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Local Immunosuppression by Single-dose of FK506 Microspheres and Clodronate Liposomes Leads to Indefinite Survival of Subcutaneously Transplanted Islets.

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ABSTRACT
Introduction
Islet transplantation is a promising way of replacing exogenous insulin therapy in type 1 diabetes. Long-term administration of immunosuppressive drugs is reported to induce several adverse effects including nephrotoxicity, opportunistic infections, and impairment of islet function. Therefore, we aimed to develop a robust immunoprotection protocol using a short-term locally-acting immunosuppressive regimen to develop transplant tolerance in a xenograft model of islet transplantation.

Methods
In this study, we report a robust protocol for local immunosuppression using single-dose of FK506 microspheres and clodronate liposomes in subcutaneous islet transplantation. Pancreatic islets were co-delivered with FK506 microspheres and clodronate liposomes into the subcutaneous space immune competent diabetic mice using injectable hydrogel.

Results and Discussion
In this study, we showed that a short-term treatment with FK506 and clodronate induces tolerance to mouse islet xenograft as indicated by indefinite survival of the transplanted islets. The tolerant mice displayed DC with a reduced percentage of CD4+ and CD8+ effector T cells in spleen, lymph nodes, graft and graft-associated region of the tolerant mice. The acceptor of the graft is associated with an impaired generation of IFN-γ producing Th1 cells, IL-17 producing Th17 cells, and an enhanced generation of CD4+CD25+FoxP3+ regulatory T cells.

Conclusion
The induction of tolerogenic DC by a short-term local therapy of FK506 microspheres and clodronate led to the generation of CD4+CD25+FoxP3+ regulatory T cells. The regulatory T cells maintained long-term graft tolerance in the islet recipients. Our results suggest a possible clinical strategy for local immunosuppression which may pave the way towards improving the therapeutic outcomes in clinical islet transplantation.

Keywords
Islet transplantation, immune rejection, local immunosuppression, immune tolerance

Decreased Production of Reactive Oxygen Species in 3D-mesenchymal Stem Cell Spheroids Leads to Increased Therapeutic Efficacy via Autophagy Induction

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ABSTRACT
Introduction
Mesenchymal stem cells (MSC) are being explored clinically as a new therapeutic agents for the treatment of several inflammatory diseases. Despite some encouraging results in treatment of various inflammatory disorders, poor viability of MSC post-transplantation is a major hurdle in MSC-based therapy. In previous studies, 3D-MSC spheroids showed enhanced anti-inflammatory effect and higher cell survival. In this study, we aimed to investigate the molecular signaling pathways responsible for the enhancement of cell viability in 3D-MSC, particularly focusing on autophagy and reactive oxygen species (ROS).

Method
3D-MSC spheroids were prepared by using hanging drop technique. Cell viability, ROS production, and autophagy activation in 3D-MSC were compared with that of 2D-cultured MSC. Furthermore, viability of transplanted 2D-cultured and 3D-cultured MSC was compared in vitro and in vivo. In addition, the effectiveness of 3D-MSC was confirmed in vivo by intraperitoneal injection of the 3D-MSC in a mouse model of experimental colitis.

Results
3D-MSC showed higher cell viability, low ROS production, and upregulation in the expression of antioxidant proteins such as catalase, SOD2, and hemooxygenase-1 (HO-1). Inhibition of HO-1 by gene silencing in the 3D-MSC led to an increase in ROS production. In addition, HO-1 induction upregulated the catalase expression and attenuated ROS production in the MSC. Interestingly, HO-1 induction further induced autophagy activation. Furthermore, inhibition HIF-1α resulted in HO-1 downregulation in 3D-MSC. This suggested HO-1/HIF-1α axis may be involved in autophagy activation and cell survival in 3D-MSC. In vivo, silencing of autophagy in 3D-MSC caused decreased effectiveness of the MSC in ameliorating colitis in mice.

Conclusion
The attenuation of ROS production in 3D-MSC led to an enhancement in MSC survival via the induction of autophagy. Therefore, the therapeutic effectiveness of 3D-MSC is at least, in part, mediated by autophagy induction.

