activator-like effector nucleases
TDP-43	: TAR DNA-binding protein 43
TEWL	: Transepidermal water loss
TGF	: Transforming growth factor
TGF beta	: Transforming growth factor beta
TGF-B	: Transforming growth factor Beta
TGFα	: Transforming growth factor-alpha
TGF-β	: Transforming growth factor beta
TGF-β1	: Transforming growth factor beta 1
TIMP	: Tissue inhibitor metalloproteinase
TIMP-1 and TIMP-2	: Tissue inhibitors of metalloproteinases 1 and 2
TNF	: Tumor necrosis factor
TNF-α	: Tumor necrosis factor-alpha
TORCH	: Toxoplasmosis, Other (such as syphilis), Rubella, Cytomegalovirus, and Herpes simplex virus
TPHA	: Treponema pallidum hemagglutination assay
TPO	: Thrombopoietin
TSPCs	: Tendon stem/progenitor cells
Txn	: Thioredoxin
UC	: Umbilical cord
UCB-CM	: Umbilical Cord Blood-Conditioned Medium
UCB-MSC	: Umbilical cord blood-mesenchymal stem cell
UCB-MSCs	: Umbilical cord blood mesenchymal stem cells
UC-MSC	: Umbilical cord-mesenchymal stem cell
UC-MSCs	: Umbilical cord mesenchymal stem cells
UCSC-CM	: Umbilical cord stem cell-conditioned medium

UPN	: Universitas Pembangunan Nasional
USD	: United States dollar
UV	: Ultraviolet
UV-A	: Ultraviolet A
UV-B	: Ultraviolet B
UV-C	: Ultraviolet C
UVR	: Ultraviolet radiation
VEGF	: Vascular endothelial growth factor
VLCFA	: Very long chain fatty acids
WHO	: World Health Organization
WMSI	: Wall motion score index
WNT	: Wingless and INT-1

Glossary

Acute Myocardial Infarction (AMI)	:	a medical condition characterized by the sudden blockage of blood flow to a part of the heart, causing heart muscle damage or death.
adult and somatic stem cells	:	found in various tissues, these cells are crucial for tissue repair and regeneration and pose unique ethical considerations in research.
adult stem cells	:	stem cells that can be isolated from various adult tissues, such as bone marrow, adipose tissue, dental pulp, hair bulb, and mobilized peripheral blood, as well as partition waste, such as umbilical cord tissue, umbilical cord blood, amnion or placenta

adverse event	:	any unfavorable or unintended disease, sign, or symptom (including an abnormal laboratory finding) that is temporally associated with the use of a medical treatment or procedure, and that may or may not be considered related to the medical treatment or procedure
adverse left ventricular	:	unfavorable conditions or dysfunction in the left ventricle
aging	:	(1) the natural and irreversible process characterized by the gradual decline in cellular and organ function over time; (2) the progressive physiological changes in an organism that lead to senescence, or a decline of biological functions and of the organism's ability to adapt to metabolic stress
agonists	:	a drug or other substance that combines with a receptor to produce a specific physiological effect
akinesis	:	loss of absence of normal muscle movement
aliquot	:	fraction
allogeneic	:	transplantation or use of cells, tissues, or organs donated from a genetically different individual of the same species
amniotic fluid	:	the clear or slightly yellow fluid that surrounds and protects an unborn baby as it grows during pregnancy
amphotericin B	:	generic name of an antimycotic agent

angiogenesis	:	the formation of new blood vessels, potentially enhancing blood supply to infarcted areas, is a crucial process in wound healing and tissue repair, mediated by factors such as the vascular endothelial growth factor (VEGF).
angiogenic	:	of or relating to the formation of vessels, esp. blood vessels
angiopoietin-1	:	a glycoprotein that activates its Tie2 receptor by inducing tyrosine phosphorylation. It promotes vessel maturation and stability
anti-aging	:	the process to tending to prevent or lessen the effects of aging
antibiotics	:	substances that can inhibit the growth or kill bacteria
antifibrotic	:	substances or treatments that inhibit or reduce the formation of fibrous tissue in organs or tissues
antimycotics	:	substances against yeast and fungi
antioxidant activity	:	MSCs' ability to enhance levels of endogenous antioxidants, protecting cells against oxidative stress.
apoptosis	:	a genetically directed process of cell self-destruction that is marked by the fragmentation of nuclear DNA, is activated either by the presence of a stimulus or removal of a suppressing agent or stimulus, is a normal physiological process eliminating DNA-damaged, superfluous, or unwanted cells, and when halted (as by gene mutation) may result in uncontrolled cell growth and tumor formation.

apoptotic	:	the process of programmed cell death, a controlled and orderly mechanism crucial for various physiological functions in multicellular organisms.
articular cartilage defect	:	damage to the cartilage that leads to poor vascularization and healing potential, potentially developing into end-stage arthritis.
aseptic technique	:	procedures used to prevent contamination by pathogens.
astrocytes	:	star-shaped glial cells in the brain and spinal cord that perform various functions, including biochemical support of endothelial cells that form the blood-brain barrier, provision of nutrients to nervous tissue, and repair and scarring process of the brain and spinal cord following traumatic injuries.
athropy	:	decrease in size or wasting away of a body part or tissue
autoclave	:	an equipment to do wet sterilization
autologous	:	(1) the use of one's own cells, tissues, or organs for medical procedures; (2) derived from the same individual; involving one individual as both donor and recipient.

autophagy	:	(1) a cellular process for recycling damaged cell parts, which can become abnormal in a hyperglycemic environment, affecting cellular functions and homeostasis. (2) the biological process that involves the enzymatic breakdown of a cell's cytoplasm or cytoplasmic components (such as damaged or unneeded organelles or proteins) within the lysosomes of the same cell
avascular	:	tissues or organs lacking blood vessels.
Avascular Necrosis of the Femoral Head	:	an orthopaedic disorder characterized by the disruption of blood vessels leading to necrosis of the subchondral bone, resulting in femoral head necrosis.
bFGF (Basic Fibroblast Growth Factor)	:	a growth factor that enhances fibroblast proliferation, promotes angiogenesis, and collagen maturation during wound healing.
biomarkers	:	a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or a condition or disease
biomedical research	:	research that aims to understand human health and disease, which can lead to the development of new treatments.

bone marrow stem cell	:	adult stem cells obtained from bone marrow
bone morphogenetic protein	:	members of the transforming growth factor-beta (TGF-b) superfamily of proteins that play important roles in inducing bone and cartilage formation. BMPs are multi-functional growth factors that signal through serine/ threonine kinase receptors and have been shown to orchestrate tissue architecture by coordinating cell differentiation, proliferation, and apoptosis
Brachial Plexus Injury (BPI)	:	damage to the peripheral nerves resulting in paralysis of the upper extremities, often due to high-energy trauma.
Brachial Plexus Injury Chemokines	:	a large family of small, secreted proteins that signal through cell surface G protein-coupled heptahelical chemokine receptors. They are known for their ability to stimulate the migration of cells, particularly white blood cells (leukocytes).
BS	:	bio-safety cabinet
buffy coat	:	white ring that appears after whole blood is centrifuged, which is located between red blood cells and plasma
cardiac myocyte	:	a heart muscle cell responsible for contracting and pumping blood
cardiac regeneration	:	the process of replacing damaged heart tissue with new, healthy tissue

catalase	:	A red crystalline enzyme that consists of a protein complex with hematin groups and catalyzes the decomposition of hydrogen peroxide into water and oxygen
CD	:	cluster of differentiation
CD34+	:	transmembrane glycoprotein expressed on early lymphohematopoietic stem cells, progenitor cells, and endothelial cells
CD34+ Cells	:	a subset of hematopoietic stem cells characterized by the expression of the CD34 protein, involved in cell adhesion and signaling.
cell	:	the smallest unit of an organism, discovered by Robert Hooke in 1665.
cell differentiation	:	the process of stem cells becoming specialized cell types in their structures and function
cell factory	:	a system used for large-scale cell culture, consisting of multiple layers or chambers to increase surface area for cell growth
cell therapy	:	a treatment or a therapeutic approach involving the use of cells in which cellular material is injected into a patient; specifically, stem cell therapy is of interest due to its potential to regenerate damaged tissues
cellular apoptosis	:	programmed cell death, which can be exacerbated by diabetes, leading to cellular and tissue injury

central nervous system	:	It is made up of the brain and spinal cord. It is one of 2 parts of the nervous system. The other part is the peripheral nervous system, which consists of nerves that connect the brain and spinal cord to the rest of the body.
cGMP	:	current Good Manufacturing Practices
cGTP	:	current Good Tissue Practice
Chemokines	:	any of a group of cytokines produced by various cells (as at sites of inflammation) that stimulate chemotaxis in white blood cells (such as neutrophils and T cells)
chimera	:	an organism containing a mixture of genetically different tissues, formed by processes such as the fusion of early embryos, grafting, or mutation.
chronic inflammation	:	long-term inflammation, which can last for several months or even years, often resulting from failure to eliminate the cause of an acute inflammation or an autoimmune response to a self-antigen.
clinical trials	:	research studies involving human participants to evaluate the safety and efficacy of new medical treatments, interventions, or drugs
collagen	:	the main structural protein found in skin and other connective tissues or the extracellular matrix of various tissues, providing strength and elasticity to the skin. It is widely used in purified form for cosmetic surgical treatments
collagenase	:	an enzyme that digests collagen

