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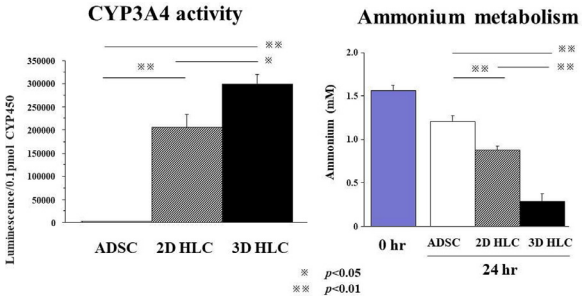
Background & Aim The aim of this study is to clarify the effectiveness of a new 3D-culture system of hepatocyte like cells (HLCs) from human adipose derived mesenchymal stem cells (ADSCs).

Methods, Results & Conclusion Methods 2.0×10^4 human ADSCs with or without 0.02 mg of a scaffold-type extracellular matrix called compound X per well were seeded in 96-well U bottom plate, then our 3-step differentiation protocol was applied. At each step, cell morphology and gene expressions were investigated. Hepatocyte functions were evaluated with albumin secretion, CYP3A4 activity and ammonium metabolism. Those parameters were compared between 2D and 3D HLCs.

Results In 2D culture, ADSCs had gradually changed those morphology, and HLCs had the shapes of hepatocytes. Definitive endoderm showed significantly higher Sox17 mRNA levels compared to undifferentiated ADSCs. Hepatic endoderm showed also higher AFP levels, and HLCs showed higher ALB levels. In 3D culture, HLCs made the forms of spheroids from day6, and the sizes were gradually increased. Gene expression levels of each step had significantly higher levels than 2D HLCs. 3D HLCs showed higher CYP3A4 activity and better ammonium metabolism compared with 2D HLCs.

Conclusion Our new 3D-culture differentiation protocol of HLCs from ADSCs might be effective and promising for clinical cell transplantation.



CYP3A4 activity and ammonium metabolism in ADSC, 2D and 3D HLC.

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Effect of cryopreservation of mesenchymal stromal cells after non-viral transfection on cell viability and transgene expression

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Background & Aim Mesenchymal stromal cells (MSCs) have been shown to exert important immunomodulatory effects in both acute and chronic diseases. In acute inflammatory conditions, an "off-the-shelf" cryopreserved, allogeneic cell product might be better suited as it can be ready for administration to patients without delay. Genetically modified cells have recently gained tremendous interest given the successes in chimeric antigen receptors (CAR T) cell therapy, in which T cells from patients can be genetically engineered to produce an artificial T-cell receptor for treating cancer. However, whether a genetically modified, allogeneic MSC can have sustained transgene expression post cryopreservation has not yet been well studied. Herein we assess the effect of non-viral transfection on cell viability and protein expression in cryopreserved-and-then-thaw MSC.

Methods, Results & Conclusion MSCs were derived from bone marrow of healthy donors and characterized using the ISCT criteria (surface marker expression and tri-lineage differentiation potentials). MSCs were transfected with non-viral plasmid using two protocols: 1) transfection 24 hours after cell seeding, or 2) transfection after 6 days of culture. Transfected MSCs were cryopreserved at 24 hours after transfection. Viability (Trypan blue), cell number recovery and transgene expression (Western blot) were examined. Prior to cryopreservation, the cell viability was 87% ± 3% from protocol 1 (n=2) and 93% ± 1.2% from protocol 2 (n=3). After one week of storage in liquid nitrogen, cell viability was 71% ± 4% and 82% ± 5% for Protocol 1 and 2 respectively, while the percentage of cell recovery post thaw was 72% ± 9% and 95% ± 2%. Thawed cells were sub-cultured to confirm no abnormality in cell growth and morphology. To determine levels of transgene protein expression, cell pellets were collected pre- and post-cryopreservation. Western blot analysis showed that `transgene protein could be detected 2 days after cells were thawed, and the protein expression was modestly reduced to 78% ± 6.7% to that of pre-cryopreservation in protocol 1 and 93%± 1.7% in protocol 2.