National Research Foundation of Korea. Korea Health Industry Development Institute (KHIDI). Gyeongsangbuk Science and Technology Promotion Center of Korea.
Introduction: Mesenchymal stem cells (MSC) are capable of multi-lineage differentiation and can be potentially used as a promising tool for tissue regeneration. They can differentiate into osteoblasts and promote bone regeneration, however, this process required a long time and often is not efficient enough. Application of MSCs attached to 3D scaffolds will increase bone regeneration capacity. We investigated the biological properties and osteogenic potential of sheep bone marrow-derived MSCs (BM-MSCs), influence of bone morphogenetic protein-2 (BMP-2) on osteogenesis and efficacy to seed them onto biocompatible scaffold.

Materials and Methods: MSCs were isolated from BM collected from sheep iliac crest (n=6). Stem cell markers were analyzed by flow cytometry. MSCs differentiation into the osteogenic lineage was examined in: complete αMEM medium supplemented with 20ng/ml FGF (control), and αMEM medium supplemented with FGF and 100ng/ml BMP-2. The differentiation potential into osteoblasts was examined by Alizarin Red S staining. mRNA expression for osteogenic genes: BMP-2, Runt-related transcription factor 2 (Runx2), Osterix (Osx), type I collagen (ColI), osteopontin (Opn) and osteocalcin (Ocn) was performed by RT-PCR. MSC were seeded at the density 1x10⁶/ml onto a polymeric scaffold. The deposition of sheep MSCs on the 3D scaffold was performed by RT-PCR. MSC were seeded at the density 1x10⁶/ml onto a polymeric scaffold. The deposition of sheep MSCs on the 3D scaffold was examined on day 3, 7 and 12 by PKH26 Red and DAPI staining.

Results: Sheep BM-MSCs were positive for CD73, CD90 and CD105 and negative for CD34, CD45 and MHC class II. Cells incubated 21 days in medium with BMP-2 revealed higher bone mineralization capacity confirmed by Alizarin Red S staining. BMP-2 enhanced the osteogenic potential of MSCs by the upregulation of mRNA for osteogenic lineage genes: BMP-2, Runx2, Osx, Coll, Opn, and Ocn compared to control. MSCs seeded onto biocompatible 3D-scaffold after 12 days of culture were attached and spread on the scaffold.

Discussion: Sheep BM-MSCs with characteristic of naive stem cell markers were able to differentiate into osteogenic lineage and to proliferate on 3D scaffold. BM-MSCs stimulated with BMP-2 had greater osteogenic differentiation capability compared to the control. Conclusions: BMP-2 improves the ability of MSCs to differentiate into osteogenic lineage at early and late differentiation steps. Ovine BM can serve as a source of MSC for bone regeneration and BMP-2 enhance their osteogenic potential, and can be effective for bone engineering in preclinical large animal model.

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Does Antinuclear Antibody (ANA) Have a Role in Lymphoma Patients?

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No specific study group.

Introduction: Association between autoimmune diseases and malignancy including lymphoma has been described by various studies, however neither definite role of immune system nor cause of this has been understood.1 Antinuclear antibody (ANA) is an important group of autoantibodies which have variable role, protective and offensive, in epithelial malignancies, however the role in lymphoma has not been explored deeply to date.2,3 We have tried to find out the occurrence of ANA in lymphoma.

Methods: Newly diagnosed lymphoma patients were recruited and clinicopathological parameters were recorded. Serum was collected before the initiation of chemotherapy. Indirect immunofluorescence (IIF) was done for the detection of ANA (On Hep2 cell line, NOVA Lite, 1:40 dilution). Reotyping of ANA patterns was based on International consensus on ANA pattern.

Results and Discussion: Out of 75 lymphoma (48 Non hodgkin lymphoma + 27 Hodgkin’s Lymphoma (HL)) patients 14 (18.67%) showed positivity for ANA (9 nuclear positivity + 5 cytoplasmic positivity) on IIF which is significantly higher (p= 0.0166) as compared to healthy control (2/50). Most of these ANA positive cases were NHL however only 2 of HL patients displayed cytoplasmic positivity. Most frequent pattern was homogenous (5/9)(Figure-1) followed by speckled (3/9) (Figure-2) and nucleolar (1/9). Among cytoplasmic positivity most common pattern was cytoplasmic fine speckled (4/5) followed by cytoplasmic reticular pattern (1/5) Figure-3). Further characterization of ANA revealed that most of ANA were against SSA (3/9) and dsDNA (2/9) followed by Scl-70 (1/9) and one ytoplasmic patterns turned out to be anti-mitochondrial antibody. None of the patients were positive for Anti-Ro, Anti-La, Anti-Jo1, MPO and PR3. 3 of the ANA pattern and 4 of cytoplasmic pattern were not categorized in particular subtypes. No significant association was seen between ANA positivity and various clinicopathological parameters. Two patients with positive ANA had rheumatological features (arthralgia and arthritis). No patients have any evidence of recurrence after the follow up of 4 months till date. Earlier studies have shown the dual role ANA, poor (in breast cancer)2 as well better (lung and colorectal carcinoma)2,3 prognostic factor, however few recent studies have found it to be associated with lesser median survival and frequent relapse in lymphoma.2,3 Further analysis of these ANA may represent specific protein alteration in cancer cells and may help in early prediction of malignancy.

