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# STABILITY CHARACTERISTIC OF CRYOPRESERVED HUMAN UMBILICAL CORD WHARTON'S JELLY-DERIVED MESENCHYMAL STROMAL CELLS

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**Background & Aim:** Mesenchymal stromal cells (MSCs) are multipotent mesodermal lineage derived stem cells that can self-replicate and differentiate into all mesodermal, some of neuroectodermal and hepatic endodermal progenies. Umbilical cord derived Wharton's jelly MSCs (WJ-MSCs) are an attractive source for cell-based therapy regenerative medicine due to the stability in large scale expansion, non-tumor formation and low immunological rejection. Therefore, WJ-MSCs are a superior banking MSCs source for further clinical application.

**Methods, Results & Conclusion:** WJ-MSCs were isolated at a 100% successful rate and characterized by flow cytometry for CD34, CD45, CD73, CD90, CD105, CD106 and STRO-1. Growth kinetics characteristics were investigated by population doublings (PD), clinical large-scale expansion and high viability maintenance. MSCs were assessed for differentiation potential to cartilage, adipose and bone. Flow cytometry analysis showed no differences in expression of CD34, CD45, CD73, CD90, CD105, CD106 and STRO-1 among all cryopreserved WJ-MSCs for six years (2013–2018;  $n=30$ ). All years of cryopreserved WJ-MSCs maintained high proliferative potential with no difference in all passages. Moreover, they were capable to differentiate into cartilage, adipose and bone structures as determined with histochemistry. WJ-MSCs have a high potential for stem cell banking from umbilical cord due to its noninvasive techniques of procurement, high successful isolation rate and low risk microorganism contamination. Furthermore, WJ-MSCs are suitable sources of stem cells for potential use in regenerative medicine in further clinical applications.

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# COMPARISON OF VIABILITY AND PROLIFERATION POTENCY OF STROMAL VASCULAR FRACTION STORED AT DIFFERENT TEMPERATURE

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**Background & Aim:** Adipose tissue provides an abundant source of stromal vascular fraction (SVF), which resides a potential regenerative medicine named mesenchymal stem cells (MSC). Storage period and temperature before isolation may impact the quality of isolated cells. Here, we identify the effect of storage periods and temperatures to SVF viability and cell number along with the MSC recovery.

**Methods, Results & Conclusion:** A total of 70 ml donor-adipose tissue was divided into two different groups, room temperature (RT) and 4-8°C. SVF was isolated using manual method. Each group was processed after stored for 4, 5, 6, 7, and 12 days. After it passed each incubation time, adipose samples were processed enzymatically until SVF was obtained. The viability of SVF stored at RT was decreasing until 40%, but relatively constant when stored at 4-8°C (>75%). Nevertheless, the sample stored at RT revealed to have better proliferation and colonization than sample stored at 4-8°C. The sample retained the proliferation capability when stored at RT up to 7 days, but only up to 5 days when stored at 4-8°C. The average of SVF number was higher in the sample stored at RT (8,552,315 cell/mL) compared to the sample at 4-8°C (4,581,111 cell/mL). The highest SVF number was observed in sample stored at 4 days before isolation. The average of MSC recovery was also higher at RT compared to sample stored at 4-8°C, which was 6% and 3%, respectively. In conclusion, adipose tissue is preferable to be stored at RT before isolation for higher SVF total number and MSC recovery. The adipose tissue should be processed immediately or stored only up to 4 days after sample collection.

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# STROMAL VASCULAR FRACTION (SVF) THERAPY FOR TREATMENT OF VARIOUS DISEASES: DELIVERING SAFETY OF THE FIRST PATENTED SVF TECHNIQUE IN INDONESIA

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**Background & Aim:** To report the safety of SVF therapy done by the first patented processing technique in Indonesia for treating various diseases via various routes of administration.

**Methods, Results & Conclusion:** Stromal vascular fraction (SVF) therapy has been performed over the past seven years to treat 493 patients by our group in five clinical centers. SVF was isolated from the lipoaspirate collected from each respective patient through a simple manual liposuction procedure. For each patients, blood was drawn as well to isolate platelet-rich plasma (PRP). SVF and PRP were administered to all subjects by intravenous (IV) injection in an autologous manner, with minority group within the population received an additional spinal, intra-articular, or subcutaneous injections, or combination of several injection sites. The top 5 common diseases were diabetes mellitus, antiaging, degenerative musculoskeletal disease, cardiovascular disease, and autism. On 2016 we patented a new technique called H-Remedy and use it for 402 patients since, 224 males (55.7%) and 178 females (44.3%) aged 1 to 86 year old (median = 55). Lipoaspirate volume was from 10 ml to 415 ml (median = 95) and total number of SVF was ranged between  $0.2 \times 10^6$  per 10 ml to  $5.5 \times 10^6$  per 10 ml (median =  $22.4 \times 10^6$  per 10 ml). The total of 87 patients (21.6%) received 4 times SVF treatments, 161 patients (40%) received 3 times SVF treatments, and 114 patients (28.4%) received 2 times SVF treatments, all during 2 month period. Only 40 patients (10%) received 1 time SVF treatment. It was determined that any complications observed during the treatments with H-Remedy had been identified as a result of spinal or intra-articular injections and not related with direct therapeutic effect from SVF or PRP. These minor side effects had been identified early and successfully treated. In conclusion, the technique and clinical protocol were considered as safe and feasible, and therefore can be used to the next phase of trial to evaluate the efficacy of SVF therapy.

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# BRAIN-DEATH DONORS AS AN ALTERNATIVE SOURCE OF HUMAN STROMAL MESENCHYMAL CELLS FOR CELL-BASED THERAPY

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**Background & Aim:** In the last decades, human mesenchymal stromal cells (hMSC) have been widely investigated, due to their capacity to extensively proliferate *in vitro* while maintaining their differentiation potential into several mesodermal lineages. Besides, they have low immunogenicity, immunomodulatory effects, ability to migrate and graft at inflammation sites and secrete bioactive molecules. These properties are of great interest for applications in cell therapy and regenerative medicine and hMSCs are currently being evaluated in several clinical trials. Various sources of hMSCs have been studied, being bone marrow (BM) the most commonly used for autologous or allogeneic clinical applications. In the latter case, one of the difficulties is the scarce availability of living donors of BM. In contrast, the alternative use of deceased donors as an allogeneic source has been little explored. The aim of our study was to evaluate the relative potential of BM from brain-death donors (BDD) as a source of hMSCs for cell therapy, obtained from two different sites: femur (F) and iliac crest (IC).

**Methods, Results & Conclusion:** Adequate procedures were developed for BM collection and processing, as well as for *in vitro* culture, immunophenotyping and differentiation. Fourteen donor-matched BM samples (IC and F) were processed from seven deceased donors (Table 1). Our results show that it is possible to obtain viable hMSC from both sites. Although the total number of mononuclear cells (MNC) per gram of BM appears to be greater in the IC than in F, no statistically significant differences were observed between both sources. Neither the total content of fibroblastoid colony-