Conditioned Medium (CM)	:	the culture medium containing the secreted factors from MSCs such as bioactive molecules, used in cell-free therapy for regenerative purposes
coronary artery disease	:	a condition especially one caused by atherosclerosis that reduces blood flow through the coronary arteries to the heart and typically results in chest pain or heart damage
coronary occlusion	:	blockage of coronary arteries, crucial vessels that supply the heart muscle with oxygen and nutrients
CPD	:	cumulative population doublings
CRISPR-Cas9	:	a gene editing technology that has transformative prospects for personalized medicine, used in stem cell advancements.
CRISPR-Cas9 Gene Editing	:	a powerful tool for editing genes within organisms with high precision, with implications for correcting genetic defects and improving health.
Critical-sized Bone Defects	:	defects that are larger than 2.5 times the bone's diameter, representing a significant challenge in orthopaedics due to the limited capacity of the fracture and its surrounding environment to heal optimally.
cryopreservation	:	the process of cooling and storing cells, tissues, or organs at very low temperatures (-196^{o}C) to preserve and maintain their viability
cryovials	:	vials to keep cells under very low temperatures (-196oC)

cumulative population doublings	:	the sum of population doublings of successive passages
cyclosporine	:	a drug with immunosuppressive properties used to prevent the rejection of grafts and transplants.
cytokine	:	secreted proteins released by cells or signaling molecules that have a specific effect on the interactions and play a crucial role in communications between cells, such as interferon, interleukin, and growth factors, which are secreted by certain cells of the immune system and have an effect on the other cell
cytokines	:	any of a class of immunoregulatory proteins (such as interleukin or interferon) that are secreted by cells, especially of the immune system
degenerative disc disease	:	deterioration of the discs in the spine, leading to inflammation and further degradation of tissue.
depression	:	a mood disorder that causes a persistent feeling of sadness and loss of interest. Also called major depressive disorder or clinical depression, it affects how you feel, think and behave and can lead to a variety of emotional and physical problems
dermal collagen staining	:	a method for assessing collagen content in skin tissue, often using Sirius Red dye.
dermis	:	a connective tissue layer of skin between the epidermis and subcutaneous tissue.

diabetes	:	a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.
Diabetes Mellitus	:	a disease in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood and urine.
diabetic complications	:	divided into microvascular (retinopathy, neuropathy, nephropathy) and macrovascular (coronary artery disease, peripheral artery disease, stroke) complications, arising from diabetes.
diabetic ulcer	:	a serious complication characterized by nonhealing wounds, often leading to minor and major amputations.
diamond concept	:	a concept introduced by Giannoudis that states four pillars must be addressed for optimal fracture healing, osteogenic cells, osteoconductive, osteoinductive, and mechanical stability.
differentiation	:	the process whereby a stem cell loses its capacity for self-renewal and becomes a mature and specialized cell type
Dimethyl Sulfoxide (DMSO)	:	a reagent of an organic compound usually used in cryopreservation medium to prevent ice crystal formation during cryopreservation.
dormant	:	not active or growing now, but having the ability to become active in the future:

drug discovery and toxicology	:	the processes of identifying new candidate medications and assessing their toxic effects on the body.
dysmyelination	:	an inborn error of metabolism affecting myelinogenesis that causes it to be abnormal, arrested, or delayed.
echocardiogram	:	a medical imaging test or an ultrasound imaging technique that uses sound waves to create a detailed picture of the heart's structure and function to view the heart, assess its function, and diagnose heart conditions
elastin	:	a protein in connective tissue that allows tissues to resume their shape after stretching or contracting.
electrocardiogram	:	a graphical recording of the heart's electrical activity
Embryonic Stem Cells (ESCs)	:	stem cells derived from early-stage embryos (the inner cell mass of a blastocyst), capable of differentiating into almost every cell type in every organ of the body, but these cells are controversial due to the ethical implications related to the moral status of embryos and their potential for harm
enzymatic method	:	a method for isolating cells by digesting the extracellular matrix and tissue structures using specific enzymes.
epidermis	:	the outermost of the three layers that comprise the skin

epidermis and dermis	:	the outermost and middle layers of the skin, respectively, each with distinct functions and cellular composition.
epigenetic landscape	:	a metaphor used to describe the process of cellular decision-making during development in which a cell can differentiate into various types.
Eppendorf tube	:	a small conical tube, usually can contain 1.5 ml of liquid/suspension
equitable access	:	the ethical principle that advancements in stem cell research and therapies should be available to all individuals, regardless of socioeconomic status.
ethical dilemmas	:	complex moral issues that arise in stem cell research, particularly concerning the use of embryonic stem cells, informed consent, and equitable access to therapies.
ethical dimensions	:	considerations related to morality, ethics, and values in the conduct of scientific research, especially concerning human subjects.
ethical guidelines	:	established principles designed to guide the conduct of research to ensure it is performed responsibly, respecting the dignity, rights, and welfare of participants.
ethical guidelines and best practices	:	frameworks developed by organizations like ISSCR, NIH, and WHO to address ethical complexities in stem cell research, covering informed consent, protection of research participants, and appropriate use of stem cell sources.

exosome	:	an extracellular vesicle that are released by cells in both physiological and pathological situations upon fusion of an intermediate endocytic compartment
exosomes	:	nano-sized vesicles released by cells, containing various biomolecules, such as proteins or nucleic acids, play a role in intercellular communication
explants method	:	a technique for cell culture where tissue pieces are placed in culture media to allow cells to migrate out and proliferate.
Extracellular Matrix (ECM)	:	fills spaces between cells and includes basement membranes. It is composed of fibrous proteins (e.g., collagen, elastin) and glycosaminoglycans. Provides support and anchorage for cells, serves as a medium for the diffusion exchange of nutrients and metabolites, and sequesters and releases various growth factors.
Extracellular Vesicles (EV)	:	particles released by MSCs containing proteins, lipids, and nucleic acids, involved in cell communication and regenerative processes.
fatwa	:	a formal ruling or interpretation on a point of Islamic law given by a qualified legal scholar.
FBS	:	fetal bovine serum
fetal bovine serum	:	serum that is taken from a bovine fetus and usually used as a supplement in a culture medium

fibroblast(s)	:	a connective-tissue cell of mesenchymal origin that secretes collagen proteins and other fibers from which the extracellular fibrillar matrix of connective tissue forms playing a crucial role in skin repair and regeneration.
fibroblast growth factor-2	:	a member of the fibroblast growth factor family that is bound to basement membranes (BM) of blood vessels; its proangiogenic actions can be activated by heparin sulfate-degrading enzymes, which causes it to be released from BMs
fibrous tissue	:	connective tissue that is composed mainly of fibers and is less differentiated than regenerative tissue
ficoll hypaque	:	a cell separation reagent as density gradient media used for isolating mononuclear cells from blood or bone marrow
free radicals	:	unstable molecules that can damage cells, contributing to aging and diseases.
fungizon	:	brand name of Amphotericin B
GABAergic Signals	:	signals related to the neurotransmitter gamma-aminobutyric acid (GABA), which is known for its inhibitory effect on neuron activity in the brain.
genome editing	:	a method of making specific changes to the DNA of a cell or organism.
Gentamycin	:	generic name of a broad-spectrum aminoglycoside antibiotic
glycine	:	an amino acid that is a key component of collagen.

Good Manufacturing Practices (GMP)	:	regulations enforced by the FDA that require that manufacturers, processors, and packagers of drugs, medical devices, some food, and blood take proactive steps to ensure the quality of drug products and medical interventions are safe, pure, and effective
Graft-versus-host disease (GVHD)	:	a condition in which the stem cells you receive during an allogeneic stem cell transplant view your body's cells as a threat and attack
Growth Factors	:	proteins secreted by MSCs that play roles in tissue regeneration, including Epidermal Growth Factor (EGF), Basic Fibroblast Growth Factor (bFGF), Transforming Growth Factor-beta (TGF-b) and Growth Differentiation Factor-11 (GDF-11) that involved in the wound healing process, influencing various phases of healing through inhibitory or stimulatory effects. It is a naturally occurring protein capable of stimulating cellular growth, proliferation, healing, and cellular differentiation
Halal Product Assurance	:	the guarantee that products comply with Islamic dietary laws and are permissible for consumption by Muslims.
hematopoietic	:	of, relating to, or involved in the formation of blood cells

Hematopoietic Stem Cells (HSCs)	:	multipotent stem cells, found in the bone marrow and blood, that can develop into all types of blood cells through the process of hematopoiesis, including myeloid-lineage and lymphoid-lineage cells
hemostasis	:	stoppage of the flow of blood
Hepatocyte Growth Factor	:	a protein that plays a significant role in stimulating cell growth, cell motility, and morphogenesis. It has been studied for its potential therapeutic applications in tissue repair and regeneration
hippocampus	:	a region of the brain associated with memory and learning, where adult neurogenesis is known to occur.
homeopathy	:	a system of treating diseases in which sick people are given very small amounts of natural substances that, in healthy people, would produce the same effects as the diseases produce
homeostasis	:	a relatively stable state of equilibrium or a tendency toward such a state between the different but interdependent elements or groups of elements of an organism, population, or group
homing	:	(1) the phenomenon whereby cells migrate to the organ of their origin; (2) the ability of stem cells to migrate toward areas of damage or inflammation.
HSCs	:	hematopoietic stem cells
huFN	:	human fibronectin

Human Embryonic Stem Cells (hESCs)	:	stem cells derived from early-stage human embryos, capable of differentiating into any cell type in the human body.
human induced Pluripotent Stem Cells (hiPSCs)	:	cells that have been genetically reprogrammed to an embryonic stem cell-like state, enabling them to differentiate into any cell type.
human leukocyte antigen	:	a group of proteins that are essential for the immune system to recognize foreign cells. They play a crucial role in immune response and are important in organ transplantation and autoimmune diseases
human pluripotent stem cells	:	particularly potent type of stem cell that normally only exists during early embryonic development
hyaluronidase	:	an enzyme that digests hyaluronic acid
hydroxyapatite	:	a naturally occurring mineral form of calcium apatite, the main mineral component of bone and teeth. It is often used as a biomaterial in bone tissue engineering due to its biocompatibility and osteoconductive properties
hyper flask	:	ten-layered flask with one opening
hyperglycemia	:	a condition of elevated blood sugar levels, contributing to microvascular and macrovascular disruptions and other complications in diabetes
hypertention	:	a medical condition characterized by high blood pressure, which can lead to increased stress on the arteries and various cardiovascular issues
hypodermis	:	the bottom layer of the skin

hypokinesis	:	a decreased or diminished ability of a part of the body, such as a muscle or the heart, to move or contract
Hypoxia	:	a condition that a deficiency in oxygen sufficient, characterized by a deficiency of oxygen in tissues, often leading to cellular dysfunction or damage, to enlist physiological mechanisms to correct the deficiency; occurs in normal and pathological states
Hypoxic	:	of, relating to, or affected with hypoxia: resulting from, causing, or experiencing inadequate levels of oxygen in the tissues and cells of the body
immunogenicity	:	immunogenic (relating to or producing an immune response) property or ability.
immunomodulation	:	the regulation of the immune system's response to modification of the immune response or the functioning of the immune system by the action of an immunomodulator and reduce inflammation or stimulate healing
immunomodulatory	:	that modifies the function of the immune system
in vitro	:	experiments or processes conducted outside a living organism, typically in a laboratory setting, taking place outside a living organism
in vivo	:	experiments or processes conducted within a living organism, providing a real-life context for scientific studies, taking place inside a living organism