Conclusion: Significantly higher prevalence of ANA has been observed in NHL. No significant correlation is noticed between ANA positivity and various clinicopathological parameters however their early detection may help in prediction of development of lymphoma. Larger study with long follow up may require to understand their prognostic significance.

We thank all the staff of various departments of our Institute.

References:
Mesenchymal Stem Cells Alleviated Palmitic Acid Induced Endothelial to Mesenchymal Transition in HUVEC by Secreting STC1

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Background: Endothelial dysfunction (ED) is an early indicator of vascular complications in type 2 diabetes. Hyperlipidemia is believed to be a key causal factor promoting ED. Our previous study showed that mesenchymal stem cells (MSCs) were able to ameliorate palmitic acid (PA) induced ED, but the mechanisms involved are unclear. Endothelial-to-mesenchymal transition (EndMT) is demonstrated mechanistically related to ED in a variety of vascular diseases. Here we explored the effects of MSCs on PA induced EndMT and disclosed the mechanism in human umbilical vein endothelial cells (HUVECs).

Methods: The primary HUVECs were treated by PA (50 μM) with or without human umbilical cord derived MSCs (UC-MSCs) for 24 h in Transwell system at a ratio of 5:1. EndMT related proteins expression were evaluated by western blot and immunofluorescence. The secretion of stanniocalcin 1 (STC1) was quantified using an ELISA kit. Recombinant STC1 (rSTC1) and siRNA targeting STC1 were used to evaluate the role of STC1 in MSCs mediated protective effects.

Result: After PA treatment, the cell morphology of HUVECs changed from an endothelial-typical cobblestone-like appearance to a mesenchymal-typical spindle-shaped pattern, suggesting the occurrence of EndMT. However, coculture with MSCs alleviated the morphological change of HUVECs (Fig.1A). Western blot analysis revealed that PA significantly decreased endothelial marker CD31 and CD144 expression and increased fibroblast markers vimentin and α-SMA expression, which could be partly reversed by coculture with MSCs (Fig.B). Consistently, immunofluorescent staining for CD31 and α-SMA revealed a significant loss of CD31 and enhancement of α-SMA in PA group in comparison of control. However, MSCs effectively antagonized PA induced toxicity by increasing CD31 and decreasing α-SMA expression in HUVECs (Fig.1C).

Next, we explored the underlying mechanisms focusing on the secretome of MSCs. We found that resting MSCs secreted more STC1 than HUVECs did, while, PA (50 μM) strongly stimulated MSCs to synthesize and release much more STC1 (Fig.2A). Furthermore, the cell viability of HUVECs declined to 49% of control after PA stimulation. However, the addition of rSTC1 showed similar protective effect of MSCs, and restored the cell viability to 89% (Fig.2B). rSTC1 also reversed the expression switch of EndMT-related proteins (Fig.2C). Additionally, silencing of STC1 gene by siRNA in MSCs abrogated above protective effects suggesting the critical role of STC1 in MSCs mediated protective effects (Fig.2D-E).

Conclusion: MSCs ameliorated PA-induced EndMT in HUVECs, in which the STC1 secreted by MSCs has played a critical role.

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Human Hepatocyte Spheroids Show Plasticity-enabling Extended Culture and Pretransplant Conditioning

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Introduction: Primary human hepatocytes are a source of unmatched medium for drug testing, basic scientific research and cell transplantation. However, it has proven difficult to maintain hepatocytes in culture for these purposes. Hepatocyte transplantation, although a successful method is still hampered by difficulties, one of which is keeping cells alive in suspension avoiding anoikis. Cryopreservation is successful to some degree in extending the viability and usefulness of primary hepatocytes but a large number of cells are lost using this method. In this study we investigated large-scale production of spheroids from primary human hepatocytes and their subsequent reversions to monolayer culture as a method for extended in vitro use and also to study the innate remodeling and plastic ability of hepatocytes.