Our results address the concerns that transgene expression might be lost during the process of cryopreservation by showing a sustained but slightly reduced level of proteins overexpression to that of pre-cryopreservation. Further optimization will be conducted to minimize the loss of protein expression and to improve viability after thaw.

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Case Report: Effect of Conditioned Medium Nasal Drop (CMND) and Intravenous Umbilical Cord-Mesenchymal Stem Cells (UC-$MSCs_{IV}$) on the function of cardio respiration after 4 years Percutaneous Coronary Intervention (PCI) in RSPAD Gatot Soebroto

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Background & Aim Coronary artery disease (CAD) is one of the leading cause of death in the world. Currently, the common therapy for this disease is to use drugs and surgical procedures however the result has been less optimum. Therefore, it is necessary to develop an alternative treatment using Mesenchymal Stem Cell (MSC) and Conditioned Medium (CM).

Methods, Results & Conclusion MSCs were obtained from the umbilical cord of eligible donor and process in a cGMP-standardized laboratory certified by the Indonesian Ministry of Health. A 52-year-old subject who experienced Coronary Artery Disease was treated with PCI four years before the stem cell therapy. The subject signed an informed consent for stem cell therapy before the injection. Allogeneic UC-MSC for CAD was packed in a syringe containing 20 ml NaCl 0.9% with cell concentration of 20×10^6. Umbilical Cord-Mesenchymal Stem Cells were administered intravenously and conducted three times over 4 months period. Meanwhile, Conditioned Medium (CM) was applied intranasally with a dose of 0.4 cc/day for a week after each UC-MSCs administrated. Assessment with Magnetic Resonance Imaging (MRI) shows improvement on the Left Ventricular Ejection Fraction (LVEF) from 55% at baseline and 75% at 3 weeks after first stem cell transplantation. Endothelial Cell Progenitor (EPC) count show decreasing the number of EPC cells from 11,893 cells/mL into 4,932 cells/mL. This shows the angiogenesis process takes place after the UC-MSC injection. On the twelve weeks after the first injection, the subject undergo spirometry test and shows improvement in the vital capacity (85% into 94%), forced capacity (85% into 94%), and forced expansion volume (93% into 103%). There are no adverse events occur and the patient experience improvement in physic, MRI, laboratory and spirometry assessment.

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Case Report: Safety profile and efficacy of allogeneic umbilical cord-mesenchymal stem cells therapy via intracoronary route for acute myocardial infarction

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Background & Aim Coronary artery diseases may lead to myocardial infarction (MI) and cause reversible or irreversible ischemic heart damage. Currently, the common therapy for this disease is to use drugs, and percutaneous coronary intervention (PCI). The application of drugs and surgery have been less optimum for Acute Myocardial Infarction (AMI). Therefore, it is necessary to develop an alternative treatment with Umbilical Cord-Mesenchymal Stem Cells (UC-MSCs). This study aims to assess the safety profile and efficacy of UC-MSCs therapy via intracoronary route for AMI.

Methods, Results & Conclusion A 78-year-old subject who experienced AMI was treated with PCI. After performed PCI, the subject signed an informed consent form for stem cell therapy. One route week after PCI, UC-MSCs were administered $\pm 50 \times 10^6$ cells to the heart via intracoronary. The observation was carried out at 2 weeks, 3 and 6 months after stem cell injection. The subject performed 6-minute walking test at 2 weeks, 3 and 6 months after injection and the result was 336 m, 408 m. and 324 m, respectively. Assessment with Magnetic Resonance Imaging (MRI) showed Left Ventricular Ejection Fraction (LVEF) improvement from 35% as the baseline and 41% at 6 months after transplantation. The subject also had an improvement in wellness blood tests after 6 months of MSCs injection. The improvement parameter was fibrinogen (769 into 233 mg/dL), antioxidant (1.31 into 1.7 mmol/L), and hs-CRP (61.9 into 1.1 mg/L). After 2 weeks of MSCs injection subject did not experience any adverse events.