Induced Pluripotent Stem Cells (iPSCs)	:	(1) a type of pluripotent stem cell that can be generated directly from adult somatic cells, capable of developing into most, if not all, of the 200 cell types of the body offering an alternative to embryonic stem cells (ESCs) without the associated ethical concerns; (2) adult cells that have been genetically reprogrammed to an embryonic stem cell-like state; (3) cells generated from adult tissues that have been reprogrammed back into an embryonic-like pluripotent state, offering a potential ethical alternative to ESCs.
infarct	:	a localized area of tissue damage or cell death, typically caused by a lack of blood supply
inflammation	:	body's protective response to injury or infection
informed consent	:	(1) a crucial ethical requirement in research where participants are fully informed about the study's purposes, risks, and benefits, and consent to participation freely; (2) fundamental to ethical research, ensuring participants are fully aware of the risks, benefits, and potential outcomes of a study, especially crucial in stem cell research.

insulin resistance	:	reduced sensitivity to insulin by the body's insulin-dependent processes (such as glucose uptake and lipolysis) that is typical of type 2 diabetes but often occurs in the absence of diabetes
Insulin-like Growth Factor	:	a hormone with structural similarities to insulin. It plays a key role in cell growth, proliferation, and differentiation, and has been investigated for its potential in tissue regeneration and repair
interleukin	:	signaling proteins that regulate immune responses and communication between cells
interleukin	:	one of a group of related proteins made by leukocytes (white blood cells) and other cells in the body that regulate immune responses
International Society for Cell Therapy (ISCT)	:	an international society dedicated to advancing the science, technology, and application of cellular therapies.
intracoronary	:	medical procedures or interventions taking place within the coronary arteries of the heart
intracoronary administration	:	a method of delivering therapies directly into the coronary arteries.
intramyocardial	:	procedures or interventions conducted within the muscular tissue of the heart
intravenous	:	(1) administration of substances directly into a vein; (2) situated, performed, or occurring within or entering by way of a vein.

intravenous administration	:	a method of delivering therapies into the body through the veins.
ischemia	:	a condition in which blood supply to a particular area of the body is reduced, potentially leading to tissue damages
ISCT	:	International Society for Cell and Gene Therapy
kanamycin	:	generic name of an aminoglycoside antibiotics
keratinocytes	:	the primary cell type of the epidermis that is responsible for forming the protective barrier of the skin and produces keratin. It is formed in the basal epidermal layer above the dermis, divides to produce more keratinocytes, and ultimately migrates into the outer protective layers of the skin and undergoes final differentiation into a corneocyte
Langerhans Cells	:	a pancreatic cell that produces hormones (e.g., insulin and glucagon) that are secreted into the bloodstream
left ventricular	:	the left lower chamber of the heart is responsible for pumping blood into the systemic circulation
Left Ventricular Ejection Fraction (LVEF)	:	a measurement of the percentage of blood pumped out of the left ventricle with each heartbeat or contraction, used as an indicator of heart function.
Leukapheresis	:	a procedure healthcare providers use to remove white blood cells from blood
Leukoplakin	:	a thick white patches on the inside surfaces of the mouth

Ligament and Tendon Healing	:	the process of repairing injuries to tendons and ligaments, which is challenged by inadequate vascularization and the tendency to form mechanically weaker scar tissue.
Lymphoprep™	:	cell separation reagent
Macrophages	:	a large white blood cell in the immune system that destroys bacteria and other harmful substances
Macrovascular	:	of, relating to the large blood vessels, esp. the aorta and its branches or the coronary arteries or both
Magnetic Resonance Imaging	:	a medical imaging technique that uses strong magnetic fields and radio waves to generate detailed images of the internal structures of the body
Malondialdehyde	:	highly reactive three-carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism
Matrix Metalloproteinase Protein	:	a family of enzymes involved in the breakdown of extracellular matrix components. They play a role in tissue remodeling and have implications in various pathological conditions, as well as in tissue engineering and regenerative medicine
melanocytes	:	cells in the epidermis of the skin and eyes that produce and contain melanin, the pigment responsible for skin color
mesenchymal	:	cells that develop into connective tissue, blood vessels, and lymphatic tissue

Mesenchymal Stem Cells (MSCs)	:	a type of adult stem cell or multipotent stromal cells or multipotent stem cells or a progenitor cells found in various tissues like bone marrow, skin, fat tissue, etc. They capable of differentiating into a variety of cell types in mesoderm-derived forming connective tissue, e.g. chondrocytes (cartilage cells), osteoblasts (bone cells), myocytes (muscle cells), and adipocytes (fat cells) with properties beneficial for skin rejuvenation. including bone, cartilage, muscle, and fat cells. It is known for its regenerative properties and immunomodulatory effects, secretion of growth factors, and promotion of angiogenesis. It is playing a crucial role in stem cell-based therapy for diabetes and wound healing, and commonly used in treating orthopaedic disorders
mesoderm	:	the middle of the three primary germ layers of an embryo that is the source of many bodily tissues and structures
microneedling	:	a cosmetic procedure that involves creating micro-injuries on the skin surface to stimulate skin repair and rejuvenation, used in conjunction with MSC-CM for enhanced effects.
microvascular	:	of, relating to, or constituting the part of the circulatory system made up of minute vessels (such as venules or capillaries) that average less than 0.3 millimeters in diameter

microvesicle	:	represent a more heterogeneous population than exosomes, typically ranging in size between 100 and 1000 nm in diameter
mitochondrial	:	of, relating to, or of the nature of a mitochondrion or mitochondria.
MNCs	:	mononuclear cells
mononuclear cells	:	cells that have one nucleus
Morbidity	:	the incidence of disease: the rate of illness (as in a specified population or group)
Morphogens	:	substances that govern the pattern of tissue development in the process of morphogenesis, guiding the development of tissues and organs.
Mortality	:	(1) the number of deaths in a population during a given time or place; (2) the state of being subject to death or the rate of deaths in a population, often expressed as a percentage or per unit of population over a specific time.
Motility	:	the ability of plants, organisms, and very small forms of life to be able to move by themselves
Mr. Frosty	:	a device to keep cells and to freeze cells gradually in a freezer
MSCs	:	mesenchymal stem cell
multi flask	:	multiple layered flasks with one opening

Multipotent Stem Cells	:	stem cells that can differentiate into more than one germ layer but not all three or cells that can develop into more than one cell type but are more limited than pluripotent cells. Examples: adult stem cells and blood stem cells, specifically mesenchymal stem cells (MSCs), which are pivotal in orthopaedic applications.
Mycostatin	:	brand name of Nystatin
myeloid	:	tissue of bone marrow
Myocardial Infarction	:	the death of heart muscle tissue due to lack of blood supply
Myocyte	:	a muscle cell, the basic structural and functional unit of muscle tissue
Necrosis	:	type of cell death is characterized by the premature death of cells in living tissue, often resulting from injury, infection, or disease
Nephropathy	:	an abnormal state of the kidney
Neural Stem Cells (NSCs)	:	stem cells located in the brain that can differentiate into various types of neural cells, including neurons and glial cells.
Neuroactive compounds	:	induce network-wide effects including gamma-aminobutyric acid, glutamate, serotonin, and adenosine, as well as cytokines, among others. These network-wide effects can exert major influences on network function in sleep states, learning disorders, and central nervous system (CNS) disorders.

Neuroblasts	:	immature nerve cells that have the potential to develop into mature nerve cells or neurons.
Neurodevelopmental and Neurodegenerative Diseases	:	disorders or conditions that affect the development and function of the nervous system, characterized by the progressive degeneration of the structure and function of the nervous system
Neurogenesis	:	the process of generating or forming new neurons from stem cells in the brain
Neurokinetic	:	relationship between neural activity and muscle movement
Neuronal Regeneration	:	the process by which neurons are renewed, restored, or regenerated in the nervous system.
Neuropathy	:	damage, disease, or dysfunction of one or more nerves especially of the peripheral nervous system that is typically marked by burning or shooting pain, numbness, tingling, or muscle weakness or atrophy, is often degenerative, and is usually caused by injury, infection, disease, drugs, toxins, or vitamin deficiency
Neurotoxic	:	the direct or indirect effect of chemicals that disrupt the nervous system.
Normoxic	:	normal oxygen levels in tissue culture flasks
Nuclear Reprogramming	:	the process by which differentiated cells are induced to return to an undifferentiated, pluripotent state.

NUNC™ EasyFill™ Cell Factory™	:	ten layered culture vessel with two openings that is developed by Thermo Fisher Scientific
Nystatin	:	generic name of an antifungal agent
Oligodendrocytes	:	a type of glial cell in the central nervous system responsible for producing myelin, which insulates nerve cell axons to increase the speed at which information travels from one nerve cell to another.
Organoid Development	:	the process of creating three-dimensional mini-organs from stem cells in vitro for research and studying on human organ development and disease, raising ethical questions about the nature of these entities, their status, and potential functions
Osteoconductive Pillars	:	scaffolds, specifically the extracellular matrix, which supports the migration and adhesion of osteoinductive and osteogenic cells at the fracture site, crucial for fracture healing.
Oxidative Stress	:	(1) physiological stress on the body that is caused by the cumulative damage done by free radicals inadequately neutralized by antioxidants and that is held to be associated with aging; (2) an imbalance between free radicals and antioxidants in the body, leading to cell damage and contributing to aging; (3) increased production of reactive oxygen species (ROS), leading to cellular damage and playing a role in the development of diabetic complications.