Materials and Methods: Primary human hepatocytes were isolated from resected tissues or donor organs not used in transplantation. Spheroids were formed using oscillating rocker technique or pendulum bioreactors and kept in suspension. Spheroids were subsequently placed on monolayer of diluted EHS and microscopy was performed using confocal and time-lapse techniques.

Results and Discussion: Large number of spheroids were rapidly produced from primary human hepatocytes. Spheroids formation was within 48 hours, which is a great advantage over conventional aggregate/spheroid forming methods requiring up to five days. The spheroids were of different sizes ranging single cells of 20 µm to large aggregates of >200 µm. Spheroids ranging 40 µm to 100 µm contained the largest ratio of viable cells and best morphology. They also proved the highest capacity for remodeling and reversion to single cell configuration in monolayer as shown by time-lapse microscopy. Spheroids retained monolayer-plateing capacity several days into suspension culture. These results suggest spheroids as a method of extending primary human hepatocytes in vitro and facilitate conditioning and modification such as gene editing/correction, evaluation of function and ultimately repeated clinical transfusions.

Conclusion: Large-scale rapid production of hepatocyte spheroids may help extend the utility of primary human hepatocytes. Suspension culture of hepatocyte spheroids can provide cells for repeated in vitro plating and provide an opportunity for pre-transplant conditioning and modifications.

Figure 1. Formation of spheroids and reversion to single cell state in primary human hepatocytes. Human primary hepatocytes formed large numbers of spheroids within 48 hours in a pendulum stirred bioreactor (A) ranging between 20–200 µm in diameter. The formed aggregate were plated on diluted EHS substrate and allowed to attach. Live confocal microscopy (B) revealed anchorage of aggregate (*) and infiltration of hepatocytes. Time-lapse microscopy (C) further revealed the plasticity of these spheroids in reverting to single cell configuration and forming conventional monolayer culture.

D}eceased Donor Adipose Tissue: An Untapped Source of Mesenchymal Stem Cells

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Purpose:
Adipose tissue from living donors is a widely accepted source of mesenchymal stem cells. Adipose tissue can also be procured from deceased donors in high volumes and routinely during the deceased donor workup.

Materials:
Adipose tissue was excised from the abdomen of research-consented deceased donors. Sections of the adipose tissue were mechanically minced and washed with saline. The mixture was then filtered, centrifuged, and resuspended in wash buffer to represent the stromal vascular fraction (SVF). The SVF was tested for viability, total nucleated cell count (TNC), and the presence of mesenchymal stem cells (MSC). The functionality of the MSC was assessed by their growth potential and their capacity to differentiate using specific differentiation media.

Results:
We observed that the mean TNC/g of samples processed fell above the living donor value ranges. The mean %MSC/TNC value fell within the living donor value range (Table 1).

Induction of adipocyte differentiation led to the morphological conversion of MSC to form lipid droplets, a characteristic of mature white adipocytes, accompanied by the expression of adipocyte differentiation genes. Similarly, differentiation of MSC into osteocytes and chondrocytes, when exposed to the respective differentiation media, was observed. Differentiation specific gene expression was also confirmed. In all cases, the undifferentiated cells did not exhibit any changes either at the morphological or gene expression level (Figure 1).

Conclusion:
The deceased donor stem cells can be routinely procured and potentially supplement the current available living donor stem cells sources. We have established that deceased donor adipose-derived MSC are similar to living donor adipose-derived MSC and can differentiate to adipocytes, osteocytes, and chondrocytes.

Figure 1. Differentiation of MSC isolated from non-enzyme treated SVF. Light microscopic images are representative of three separate experiments. (A) Adipocyte differentiation. (B) Osteocyte differentiation. (C) Chondrocyte differentiation. (B,D,F) Semi-quantitative qRT-PCR analysis of lineage-specific gene expression.
Procurement of Stem Cells from Deceased Donor Bone Marrow: A Viable Alternative to Living Donors

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Background:
Deceased donor bone marrow can be procured, substantially increasing the supply and access to stem cells without the pain, morbidity, and mortality associated with living donor stem cell collections. Obtaining organs and tissues for transplantation from deceased donors is a widely accepted strategy; however, during the routine deceased donor process, procuring the bone marrow is not performed. Our study outlines a process to isolate hematopoietic stem cells from deceased donors. Deceased donor stem cells can be routinely procured and potentially supplement the current available living donor stem cells sources.