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Single infusion of allogeneic neonatal Mesenchymal stromal cells to manage refractory feline gingivostomatitis- A clinical pilot study

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Background & Aim Refractory feline chronic gingivostomatitis (FCGS) is a debilitating oral mucosal inflammatory disease. Two perfusions of 20.10E6 of fresh Adipose Mesenchymal Stromal Cells (MSCs) are necessary to dampen inflammatory clinical signs (Arzi 2017). Neonatal MSCs have potent immunomodulatory functions. In this pilot study, we investigated the feasibility of one infusion of lower dose of cryopreserved neonatal allogeneic MSCs for the treatment of this condition.

Methods, Results & Conclusion Methods 8 cats suffering from refractory GSCF (not responding to tooth extraction, total (n=6) or partial (n=2)) were treated by board-certified veterinary dental specialists at 3 medical centers. All cats received a single IV perfusion of 10.10E6 cryopreserved neonatal MSCs. Follow up evaluations were scheduled at 15 days, 2,3, and 6 months following cells administration. Clinical disease severity was evaluated by dental specialists using validated stomatitis disease activity index (SDAI) scoring system (ranging from 0 (no disease) to 24 (severe disease)). Owners completed a questionnaire scoring appetite, wellness, activity level, along with general behavior, on a scale of 0-3 for each parameter.

Results All 8 cats completed the study. No adverse effect has been reported during the 6 months follow up, except one case of mild vomiting during perfusion, a common immediate transfusion reaction in cats. Animals with total tooth extraction (n=6) responded all positively to the treatment with substantial clinical improvement, as assessed by a significant improvement of the SDAI score up to 3 months post-treatment (SDAI score range $[9\text{-}13]_{D0}$ vs $[2\text{-}9]_{M3}$; $p<0,05$; Friedman test). Similar results were observed with the 2 cats with partial tooth extraction (SDAI score decreasing from 14 (D0) to 2 (M6) for both animals). Owners accordingly reported also a satisfactory evolution of their animals.

Conclusion These data reported for the first time that single perfusion of cryopreserved neonatal MSCs is safe and effective for long-term clinical improvement (up to 6 months) in cats suffering from GSCF with total or partial tooth extraction. It paves the way of new therapeutic approaches for the treatment of refractory severe oral lesion and potentially to the translation to human invalidating stomatitis.

Arzi B et al., (2017) Therapeutic Efficacy of Fresh, Allogeneic Mesenchymal Stem Cells for Severe Refractory Feline Chronic Gingivostomatitis. Stem Cell Translational Medicine

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A novel cell-based strategy based on MHCI/CD90 expression levels for highly proliferative MSC identification

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Background & Aim Mesenchymal stromal cells (MSCs) are of clinical interest because of their validated safety profile and their tremendous biological properties. Nonetheless, variable clinical outcomes have been reported in the literature. Non-reproducible results are attributed in part to the cellular heterogeneity of MSCs, which makes consistent conclusions about MSC therapeutic potential difficult. The identification of new characteristics aiming to select homogeneous and functional MSCs batches is a real challenge to spread this therapeutic approach. This is particularly important for complex tissue sources like placenta.

Methods, Results & Conclusion Canine placenta-derived MSCs (P-MSC) populations were isolated from 18 placentas collected at full-term and evaluated according to the ISCT minimal criteria, including morphological assessment, phenotypic evaluation, immunomodulatory properties and in vitro differentiation ability. No significant difference was found. Nonetheless, dynamic variations of the expression levels of the CD90 and MHCI markers were reported among the cellular populations during the cell passages. In particular, our data showed that the $MHCI^{low}/CD90^{high}$ sub-population display high proliferative activity, along with high chondrogenic differentiation ability.

These results suggest that a better characterization of the MSCs bulk populations, based on a multiparametric data evaluation may help to standardize the cellular products. This could help to respond to industrial challenges for drug development

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Coating strategies for expansion of mesenchymal stromal cells in a hollow-fibre bioreactor

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Background & Aim Background The increasing request for culture expanded mesenchymal stromal cells (MSC) in the last years discloses the need to reduce costs and man power in the manufacturing process. This can be achieved by the use of closed, (semi-)automated bioreactor systems. We have decided to test a functionally closed hollow-fibre bioreactor system (Quantum®). Surveillance of metabolic parameters and adaptation of feeding in dependence of these is possible. However, the