Paracrine Activity/ Signaling	:	communication or cell signaling in which a cell produces signaling molecules between cells (MSCs) where signals affect nearby cells through the release of secreted factors, influencing tissue repair and regeneration.
Penicillin	:	generic name of antibiotics that are produce by certain blue molds
Percutaneous Coronary Intervention	:	a medical procedure used to treat coronary artery disease by opening narrowed or blocked coronary arteries. It typically involves the use of a catheter and may include techniques such as angioplasty and stent placement
Peripheral Artery Disease	:	damage to or dysfunction of the arteries outside the heart resulting in reduced blood flow, a common complication in diabetes associated with diabetic peripheral nerves, leading to nonhealing foot ulcers
Phosphate Buffered Saline (PBS)	:	a buffer solution commonly used in biological research.
Photoaging	:	(1) premature aging of the skin caused by repeated exposure to ultraviolet radiation, primarily from the sun, characterized by wrinkles, uneven skin texture, and decreased elasticity; (2) the cumulative detrimental effects (such as wrinkles or dark spots) on skin that result from long-term exposure to sunlight and especially ultraviolet light
Placebo	:	inactive substance or treatment that resembles a real medical intervention

Plasticity	:	(1) the ability of stem cells to cross lineage boundaries and differentiate into unrelated cell types; (2) a phenomenon used to describe a cell that is capable of becoming a specialized cell type of different tissue
Platelets	:	a very small cell in the blood that makes it thicker and more solid to stop bleeding caused by an injury
Pluripotency (noun)	:	the ability of a stem cell to develop into nearly any type of cell in the body, a characteristic or a unique feature of embryonic stem cells and iPSCs that allows them to transform into various cell types, offering significant potential for regenerative medicine and disease research
Pluripotent (adj.)	:	the ability of a stem cell to develop into all types of cells in the body
Pluripotent Stem Cells	:	cells that can give rise to almost all types of cells and transform into three embryonic layers, endoderm, and mesoderm, and ectoderm in the body but not an entire organism. They originate from embryonic cells after the blastocyst phase.
Polymyxin B sulfate	:	generic name of polypeptide bactericidal antibiotics
population doubling time	:	the time that is required for certain cells to double their population

Prevalence	:	the number of cases of disease, injury or illness present in a defined population during a particular point in time
Progenitor cell	:	a transitional form of stem cell that are derived from stem cells and have the ability to differentiate into a specific type of cell, can no longer renew itself, cannot divide and reproduce indefinitely
Prognosis	:	anticipated course or outcome of a medical condition, indicating the likelihood of recovery or progression based on various factors
Proliferate	:	multiply or increase rapidly in number
Proliferation	:	(1) an increase in the number of cells due to cellular growth and cell division; (2) expansion of the number of cells by the continuous division of single cells into two identical daughter cells; (3) to grow by rapid production of new parts, cells, buds, or offspring; to increase in number as if by proliferating
Prophylactic	:	guarding from or preventing the spread or occurrence of disease or infection
Proteinopathies	:	a family of diseases caused by misfolded proteins and featured by the presence of aberrant protein aggregates in the cell.
Public Health and Cultural Sensitivity	:	in contexts like Indonesia, balancing individual rights with collective well-being and respecting cultural and religious values in medical decision-making.

Quantum Bioreactor	:	a system of culture vessel, which contains hollow fibers, for expanding cells to enable a continuous flow of culture medium in a controlled environment
Regeneration	:	the process of renewal, restoration, and growth that makes genomes, cells, organisms, and ecosystems resilient to natural fluctuations or events that cause disturbance or damage.
Regenerative Medicine	:	a field of medical research focused on developing methods to regrow, repair, or replace damaged or diseased cells, organs, or tissues, utilizing the capabilities of stem cells
Regenerative Therapies	:	medical treatments that restore lost, damaged, or aging cells and tissues in the human body, often utilizing stem cells.
Regional Wall Motion Abnormalities (RWMA)	:	(1) changes in the movement of the heart's walls during the cardiac cycle, often indicative of underlying heart muscle damage or dysfunction (2) localized impairment in the movement of a specific area of the heart muscle
Regulation cells	:	any process that controls the series of events by which a cell goes through the cell cycle
Reperfusion	:	the restoration of normal blood flow to an area previously experiencing ischemia or infarction

Retinopathy	:	any of various noninflammatory disorders of the retina including some that cause blindness
Secretome	:	bioactive molecules or substances secreted by cells into the extracellular space, including proteins, peptides, lipids, and other molecules, like cytokines, chemokines, and growth factors, that mediate intercellular communication and are involved in tissue repair and regeneration
Sedentary	:	not physically active
Self-renewal	:	the process by which stem cells divide to make more stem cells, perpetuating the stem cell pool throughout life
Six-Minute Walk Test (6MWT)	:	a simple exercise test that measures the distance an individual can walk on a flat, hard surface in six minutes, used to assess functional capacity and response to treatment in heart failure patients.
Skin Aging	:	(1) a complex, inevitable process influenced by intrinsic and extrinsic factors, including ultraviolet radiation, leading to photoaging (2) changes in skin appearance and function due to both intrinsic (genetic, hormonal) and extrinsic (UV exposure, pollution) factors
Somatic Cell Nuclear Transfer (SCNT)	:	a laboratory strategy for creating a viable embryo from a body cell and an egg cell.
Somatic Cells	:	any cell of a living organism other than the reproductive cells.

Somatic Stem Cells	:	also known as adult stem cells, these are found in various tissues of the body and can regenerate into different cell types from their originating organ.
spinner bioreactor	:	a culture vessel, which has impellers, to enable stirring or spinning motion
ST-Elevation Acute Myocardial Infarction	:	a type of heart attack characterized by ST-segment elevation on the electrocardiogram, indicating significant myocardial damage
Stem Cell Research	:	(1) the study of stem cells aimed at understanding their properties and potential applications in medicine, including regenerative therapies and disease treatments (2) a groundbreaking field with the potential to revolutionize medicine and biotechnology through regenerative therapies and disease treatments
Stem Cell Therapy	:	a regenerative medicine or treatment approach involving the use of stem cells to repair or replace damaged cells, tissues, and organs
Stem Cell(s)	:	undifferentiated cells with the potential to develop into many different types of cells in the body (blood cells, nerve cells, muscle cells, etc.) that capable of dividing, self-renewing, and differentiating into various types of body cells. They serve as a repair system for the body and play a crucial role in regenerative medicine, especially in orthopaedics for treating bone abnormalities and regenerating nerves, tendons, ligaments, and cartilage

Streptomycin	:	generic name of an aminoglycoside antibiotic
Stromal cells	:	a highly heterogeneous class of connective tissue cells that build the infrastructure of any organ and fulfill a variety of fundamental roles in health and disease
Subcutaneous Administration	:	the method of delivering substances into the fatty tissue layer beneath the skin.
Subcutaneous Injection	:	a method of administering MSCs or MSC-CM into the fatty tissue beneath the skin for therapeutic purposes.
Subventricular Zone (SVZ) and Dentate Gyrus (DG)	:	regions in the brain known for being neurogenic zones, where the generation of new neurons (neurogenesis) occurs throughout life.
T150	:	culture vessel or flask that has 150 cm2 surface area
T175	:	culture vessel or flask that has 175 cm2 surface area
T225	:	culture vessel or flask that has 225 cm2 surface area
T25	:	culture vessel or flask that has 25 cm2 surface area
T75	:	culture vessel or flask that has 75 cm2 surface area
Teratoma Formation	:	a potential risk of stem cell therapies where the implanted stem cells develop into a type of tumor that can contain various tissues, including hair, muscle, and bone.

Thrombosis	:	formation of a blood clot within a blood vessel, which can obstruct blood flow and lead to various health issues
Tissue Engineering	:	a field of biomedical engineering that creates complex human tissues or organs using stem cells and scaffold materials, that use a combination of cells, engineering, materials methods, and suitable biochemical factors to restore, maintain, improve, or replace different types of biological tissues, and raising ethical questions about the moral status of these engineered tissues and the potential for commodification
Totipotent Stem Cells	:	stem cells that can differentiate into any type of human cell, including placental and extraembryonic cells or cells with the potential to develop into a whole organism. However, their use in clinical practice has been limited due to ethical concerns.
Transcription Factors	:	proteins that help turn specific genes on or off by binding to nearby DNA.
Transdifferentiation	:	the process by which one differentiated cell type transforms into another cell type without first becoming a pluripotent stem cell.
Transepidermal Water Loss (TEWL)	:	the amount of water that passively evaporates through skin to the external environment due to water vapor pressure gradient on both sides of the skin barrier

Transforming Growth Factor (TGF)	:	a member of a family of polypeptide growth factors, which together with their receptors are involved in the induction of neural tissue from ectoderm
Transforming growth factor-beta (TGFβ)	:	a multifunctional cytokine that exists in three subtypes in humans (TGFβ1, TGFβ2, TGFβ3), involved in various cellular processes, can induce transformation of some cell types, including cell growth and differentiation, embryonic development, regulation of the immune system, and tissue regeneration. It plays a crucial role in tissue homeostasis and has been studied for its potential therapeutic applications in regenerative medicine
Triple flask	:	triple layered culture flask with one opening
TrypLE Select	:	an enzyme used for cell dissociation; considered to be gentler than trypsin.
trypsin	:	an enzyme that can digest proteins
Tumor Necrosis Factor (TNF)	:	proinflammatory cytokine that is produced by white blood cells (monocytes and macrophages); has an antineoplastic effect but causes inflammation (as in rheumatoid arthritis)
Ulcer	:	a break in the skin or mucous membrane with loss of surface tissue, disintegration and necrosis of epithelial tissue, and often pus

Ultraviolet (UV) Light	:	a type of electromagnetic radiation with wavelengths shorter than visible light, classified into UV-A, UV-B, and UV-C rays.
Umbilical cord blood stem cells	:	stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body (hematopoietic)
Umbilical Cord Mesenchymal Stem Cell	:	stem cells derived from the umbilical cord tissue. These cells have shown promise in various regenerative medicine applications due to their multipotency and immunomodulatory properties
Unipotent Stem Cells	:	cells that can differentiate into only one cell type
Vascular Endothelial Growth Factor	:	a signaling protein that stimulates the formation of blood vessels. It is crucial for angiogenesis and has been studied for its potential therapeutic applications in promoting vascularization and tissue regeneration
VEGF (Vascular Endothelial Growth Factor)	:	a growth factor known for its angiogenic properties, promoting blood vessel formation and improving blood metabolism in wounded tissues.
VEGF-A	:	vascular endothelial growth factor type A (also called VPF, vascular permeability factor) is a key proangiogenic growth factor