Materials/Methods:
The extraction of iliac crest bone marrow from research-consented deceased donors was performed using an aspiration needle, a heparinized syringe, and a collection bag system with various filters. The procurement was performed within 2 hours of cardiac death. Theuffy coat was isolated and used for immunostaining to detect expression of cell surface markers CD34 and CD45, and viability. The functionality of the isolated hematopoietic stem cells was determined by using a Colony Forming Units assay. The deceased donor buffy coat was plated at a density of 1.25 x 10⁴ cells per well and incubated in a CO2 incubator set at 37°C and 5% CO2 for 14–16 days. After the incubation period, each distinct colony was counted and identified by its specific morphological characteristics using an inverted phase contract microscope.

Results:
We observed that the mean values of hematopoietic stem cells that we procured from deceased donor bone marrow were well within the range of the corresponding values from living donors. We observed growth of cell colonies after 14 days of incubation. These colonies had a characteristic growth pattern that was identical to the colonies observed from similar samples obtained from living donors.

Conclusion:
Together, our data demonstrates that our novel approach to isolate stem cells from deceased donors could be a routine practice to provide a viable alternative to living donor stem cells.
Mesenchymal Stem Cells (MSCs) Ameliorate Acute Kidney Injury Through Enhancing mtDNA transcription and Mitochondrial Biogenesis

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Background
Acute kidney injury (AKI) is a serious worldwide health issue with high mortality and morbidity. Kidney is a high energy-demanding organ that requires well-functioning mitochondria. MSCs have shown multiple benefits to AKI, but their specific role on renal mitochondria is not clear. In this study, we evaluated the effects of MSCs on renal function and mitochondrial injury in AKI mice.

Methods
MSCs isolated from C57/BL6 mice were infused into ischemic-reperfusion (I/R)-induced AKI mice via tail vein injection. Mice were sacrificed at 5 days after surgery, and blood/kidney samples were collected for clinical biochemistry, real-time PCR, TEM and histological examination.

Results
MSCs significantly improved renal function with decreased levels of serum BUN/CREA and renal necrotic tubules, and reduced KIM-1 (renal injury marker) and inflammatory cytokines (IL-1β/ICAM-1) in AKI mice. Moreover, BMSCs significantly rescued renal mitochondrial lesions with reduced mtROS, increased levels of ATP, mtDNA copy number, TFAM (master mediator of mtDNA transcription), mitochondrial biogenesis-related genes (PGC1-α, ATP5a-1, NDUFS8), and elevated mitochondrial cristae density and length/width ratio in AKI mice.

Conclusion
This study indicated that MSCs therapy could ameliorate renal dysfunction after AKI, and their renal protective effects were through enhancing mtDNA transcription and mitochondrial biogenesis.

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References:

Enhancing In Vitro Islet Function Using a Novel Necroptosis Inhibitor

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Introduction
Necroptosis was previously found as a mechanism of beta-cell death in diabetes and in human islet culture. Necrostatin-1 (Nec-1), a potent inhibitor of necroptosis, could reduce human islet necroptosis. This study aims to assess whether Nec-1 could improve the survival of pre-weaned porcine islets (PPIs) during maturation culture, and whether it affects their insulin secretory function.

Methods
PPIs were isolated from pancreata of 4-11-day-old, pre-weaned Yorkshire pigs and cultured in optimized islet culture media (37°C, 5% CO2) for 3 days. At day 3, the islets were divided into 2 groups, in islet culture media alone (control) or supplemented with Nec-1 (100µM, Sigma, n=3), and then cultured until day 7. Islet recovery was calculated as the percentage of IEG per gram of pancreatic tissue on day 7 compared to day 3. Flow cytometry (NovoCyte 3000/VIYB) was used to determine islet cellular viability, composition, GLUT2 expression, and differentiation. In vitro function was determined by glucose-stimulated insulin release assay. Data was expressed as mean ± SEM and evaluated using ANOVA with p<0.05 considered statistically significant.

Results & Discussion
Islet recovery was significantly higher in Nec-1 treated group (control=51.3±10.2% vs. Nec-1=112.8±6.4%, p<0.01, Figure 1). Nec-1 treated islets had a two-fold increase in the percentage of β-cells compared to untreated islets (control=8.9±1.3% vs. Nec-1=17.2±1.4%, p<0.01). Additionally, GLUT2 expression in β-cells was significantly elevated in Nec-1 treated group (control=29.9±3.2% vs. Nec-1=55.1±0.7%, p<0.05). A substantial increase in insulin secretion in response to glucose challenge was observed when the islets were cultured with Nec-1 (2.8 mM glucose: control=0.9±0.2 pg/ng vs. Nec-1=13.8±2 pg/ng, p=0.01; 28 mM glucose: control=2.3±1.6 pg/ng vs. Nec-1=38.8±5.2 pg/ng, p<0.01; Si: control=2.5±0.3 vs. Nec-1=5.3±0.6, p<0.01).

Conclusions
Identifying cytoprotective agents that could maintain islet quantity and quality during extended culture is critical to optimize the current pre-transplant protocol. Our data showed that Nec-1 improved islet recovery, increased proportion of endocrine cells, increased GLUT2 expression in β-cells, and improved insulin response to glucose. We believe that Nec-1 supplementation in culture media will enhance islet quality before transplantation. Future studies will be done to titrate the most effective dose of Nec-1, and evaluate these islets in rodent transplant models of Type 1 diabetes. Juvenile Diabetes Research Foundation.

Abstracts

Figure 1. Islet Recovery - The percentage of islet recovered as calculated by the IEG per gram of pancreas counted on day 7 compared to day 3. Data was expressed as mean ± SEM. * denotes a significant value of less than 0.05.
MSCs Protect Hepatocyte from Saturated Fatty Acid Induced Lipotoxicity through Alleviation of ER Stress by Enhancing SERCA Activity

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Background: Non-alcoholic fatty liver disease (NAFLD) has become one of the most prominent forms of chronic liver disease worldwide in recent years. Although the intense lifestyle change aiming at weight loss is the main therapeutic way for hepatic steatosis, it is non-effective when NAFLD has progressed to NASH and further liver damages. Mesenchymal stromal cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types. The increasing number of evidence suggested that MSCs have great potential to intervene NAFLD or T2DM. Nevertheless, how MSCs modulate metabolic balance and attenuate hepatic lipotoxicity in the context of NAFLD is still elusive. The sarco/endoplasmic reticulum (ER/SR) Ca²⁺ ATPase (SERCA) transports Ca²⁺ from the cytosol to the ER or SR lumen, maintaining the resting calcium concentration. Previous studies have demonstrated that SFAs inhibited the activity of SERCAs, which lead to calcium release from the ER lumen and a heavy calcium overload in the cytosol, and ultimately, result in the disruption of ER homeostasis and cell function. The aim of this study was to explored the relevant mechanism of MSCs on palmitic acid (PA) treated hepatocytes, and focusing on SERCA function.

Methods: Human hepatoma cell line HepG2 cells were exposed to palmitic acid (PA) for 24 h to induce lipotoxicity in vitro. Human umbilical MSCs were co-cultured with HepG2 by trans-well at 1:5 proportion. For further exploration of whether SERCA2 was essential for MSCs mediated protection, the HepG2 cells were treated with thapsigargin, an inhibitor of SERCA2, or transfected with SERCA2-specific siRNA.

Results: PA induced lipotoxicity and ER stress in HepG2 cells, and MSCs were able to restore the cell viability, alleviate the hepatic steatosis, and decrease the expression of ER stress markers, such as Bip, ATF6, ATF6, p-eIF2α and CHOP. In addition, PA remarkably impaired the protein expression as well as activity of SERCA2 in HepG2 cells, leading to imbalanced intracellular calcium homeostasis during ER stress. However, these disturbances were effectively rescued in MSCs treated groups. Furthermore, MSCs ameliorated the ER stress and cell damage induced by the SERCA inhibitor (thapsigargin) in HepG2 cells, accompanied by improved SERCA expression (Fig. 1). Meanwhile, SERCA2 silencing obviously abolished the ability of MSCs to reverse the PA induced cell damage, including the impaired cell viability, glucose utilization, ATP content and the disturbances in calcium homeostasis of HepG2 cells (Fig. 2).