VEGF-C	:	vascular endothelial growth factor type C induces selective hyperplasia of the lymphatic vasculature, i.e., causes lymphangiogenesis. Overexpression of VEGF-C in the skin of transgenic mice results in lymphatic (but not vascular) endothelial proliferation and vessel enlargement
Wall Motion Score Index (WMSI)	:	a quantitative measure of regional heart wall motion abnormalities, used in cardiology to assess and quantify the overall function and movement of the heart's walls. and to assess the severity of cardiac muscle damage.
Wharton's jelly	:	a soft connective tissue that consists of large stellate fibroblasts and a few wandering cells and macrophages embedded in a homogeneous jelly-like intercellular substance or material at the center of an umbilical cord
Wistar	:	an albino rat widely used in biological and medical research
Xenografts	:	a graft of tissue taken from a donor of one species and grafted into a recipient of another species

About the Editors

Basuki Supartono

Basuki Supartono was born in Jakarta. He completed his secondary education at SMA Negeri 8 Jakarta (1980), earned his Bachelor's degree in Psychology from UI (1983), graduated as a General Practitioner from FK Unair (1989), specialized in Orthopaedics and Trauma at FK UI (2000), obtained a Master's in Hospital Administration from FKM UI (2006), and earned his Doctorate from FK UI (2013) with honors as the best PhD graduate. Since 1990, he has held the rank of Senior Doctor (IVe)/Principal. He also served as the Expert Staff to the Minister of Youth and Sports of the Republic of Indonesia and as the Director of the National Sports Hospital (RSON). His expertise is in the musculoskeletal field. He has served as the Vice Dean II of the Faculty of Medicine at UPN Veteran Jakarta and currently chairs the Senate of the same university.

He teaches undergraduate and master degrees program at UPN Veteran Jakarta, master degree program at UI and ITB, and PhD program at UI. He actively conducts research and publishes scientific papers. He serves as a reviewer for the Heliyon Journal, the Wijaya Kusuma Scientific Journal, and an editor for the IRCHum Journal and BRIN Publishing (this book). He is a member of IDI, PABOI, ISAKOS, and the Health Research Ethics Committee. Satya Lencana Karya Satya XX, Satya Lencana Kebaktian Sosial for Disaster Relief Efforts during the Tsunami in NAD/North Sumatra Province from the President of the Republic of Indonesia, Humanitarian Award for Health Services in Maluku and North Maluku Provinces from the Minister of Health of the Republic of Indonesia, Recognition from the Minister of Youth and Sports of the Republic of Indonesia for his dedication, service, and achievements as the Director of RSON and in the development of RSON, Satya Lencana Karya Satya XXX. Scientific Awards: The Best Poster at the 20th National Congress of Indonesian Orthopaedic Association, Jakarta 2016; The 3rd Winner for Oral Presentation in National Symposium and Workshop Stem Cell for The Future Medicine from Basic to Clinic, Surabaya 2017. Email: basuki@upnvj@ac.id

Ariyani Noviantari

Ariyani Noviantari is a researcher at The Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency (BRIN) since March 2022. Between March 2010 and February 2022, she worked as a researcher at the Center for Research and Development of Biomedical and Basic Health Technology, Health Policy Agency (Badan Kebijakan Pembangunan Kesehatan—BKPK), Ministry of Health, previously known as National Institute of Health Research and Development (Balitbangkes), Ministry of Health of Indonesia. Before this, she worked as a Microbiologist in PT. Universal

Robina Corporation (URC) Indonesia (Jack 'n Jill) (2005–2007) and GlaxoSmithKline Indonesia (2007–2010). She is identified by her Scopus ID 57208311735, Orcid ID 0000-0001-7852-6983, and Sinta ID 6630099. She finished her undergraduate studies with cum laude predicate at the Faculty of Biology, Universitas Gadjah Mada (UGM). She then pursued her master's degree at the Master Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia. She is currently obtaining a doctorate at the Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia.

Noviantari is a member of Perhimpunan Periset Indonesia, formerly known as the Himpunan Peneliti Indonesia (Himpenindo), the Indonesian Medical Biology Association (Perkumpulan Biologi Medik Indonesia—PBMI), and worked as the Editorial Board of the Indonesian Biotech Medisiana Journal (Jurnal Biotek Medisiana Indonesia—JBMI) (2018–2022). She received the award for Best Moderated Poster at the 4th Annual International Conference and Exhibition on Indonesian Medical Education and Research Institute (ICE on IMERI) in 2019 and Satyalancana Karya Satya 10 years award. Additionally, she received the award as The Best and Most Productive Writer from Widina Media Utama Publishers. She has published books and articles in various national and international scientific publications and proceedings. Email: ariy007@brin.go.id

About the Authors

Ahmad Faried

Ahmad Faried currently works as the head of the study program at the Department of Neurosurgery and Oncology and Stem Cell Working Group, Faculty of Medicine, Universitas Padjadjaran-Dr. Hasan Sadikin Hospital, Bandung, Indonesia. He completed his PhD in Medicine at Gunma University, Maebashi, Japan, under the supervision of Prof. Hiroyuki Kuwano and Prof. Hiroyuki Kato. He received his post-doctoral grant from JSPS Japan at the same university. He continued his clinical fellowship in Department of Neurosurgery at the University of Tokyo, Japan, under the supervision of Prof. Nobuhito Saito and Prof. Hideaki Imai.

He is a neurosurgeon with cell biology as his expertise. He has a great deal of interest in neuroscience research such as neurotrauma, brain tumour malignancy, brain microvessel endothelial cells, placenta stem cells, neural stem cells, iPSC, cancer stem cells, neurosurgery, biomedical engineering, and the medical information communication and technology (ICT); especially instrumentation as well as telemedicine and integrated medical services using cloud computing system.

He received many awards in both professional and academic and industry. Among them are Innovation Award II-2018 from Indonesian Health Care Forum, Jakarta; the Young Neurosurgeon Award 2017 from the World Federation of Neurosurgical Societies in the World Congress 2017, Istanbul, Turkey; The National Outstanding Lecturer and Education Personnel Year 2012 from the Indonesian Ministry of Higher Education, Research Technology; and Ristek-Kalbe Science Award 2010 from Kalbe Pharmaceutical Company supported by Indonesian Ministry Research and Technology. He is a member of the Indonesian Young Academy of Sciences (Akademi Ilmuan Muda Indonesia, ALMI)

Ahmad Faried is also a key participant in the Konsorsium Pengembangan Sel Punca and serves on the Adhoc Team for Health Minister Regulations (Tim Adhoc Peraturan Menteri Kesehatan–Permenkes). He is actively involved in The Indonesian Association of Tissue Engineering and Cell Therapy (REJASELINDO) and contributes his expertise to the organization, specializing in application of stem cell therapy. Additionally, he also a Board Certified and as a member of the American Board of Regenerative Medicine (ABRM) and Head of Sub-Commission II, National Committee for Stem Cell and Cell Development, Ministry of Health of The Republic of Indonesia. Email: ahmad.faried@unpad.ac.id

Aisha Aziz

Aisha Aziz is an Assistant Professor, Pharmacology, Faculty of Pharmacy, Jinnah University for Women. She received her PhD in Pharmacology in 2019. Her research was on the in vitro screening of acetamide and benzimidazole derivatives on breast cancer cell lines. She has been trained in various in vitro and in vivo research techniques. Her research experience includes in vitro screening of novel drugs and nanoparticles on cancer cell lines, such as breast cancer, glioblastoma, and lung cancer cell lines. Aisha Aziz has been supervising more than 10 MPhil students and is currently working on in vivo and in vitro models of neurodegenerative diseases, inflammation, and drug-induced toxicity. Email: dr.aishaaziz@live.com

Angliana

Angliana pursued her doctoral studies at the Faculty of Pharmacy, Universitas Padjajaran. Her groundbreaking dissertation, *Effect of Mesenchymal Stem Cell-Derived Conditioned Medium Co-cultured with Mononuclear Cell from Type 2 Diabetes Mellitus Patient on Insulin Resistance Model HepG2 Cell*, exemplifies her commitment to advancing scientific knowledge. Angliana currently applies her expertise as the Quality and Development Supervisor at Prodia StemCell Indonesia. Email: angliana@prostem.co.id

Atikah Anwar Hasibuan

Atikah Anwar Hasibuan earned her bachelor's degree in biology from Universitas Indonesia. She currently serves as the Advisor for Stem Cell Therapy at PT Prodia StemCell Indonesia, focusing on internal and skin diseases. In addition to her advisory role, she has responsibilities related to toll manufacturing. Several of her writings have been published in *Cytotherapy*, the official journal of the International Society for Cell and Gene Therapy (ISCT). Email: atikahanwarhasibuan@gmail.com

Aw Tar Choon

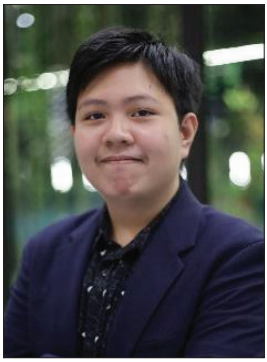
Aw Tar Choon is an expert in the field of medicine and clinical chemistry. He completed his medical education at the University of Malaya Medical School, followed by a master's degree in public policy at Harvard-NUS. He underwent training in internal medicine at Singapore General Hospital and King's College Hospital & Medical School. Additionally, he pursued training in clinical chemistry at the Department of Pathology & Laboratory Medicine at the Hospital of the University of Pennsylvania. Aw Tar Choon is the Director of Chemical Pathology at the Department of Laboratory Medicine at Changi General Hospital and the Head of Biochemistry Service at Seng Kang Hospital. Despite his busy schedule, he holds academic appointments as a Clinical Senior Lecturer (Medicine) at the National University of Singapore (NUS) and Clinical Professor (Pathology) at Duke-NUS Graduate Medical School. He was elected as Fellow of the Royal College of Physicians of Edinburgh (1999), Fellow of the Chapter of Pathologists Academy of Medicine Singapore (2004), and International Fellow College of American Pathologists (2020). His

professional qualifications include a Board Certification in Internal Medicine from NUS and The Royal College of Physicians (UK), as well as a Sub-specialty Board Certification in Chemical Pathology from the Royal College of Pathologists of Australasia. He is a member of the Royal College of Physicians in Edinburgh and Ireland and The Academy of Medicine Singapore.