Conclusion: Taken together, this study demonstrated that MSCs ameliorated lipotoxicity in PA treated HepG2 cells via the regulation of ER stress and the calcium homeostasis, in which the sarco/endoplasmic reticulum Ca²⁺-ATPase SERCA has played a key role.
Peripheral Infusion of Human Umbilical Cord Mesenchymal Stem Cells Improves Acute Liver Failure in Monkeys: A Preclinical Study

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Introduction: Acute liver failure (ALF) is associated with high mortality, and liver transplantation is the only treatment option. We have previously reported the pathophysiological mechanisms of ALF in a non-human primate model challenged by α-toxin. Our data highlighted the critical role of circulating monocyte-derived IL-6 in the initiation and acceleration of cytokine storm and ALF. We also reported that early peripheral infusion of human umbilical cord mesenchymal stem cells (hUC-MSCs) disrupts the development of cytokine storm by inhibiting the activation of circulating monocytes, offering a promising therapeutic strategy to this lethal syndrome. However, it is unknown whether this treatment is still effective when the cytokine storm is fully developed. This study was designed to evaluate the efficacy of hUC-MSC treatment in rhesus monkeys with fully developed ALF. Methods: Sixty healthy male rhesus monkeys, aged at 4–6 years, were randomly and averagely divided into two groups and the monkeys in each group were intraperitoneally administered α-toxin at either 20 or 40 μg/kg, respectively. Forty-eight hours after toxin injection, monkeys in each group were further averagely divided into 3 groups and were treated either 1x10⁷ hUC-MSCs, 2x10⁷ hUC-MSCs, or equal value of saline. Blood and liver specimens were harvested and imaging examination was performed at indicated time points. Results: 48 hours after toxin exposure, the liver indices of the monkeys increased significantly. All the monkeys received 20 μg/kg toxin survived and their sera indices returned to normal levels at 312 h, 84 h or 60 h following infusion of saline or 1U cells or 2U cells, respectively. However, in animals received 40 μg/kg toxin, 1, 2, or 3 animals survived when they were treated with saline, 1U cells or 2U cells, respectively. Despite the high mortality, hUC-MSC therapy has also shown potential to protect liver histology and improve systemic aberrance. Discussion and conclusion: Although systemic inflammation has detrimentally hampered the development of implantable devices. We have previously observed the myriad of immune activation following alginate hydrogel implantation, with macrophages being in the forefront of such immune responses [2–4]. We have previously observed the anti-inflammatory interaction of condition media (CM) from MSCs with macrophages. We hypothesized that alginate capsules loaded with MSC condition media, hereafter called anti-inflammatory capsules, could minimize the foreign body response (FBR) against alginate capsules.

Methods
UPLVG alginate (NovaMatrix®, Sandvika, Norway) were made with endotoxin-free sterile water and mounted. The alginate solution was added drop-wise into a gelling solution composed of sterile 120 mM barium chloride to generate circular microcapsules. Anti-inflammatory hydrogels were also prepared by dehydration-rehydration method, where rehydration step was conducted by MSC CM. Macrophages were cultured in RPMI with 10% Heat Inactivated FBS and 1% L-glutamine. Data was expressed as mean ± SD and evaluated using ANOVA with p<0.05 considered statistically significant.

Results and Discussion
We activated macrophages using Lipopolysaccharide (LPS) as positive control. Figure 1 shows that the alginate polymer upregulates the CD68 expression in macrophages (Figure 1A). Addition of MSC CM significantly reduces the upregulation of CD68 in macrophages activated with alginate (Figure 1A, B) or LPS (Figure 1A, C). We next implanted both control and anti-inflammatory hydrogels subcutaneously to immunocompetent mice (C57). We found that total cell infiltration around anti-inflammatory hydrogels is significantly less that control samples (Figure 2).

Conclusion
UPLVG alginate inherently activate macrophages to produce inflammatory cytokines and upregulate their CD68 marker, which follows the same pattern as LPS induced macrophage activation. We found that MSC CM could remarkably prevent such activation either in LPS- or in alginate-activated macrophages. These observations suggest that anti-inflammatory capsules could serve as long-term functional implants for cell transplantation.

References:

Figure 2. log-logistic distribution survival functions (A) and the corresponding hazard functions (B). The black curve represents the hazard function of no prophylaxis group and the grey curve represents the hazard function of early prophylaxis group. No prophylaxis group has a higher risk of viremia than early prophylaxis group with a peak of hazard at 71 days.
Hematopoietic Stem/Progenitor Cell Dependent Participation of Innate Lymphoid Cells in Low-Intensity Sterile Inflammation