Additionally, he is on the Executive Council of several global scientific institutions, including the Singapore Association of Clinical Biochemists, the Singapore Society of Pathology, and The Endocrine & Metabolic Society of Singapore. He has published over 200 journal articles, including 17 on Covid-19, and delivered over 370 lectures in 25 countries, including 11 webinars on Covid-19. He is a recipient of several distinguished national and international awards. He also serves on the editorial boards of several journals, including *Nature Scientific Reports*. Email: tarchoon@gmail.com

Bayu Winata Putera

Bayu Winata Putera completed his bachelor's degree in pharmacy, followed by a pharmacist profession at Universitas Padjajaran. He pursued his postgraduate education at Hasanuddin University, focusing on Biomedics, specifically a concentration in Clinical Chemistry. His research focused on the role of visfatin obestatin in the proliferation of pancreatic beta cells and was published in the Indonesian Biomedical Journal. He pursued his doctoral studies at the Faculty of Medicine, Hasanuddin University. His dissertation, *Stem Cell Dynamics in Stroke Patients who have undergone Intra-Arterial Heparia Flushing* explores hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and very small embryonic like cells (VSELs). Bayu Winata Putera currently serves as the Operational Manager at Prodia StemCell Indonesia. Email: bayu.winata@prostem.co.id



Billy Yosua Costantin Pongajow

Billy Yosua Costantin Pongajow graduated with a bachelor's degree in biotechnology from the University of Pelita Harapan. In 2023, he garnered recognition through the Student Creativity Program-Scientific Article, showcasing his commitment to academic excellence. Additionally, Billy brings practical experience to the table, having served as an intern in the Research-Based Therapy Department at Prodia StemCell Indonesia in 2022. Email: billypj977@gmail.com

Cita Rosita Sigit Prakoeswa

Cita Rosita Sigit Prakoeswa is the Director of Dr. Soetomo General Hospital in Surabaya, Indonesia. She earned her doctorate from Universitas Airlangga in 2007 and was inaugurated as a Professor at Universitas Airlangga in 2019. Her expertise is in dermatology and venereology. Email: cita-rosita@fk.unair.ac.id

Cosphiadi Irawan

Cosphiadi Irawan, a distinguished medical professional, completed his medical education at Universitas Indonesia. He further pursued specialized training in internal medicine through the Internal Medicine Specialist Education Program at the same institution. In 2011, he achieved a subspecialist designation in Internal Medicine, demonstrating his commitment to

excellence in his field. In 2015, Cosphiadi Irawan earned a doctorate from the Universitas Indonesia, solidifying his academic prowess and dedication to advancing medical knowledge. Currently serving as a Clinical Educator at the Faculty of Medicine, Internal Medicine Study Program, he holds the esteemed position of Lector. Cosphiadi Irawan's expertise lies in the realm of Hematology-Oncology, where he contributes significantly as a staff member in the Hematology-Oncology Division within the Department of Internal Medicine at the Faculty of Medicine, Universitas Indonesia, and Dr. Cipto Mangunkusumo National General Hospital (FKUI/RSCM). His professional journey reflects a remarkable commitment to education and clinical excellence. Email: cosphiadi@gmail.com

Cynthia Retna Sartika

Cynthia Retna Sartika completed her doctoral degree in the Faculty of Medicine at Universitas Hasanuddin in 2009. She has engaged in a multifaceted and distinguished career and is also actively involved in various professional organizations and committees, which contribute significantly to the fields of atherosclerosis and vascular diseases. Her notable affiliations include memberships in the Persatuan Atherosclerosis dan Penyakit Vascular Indonesia (PAPVI), the International Society of Stem Cell Research (ISCCR), the International Society of Cell & Gene Therapy (ISCT), and the International Society of Extracellular Vesicles (ISEV). Cynthia Retna Sartika is also a key participant in the Konsorsium Pengembangan Sel Punca and serves on the Adhoc Team for Health Minister Regulations (Tim Adhoc Peraturan Menteri Kesehatan–Permenkes). Additionally, she is also actively involved in the Asosiasi Sel Punca Indonesia (ASPI) or Indonesia Stem Cell Association and contributes her expertise to the team, specializing in the Good Manufacturing Practice for Pharmaceuticals (Tim

Ahli Cara Pembuatan Obat yang Baik—CPOB). As a member of the Asian Cellular Therapy Organization (ACTO) and the Research and Networking Commission at the Committee for Stem Cell and Cell Development, Ministry of Health of the Republic of Indonesia, Cynthia Retna Sartika demonstrates a strong commitment to advancing scientific research and healthcare practices in Indonesia. Email: c.sartika@gmail.com

David Victorious Lukas

David Victorious Lukas is a general practitioner who completed his training at the Faculty of Medicine of Universitas Pelita Harapan, Indonesia. He is now pursuing his Master's in Nutrition and Population Health at Deakin University, Australia. He has various interests and has helped in several other teams, from internal medicine to telehealth. He is committed to providing holistic quality care, particularly in terms of prevention through lifestyle and nutrition. Email: davidvlukas@gmail.com

Dewi Wulandari

Dewi Wulandari earned her doctoral degree from the Universitas Indonesia in 2022. Currently, she serves in the Department of Clinical Pathology at the Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo National General Hospital (FKUI/RSCM). She holds the position of Doctor in Charge at Prodia Stemcell Indonesia (ProSTEM). Email: dewi.wulandari@ui.ac.id

Dito Anurogo

Dito Anurogo is a distinguished scholar specializing in stem cells, regenerative medicine, molecular medicine, immunology, neuroscience, nanotechnology, medicine, health, and herbal research. He holds a PhD from the International PhD Program in Cell Therapy and Regeneration Medicine (IPCTRM), College of Medicine, Taipei Medical University, Taiwan. He serves as an Assistant Professor at the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, Indonesia. His research centers on nanoimmunobiotechnomedicine. An accomplished author, certified trainer, and reviewer for numerous national and international journals, he has received multiple prestigious awards. Email: dito.anurogo@med.unismuh.ac.id; d151109004@tmu.edu.tw

Ditta Kalyani Devi

Ditta Kalyani Devi achieved her master's degree at Universitas Padjajaran with a research focus on the effect of UC-MSC and CM UC-MSC administration on IL, MMP, and COMP levels in osteoarthritis SF-MSC. Currently, she serves as the Marketing Therapy Supervisor at Prodia StemCell Indonesia. Her academic dedication and research experience uniquely position her in a crucial capacity within the organization, overseeing marketing strategies related to therapy. Email: ditta.kalyani@prostem.co.id

Eka Ginanjar

Eka Ginanjar earned his doctorate from the Universitas Indonesia in 2019. With extensive experience as a clinician and academician, Ginanjar has made significant contributions as the Head of the Integrated Heart Service (Pelayanan Jantung Terpadu–PJT) at Dr. Cipto Mangunkusumo National General Hospital (RSCM) from 2014 to 2023. Serving as a Specialist in Internal Medicine and Consultant Cardiologist at the same institution, he honed his skills through an Interventional and Clinical Cardiology Fellowship Training Program at the National Heart Institute of Malaysia in 2012. His outstanding expertise is recognized through fellowships at leading organizations, such as the Indonesian Society of Internal Medicine (FINASIM), the American College of Physicians (FACP), and the International College of Angiology (FICA). Actively engaged in professional associations, such as PAPDI (Perhimpunan Dokter Spesialis Penyakit Dalam Indonesia/Indonesian Society of Internal Medicine) and IKKI (Ikatan Keseminatan Kardioserebrovaskular Indonesia/Indonesian Cardiocerebrovascular Society), Eka Ginanjar demonstrates his dedication to advancing cardiovascular care in Indonesia. Email: ekginanjar@gmail.com

Elizabeth Merry Wintery

Elizabeth Merry Wintery is a specialist in internal medicine who provides various medical services, such as internal medicine consultation and immunology consultation. One of her practice locations is Medistra Hospital in Jakarta. She completed her specialization degree in Internal Medicine at the Universitas Indonesia. She is registered

as a member of the Indonesian Association of Specialists in Internal Medicine. Email: merry.wintery@gmail.com

Ida Sri Iswari

Ida Sri Iswari, born in Bandung on 5 May 1961, was the first child of six brothers from partners Mr. Drs. M. Soeparman and Mrs. Ida Ayu Kartini. Finishing education from kindergarten until College Tall in Institute Teaching Knowledge Education (IKIP) or University Indonesian Education (UPI) in Bandung.

In 1981, she got to draw knowledge at the Faculty of Medical, Udayana University, and graduated in 1989. While going through education, the doctor met with their partner life, Dr. Dr. I Made Bagiada, Sp.PD -KP, FINASIM got married on 1986. She has two children, I Putu Eka Krishna Wijaya, Sp.PD -KP and Ms. Ni Made Dwi Ayu Martini, S.Kep . M.Kes. In 1990, she was appointed to Civil Service Candidates Health Bali Province and placed in Part Microbiology Clinic - Faculty Medical, Udayana University.

In 1993, Ida Sri Iswari followed their husband to continue their education at Padjadjaran University – Bandung. Master of Health obtained in 1996 and Doctor completed in 2001. When that's the Study Program Doctor at UNPAD it is a mandatory dissertation based on molecular, with various efforts as well as support from promoter and Co-Promoter writing about the Role of Serenity and Alanine on Salmonella typhi Resistant Chloramphenicol can completed.

Return to Denpasar in 2001 is believed to be PIC Development Biology Molecular – Due like Program at FK Unud 2002–2006. In 2006, She became involved in the Study Program Doctor and Masters Study Programs, especially the Field Anti-Aging Medicine Studies – Post Graduate Program at Udayana University. In 2006, She joined the Research and Development Unit of the Faculty of Medicine, Udayana

University - Sanglah General Hospital, which in 2013, in recognition by FERCAP function, became Committee Ethics Study Health Faculty of Medicine, Udayana University–Sanglah General Hospital, had the opportunity become Representative Chairman. Until now, this is still active. She is a Member of KEPK FK Unud – Ngoerah Hospital as a reviewer.

In service health, Ida Sri Iswari can become a sub-lab supervisor. Microbiology Clinic 2001–2006, then became a KSM Microbiology member Ngoerah Hospital Clinic in 2013 until now. From 2020 to 2024, Ida became chairman of the installation laboratory at Integrated Ngoerah Hospital. COVID-19 is spreading, and the pandemic started in 2020, as well as the identification of the SARS-Cov 2 virus. This Human Metapneumovirus (HMPV) began to spread in China. Hopefully, there will be no seed plague-like moment then. Email: idasriiswari@yahoo.co.id

Idrus Alwi

Idrus Alwi is a distinguished Cardiology Consultant at the Universitas Indonesia and is recognized for his exceptional expertise and significant contributions to the field of interventional cardiology and stem cell research. Idrus Alwi embarked on his academic journey at Universitas Indonesia, graduating as a specialist in internal medicine in 1996 and as a cardiology consultant in 2001. His passion for advancing medical knowledge led him to pursue a PhD at the same institution, which he completed in 2006. In 2012, with an unwavering commitment to academic excellence and patient care, Idrus Alwi was appointed as a Professor of Internal Medicine at Universitas Indonesia, a position he continues to hold with dedication and distinction. Throughout his illustrious career, Idrus Alwi has demonstrated a profound dedication to advancing the field

of interventional cardiology and stem cell applications, pioneering novel techniques and methodologies that have been instrumental in exploring innovative treatment modalities. Email: idrus_a@hotmail.com

Ika Prasetya Wijaya

Ika Prasetya Wijaya completed his medical education at Universitas Indonesia in 1992. He then continued his specialist education in internal medicine at the Specialist Doctor Education Program at Universitas Indonesia and graduated in 2003. In 2011, Ika Prasetya Wijaya completed his subspecialist education in Internal Medicine at the same university. Now, he serves as a clinical educator at the Faculty of Medicine, Internal Medicine Study Program at Universitas Indonesia, where his current functional position is as a lector. He is an expert in the field of cardiology. He is listed as a member of the staff of the Cardiology Division, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo National General Hospital (FKUI/RSCM). He is a member of the Greater Jakarta Branch of the Indonesian Society of Internal Medicine (PAPDI). Email: ipwijaya@gmail.com

Ismail Hadisoebroto Dilogo

Ismail Hadisoebroto Dilogo is a Professor of Orthopaedics and Traumatology at the Faculty of Medicine, Universitas Indonesia. Within his expertise in Orthopaedics and Traumatology, Ismail focuses on adult hip and knee reconstruction cases and advanced trauma. He completed his medical education at Universitas Airlangga and continued his specialist and doctoral education at Universitas Indonesia. Ismail also serves as the President of the

Indonesian Orthopaedic Association (PABOI) for the 2023–2025 term. In addition, he is the Head of the Stem Cell Medical Technology Installation at RSCM and the Stem Cell and Tissue Engineering Research Center at IMERI, Faculty of Medicine, Universitas Indonesia. Email: ismailortho@gmail.com

Jeanne Adiwinata Pawitan

Jeanne Adiwinata Pawitan earned her doctorate at Faculty of Pharmaceutical Sciences, Osaka University. Her current affiliation is Faculty of Medicine, Universitas Indonesia. Jeanne's expertise is on histology, stem cells, and tissue engineering. Email: jeanneadiwip@gmail.com

Jennifer Lheman

Jennifer Lheman is a senior researcher at Tarumanagara Human Cell Technology (THCT) Laboratory, Jakarta, Indonesia. She completed her master's degree at Atma Jaya Catholic University, Jakarta, Indonesia, in 2019. She is currently the deputy head of the THCT Laboratory and has published more than five papers in international journals. She specializes in tissue engineering and biomaterials development. Along with Dr. Siufui and the THCT team, she has conducted numerous laboratory trials and several animal studies. Her research works currently focus on developing secretomes derived from mesenchymal stem cells and their therapeutic applications, especially for diabetic treatment, liver cirrhosis, and hernia repair. Email: jenlheman@hotmail.com

Marsya Nilam Kirana

Marsya Nilam Kirana holds a Master of Science in Stem Cell Engineering for Regenerative Medicine from the University of Glasgow. With expertise in stem cell research and biomaterials, her background aligns well with her previous role as a scientist and advisor for stem cell therapy at PT. Prodia StemCell Indonesia. Marsya actively contributes to scientific discourse and innovation in her areas of specialization. Email: marsyankirana@gmail.com

Mochamad Syaifudin

Mochamad Syaifudin was born in the small town of Lamongan on August 16, 1963. He completed his medical doctorate at Universitas Airlangga in 1990. He pursued his master's degree in Hospital Admissions at Airlangga University, graduated in 2000. In 2015, he earned a Master of Biomedical Science degree at Udayana University. He then pursued his education at the Doctoral Medical Science Study Program, focusing on bone marrow transplantation in thalassemia major at Universitas Airlangga, Surabaya, in 2021. He is a doctor and director of the Clinic Regenerative Medicine MMC Lamongan. In 2012, he completed his Master of Biomedical Sciences, and by 2015, he had completed a doctoral program in stem cell applications for degenerative diseases. He began developing a service of excellence at the Regenerative Medicine Clinic under the name of Cell Rejuvenation Therapy (Terapi Peremajaan Sel), focusing on facial rejuvenation, hair growth, and degenerative diseases such as diabetes mellitus. Email: msyaifudin1608@gmail.com

Mochamad Wildan

Mochamad Wildan was born in Surabaya, on November 1, 1997. He enrolled in the Medical School of Universitas Airlangga, Surabaya, in 2016 and finished his studies in 2020. During this period, he also attended Clinical Observation at Juntendo University Hospital in Tokyo. His final Thesis, titled "Correlation between the History of Lower Extremity Amputation (LEA) as a Risk Factor for the Subsequent LEA in Diabetic Patients among RSUD Dr. Soetomo Patients in 2018," was published in 2020.

As per his working experience, he has practiced in RSAD Tk.III Brawijaya, Surabaya as well as Simomulyo Primary Health Center before enrolling into Majapahit Medical Center (MMC) Regenerative Medicine Clinic, Lamongan. Since then, he has been involved in the development of Comprehensive Patient Care, primarily focusing on the distinction service of MMC Clinic, which is Cell Rejuvenation Therapy (Terapi Peremajaan Sel).

Mohamad Syahrir Azizi

Mohamad Syahrir Azizi completed his education at the University of Indonesia in 2019. He has established a noteworthy presence in the field of cardiovascular imaging. As a dedicated member of the Asian Pacific Society of Cardiovascular Imaging (ASCI), he actively contributes to advancing knowledge and practices in cardiovascular health. Additionally, he

holds membership in the American Society of Echocardiography (ASE), reflecting his commitment to staying at the forefront of international developments in cardiovascular imaging. He has been honored with a fellowship in the International College of Angiology (ICA), denoted by the prestigious title FICA. Further showcasing his recognition within the medical community, Mohamad Syahrir Azizi holds fellowships in PAPDI (Perhimpunan Dokter Spesialis Penyakit Dalam Indonesia/Indonesian Society of Internal Medicine) and FINASIM (Fellowship of Indonesian Society of Internal Medicine). He is also an esteemed member of IDI (Ikatan Dokter Indonesia/ Indonesian Medical Association) and actively participates in the IKKI (Ikatan Keseminatan Kardioserebrovaskular IndonesiaIndonesian Cardiocerebrovascular Society), emphasizing his dedication to the advancement of cardiovascular care and expertise in Indonesia. Email: dr.syahrir@gmail.com

Nabilla Farah Naura

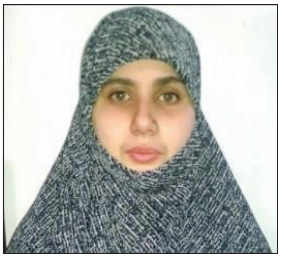
Nabilla Farah Naura is a Master of Science student at University College London studying the Manufacture and Commercialization of Stem Cell and Gene Therapies. She received a full-ride scholarship for her master's study from the Indonesia Endowment Funds for Education (LPDP), Ministry of Finance. Previously, she was an Advisor for Stem Cell Therapy at PT. Prodia Stem Cell Indonesia for 2,5 years managing stem cell clinical trials and scientific publications. Several of her writings have been published in cytotherapy, the official journal of the International Society of Cell and Gene Therapy (ISCT). Email: nabillafarahnaura@yahoo.com

Nadya Karina

Nadya Karina completed her biotechnology studies at Al Azhar University in 2017. She has been working for four years in the field of stem cells as an advisor for stem cell therapy, mainly focusing on stem cells in the areas of the heart and neurology. Email: nadyakrn8@gmail.com

Rima Haifa

Rima Haifa completed her bachelor's degree in biology at Universitas Indonesia. She pursued her master's degree at Universitas Padjadjaran, focusing on Pharmacy, specifically a concentration in Clinical Pharmacy. Her thesis focused on the role of dendritic cells derived from autologous peripheral blood or allogeneic umbilical cord blood in T cells of nasopharyngeal carcinoma patients. She is an esteemed member of various professional associations, including the Asosiasi Sel Punca Indonesia (ASPI/ Indonesian Stem Cell Association), the International Society of Cell & Gene Therapy (ISCT), the International Society of Extracellular Vesicles (ISEV), and the Asian Cellular Therapy Organization (ACTO). These affiliations underscore her commitment in being abreast of cutting-edge developments in her field. Presently, Rima Haifa holds the position of Research-Based Therapy Supervisor at Prodia StemCell Indonesia. Email: rima.haifa@prostem.co.id



Saba Majeed

Saba Majeed is an Assistant Professor of Pharmacology, Faculty of Pharmacy, Ziauddin University. She graduated from the field of Pharmacology and Neuroscience from the International Center for Chemical and Biological Sciences, Faculty of Pharmacy, University of Karachi. Her primary interest is neural stem cell research, which aims to modulate neurogenesis to treat neurodegenerative disorders. She has a great passion for exploring novel technologies and therapeutic treatment options to control neurological deficit-related orders; she has worked on neurogenesis and is involved in establishing novel models of co-culturing stem cells and neurons. Her open and contextual evaluation model is based on co-culturing techniques and promotes neurogenesis by exploring new signaling pathways for improving healthcare. She has patented her Ph.D research in neurogenesis for US Patent Isoxylitones mediated neurogenesis. Currently, she works as an assistant professor at Ziauddin University's Department of Pharmacology and is involved in research area of neuroinflammation targeting various signaling mechanisms modulating neurodegenerative diseases like alzhiemer's, stroke, parkinson's, etc. She has an excellent command on all molecular and imaging techniques. Email: saba.majeed@zu.edu.pk

Siufui Hendrawan

Siufui Hendrawan is an Associate Professor at the Faculty of Medicine, Tarumanagara University, Jakarta, Indonesia. She is also the head of Tarumanagara Human Cell Technology (THCT) Laboratory, a research laboratory founded in 2011 in collaboration with Baermed, Switzerland. She completed her PhD at Hassanuddin University, Makassar,

Indonesia, in 2017 and has published more than 15 papers in national and international journals. She specializes in tissue engineering, cell biology, and biomaterials science. She has conducted numerous pre-clinical studies and some clinical trials. Her research works specialize in tissue engineering, cell biology, and biomaterials science. She pioneers research focused on the development of mini-organs and cell-matrix implants, as well as the therapeutic application of secretome from mesenchymal stem cells. Email: siufui@fk.untar.ac.id

Somia Gul

Somia Gul is a professor at Department of Pharmaceutical Chemistry Faculty of Pharmacy, Jinnah University for Women. She has completed her specialization in Pharmaceutical Chemistry and earned her Ph.D as an HEC Indigenous Ph.D fellowship Scholar from the University of Karachi in 2011. Somia has over 17 years of teaching, research, and industrial experience as a supervisor of various M. Phil. and PhD. research scholars, research projects, and training sections. She has more than 103 research publications in reputed journals with good impact factors and one book published.

Her specialization is in medicinal chemistry, and her expertise and research interests mainly focus on drug-drug interactions of different classes of drugs, organic derivative synthesis and metal complexes, natural product chemistry, formulation development, validation and biological evaluation of herbal origin, methods development, and validation for analysis of drugs through HPLC and UV/Vis. Spectroscopy.

Somia's current research focuses on in-silico drug design, synthesis, biological evaluation, and molecular docking studies of novel analogues of biological interest, especially antimicrobials

and anti-tuberculosis agents against resistant pathogens. Email: drsomi1983@yahoo.com & SomiaGul@juw.edu.pk

Sukmawati Tansil Tan

Sukmawati Tansil Tan is a dermato-venereology doctor with more than 20 years of experience. She is currently the head of dermatovenereology department at Mayapada Hospital, Tangerang, Indonesia, and an assistant professor at the Faculty of Medicine, Tarumanagara University, Jakarta, Indonesia. She completed her PhD at Hassanuddin University, Makassar, Indonesia in 2012. She is a member of Fellow of The Indonesia Society of Dermatology and Venereology (FinsDV) and Fellow of the Asian Academy of Dermatology and Venerology (FAADV). She has published more than 15 papers in both national and international journals. She is currently focusing on research on the application of secretomes to chronic wounds, especially for diabetic ulcer treatment. Email: sukmawati@fk.untar.ac.id

Teguh Santoso

Teguh Santoso is a specialist in internal medicine, especially in cardiovascular, and a cardiology specialist who provides various medical support services, including heart examinations, thoracic, and cardiovascular surgery. One of his practice locations is at Medistra Hospital in Jakarta. He pursued specialized education as a cardiovascular consultant in internal medicine in 1975, and in 1978, he completed his education as a heart and blood vessel specialist. He then continued his Doctor of Philosophy degree at Erasmus Universiteit Rotterdam in 1980. He is

registered as a member of the Indonesian Association of Specialists in Internal Medicine and the Indonesian Association of Cardiovascular Specialists. Email: tsantoso@cbn.net.id

Wimpie Pangkahila

Prof. Dr. Wimpie Pangkahila, SpAnd-K, is a prominent professor at the Faculty of Medicine, Udayana University, Bali, Indonesia. He is a leading figure in Anti-Aging Medicine and Sexuality, actively serving as the Head of the Master's Program in Anti-Aging Medicine and the Chairman of the Center for Anti-Aging Medicine Studies at Udayana University. As an academic, Prof. Wimpie demonstrates extraordinary dedication to advancing knowledge, contributing significantly to anti-aging, sexology, and public health. He is also actively involved in various medical organizations, including serving as President of the Indonesian Andrology Association (Persandi), the Indonesian Sexology Association (ASI), the Indonesian Center for Anti-Aging Medicine (INCAAM), and the Indonesian Anti-Aging Medicine Association (InAAAM).

Prof. Wimpie has authored more than 240 scientific papers in national and international journals. His works cover diverse topics, particularly in anti-aging medicine and sexuality, enriching the scientific literature in Indonesia. He is also a prolific author, with five books on anti-aging medicine, one of which is published in English, and 23 other books on sexuality, two of which are also published in Malaysia. Additionally, he has contributed to the International Encyclopedia of Human Sexuality and the book Social Obstetric Gynecology, reflecting his profound expertise in the medical and social sciences.

Beyond his academic pursuits, Prof. Wimpie is known for his literary works. He has penned two novels, two poetry collections, and a book discussing social issues within society. His scholarly endeavors

reveal his profound reflections on various aspects of human life. Furthermore, he frequently speaks at scientific and public events, both nationally and internationally, to share his knowledge and insights in the fields of medicine, sexuality, and anti-aging.

Prof. Wimpie has received numerous prestigious awards for his contributions to medicine and academia. Among these, he holds a MURI (Indonesian Museum of World Records) World Record as the initiator of the world's first postgraduate program in Anti-Aging Medicine. This accolade underscores his role as a pioneer in the field. He has also been awarded the Leadership Award from the American Academy of Anti-Aging Medicine for founding the world's first postgraduate program in Anti-Aging Medicine. Other honors include the Satya Lencana Karya Satya Medal from the President of the Republic of Indonesia for his dedicated service in medicine and the Udayana Award for his integrity in developing the Postgraduate Program in Anti-Aging Medicine at Udayana University.

As an academic, leader, and author, Prof. Dr. Wimpie Pangkahila has had a profound impact on the field of medicine, both nationally and internationally. His dedication to enhancing the quality of life through education and research in anti-aging medicine and sexuality has earned him immense respect within the medical community. Email: wimpie.pangkahila@gmail.com

Winawati Eka Putri

Winawati Eka Putri is a lecturer at the Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya. She completed her doctoral program at Universitas Airlangga in 2021. She is an expert in Dermatology and Venereology. Her research is currently focusing on the utilization of stem cells in addressing skin problems. Email: winawatiekaputri@gmail.com

Yanni Dirgantara

Yanni Dirgantara completed her master's degree at Universitas Padjajaran with a research focus on mesenchymal stem cells derived secretome comparison between umbilical cord vs adipose tissue. Currently excelling as the Production Laboratory Supervisor at Prodia StemCell Indonesia, she brings her expertise and leadership to the organization. Yanni commits to advancing scientific knowledge, coupled with her adeptness in overseeing production processes within the stem cell laboratory. Email: yanni.dirgantara@prostem.co.id

Yulius Hermanto

Yulius Hermanto is a neurosurgery resident at Dr. Hasan Sadikin Hospital who aspires to become a hybrid vascular neurosurgeon. His primary interests are skull base and complex vascular reconstruction. This knowledge is crucial for treating patients with conditions involving both the brain and blood vessels, such as brain aneurysms, arteriovenous malformations (AVMs), and tumors located at the skull base. To address challenging conditions requiring advanced surgical techniques and multidisciplinary approaches, which he has demonstrated by pursuing mentorship from Dr. Rokuya Tanikawa and Prof. Yasuhi Takagi, well-known experts in complex vascular neurosurgery. With continued dedication and advanced training, a significant contribution to the field of neurosurgery, especially in the intersection of vascular and skull base surgery can be achieved. Email: hermanto_yulius@yahoo.com

Index

"Discovering the Miracles of Stem Cells" offers an exhaustive exploration of stem cell research, underscoring the profound transformative potential of these cells across various medical and scientific domains. This scholarly work meticulously delineates the fundamental characteristics of stem cells, including their types, functions, and pivotal roles in tissue regeneration and therapeutic interventions. The text is thoughtfully structured into multiple chapters, each focusing on distinct applications and areas of research.

The opening chapter provides a detailed exposition of the culture techniques for mesenchymal stem cells (MSCs), crucial for advances in regenerative medicine. Subsequent chapters delve into the application of stem cells in orthopedics, showcasing their effectiveness in treating bone and cartilage disorders. Further discussions cover the use of MSCs in healing diabetic wounds, the advantages of MSC-conditioned mediums in combating skin aging, and a critical evaluation of the safety and efficacy of stem cells in managing acute myocardial infarction. A particular emphasis is placed on the potential of CD34+ hematopoietic stem cells in skin rejuvenation following UV exposure, supported by robust empirical research on animal models. Later sections explore the therapeutic implications of stem cells in neurological disorders, highlighting their utility in managing neurodegeneration and other cerebral conditions. Additionally, the book examines the ethical dimensions of stem cell research, advocating for stringent ethical standards to navigate the complex moral landscapes encountered in the use of stem cells for therapeutic purposes. It also addresses the unique opportunities and challenges associated with stem cell research and application in Indonesia, reflecting on the country-specific context that shapes these endeavors.

Overall, this text serves as a comprehensive resource that elucidates both the scientific and therapeutic dimensions of stem cells while addressing the ethical, regulatory, and practical challenges in the field. Designed to equip readers with a thorough understanding of how stem cells can be utilized to enhance health outcomes and treat a wide array of diseases, this book positions stem cell research as a beacon of hope within the medical and biotechnological domains, yet acknowledges the intricate ethical, technical, and regulatory challenges that must be navigated with precision and prudence.

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