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Innate lymphoid cells (ILCs) are increasingly acknowledged as important mediators of immune homeostasis and pathology. They act as early orchestrators of immunity and as such they make up interesting therapeutic targets for several diseases and possibly a means to peripheral tolerance. Taking advantage of an animal model of sterile peritonitis, induced by intraperitoneal injection of zymosan, a fungal derived TLR-2 ligand we studied the progenitor–successor relationship between the hematopoietic stem/progenitor cells (HSPC) and ILCs at the site of sterile inflammation. Our results demonstrate that the strength of the inflammatory signal affects the capacity of BM-derived HSPCs to migrate and give rise in situ to ILCs. Both low- and high-dose of zymosan injections triggered the appearance of mature ILCs in the peritoneal cavity. Only in low-dose injected mice, the recovered ILCs seemed to be dependent on an in situ differentiation of BM-derived HSPCs and/or ILC2 precursors (ILC2P) wherein high-dose, the stronger inflammatory environment seemed to be able to induce the emergence of ILCs independently of BM-derived HSPCs.

To further elucidate the importance of the inflammatory environment per se for the attraction of mature ILCs, an anti-inflammatory drug was used. Treatment of mice with alpha-1 anti-trypsin (AAT) 15 min after injection of either low- or high- dose of zymosan, led to the blockade of the migration of HSPCs into the peritoneal cavity resulting in a loss of even mature ILCs in mice injected with the low-dose of zymosan. Contrary, in mice injected with high-dose, progenitor cells of type 2 of ILCs could be still recovered, but not mature ILC2 cells.

We suggest that the relationship between HSPCs and ILCs seem to be affected by the strength of the inflammatory stimuli and that the use of anti-inflammatory molecules like A1 antitrypsin may attenuate the inflammation and to some extent prevent the migration of HSPCs to the site of inflammation and the emergence of mature ILCs.
40 Noninvasive Monitoring of Transplanted Hepatocytes by Deep Red Imaging in Rats

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Technologies for in vivo imaging gain significance to monitor distribution and integration of cells for the use in cell therapies during the regeneration of diseased organs. The aim of the study was to identify NIR dyes for visualization of transplanted hepatocytes in vivo in rats. Wild type and CD26-deficient hepatocytes and livers from wild type Fisher rats were used. A total of six dyes (DY676, 681, 776, 777, 782, 800 emission range 699-801nm) were analyzed using a multispectral imager. For quantification, the Regions-of-Interests (ROI) of defined mixtures of CD26-positive and negative cells were detected. Results were verified by flow cytometry.

DY676, 776, 777, 782, 800 yielded non-specific conjugates with undefined cellular components. DY681 linked to the CD26-antibody specifically detected CD26-positive hepatocytes. DY681 displayed ROIs of 372, 375, 244, 102 and 9 [average signal] using defined mixtures of 100%, 85%, 50%, 15% and 0% CD26-positive cells. Flow cytometry confirmed 99%, 78%, 56%, 21% and 0.2% CD26-positive cells. Wildtype livers treated in situ with DY681-CD26 antibody displayed positive signals throughout the whole organ.

The DY681-linked antibody is eligible for the non-invasive labeling of transplanted hepatocytes. Thus, the hepatic distribution of the cells and repopulation of the liver might be observed, which helps to reduce the amount of animal experiments.

Wilhelm Roux Program 19/38.

42 Molecular Pathways in Support of Allogenic Human Amnion Epithelial Cell Transplantation for Liver Disease Without Immunosuppression

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Placenta is a non-controversial and readily available source of stem cells for regenerative medicine. We previously reported that human amnion epithelial cells (hAEC) from term placenta are not tumorigenic, have immunomodulatory and anti-inflammatory properties and are able to differentiate into functional hepatocyte-like cells. In preclinical studies with immune-competent mice, hAEC engrafted and survived without administration of immunosuppressive drugs, resulting in correction of metabolic liver diseases or the reversal of acute liver failure. In clinical settings allogenic hAEC have been transplanted without immunosuppression, and during the last year we studied and identified molecular pathways through which hAEC can drive immunooacceptance by the host immune system.

We performed a complete surface screening of hAEC, profiling all the molecules commonly described on other stem as well as somatic cells. Amnion characteristically lacks HLA class 2 expression and expresses both class 1a and non-polymorphic class 1b (responsible for maternal immune-toleration of the fetus). We quantified the level of expression of HLA-G and HLA-E molecules both as membrane-bound and soluble forms. Recently, purinergic mediators, hydrolyzed by plasma membrane nucleotidases, have also been identified to regulate immune cell response, thus we quantified the level of expression of all ecto-enzymes in hAEC preparations.

hAEC constitutively express all known ecto-enzymes. In addition, we proved constitutive expression of cd47 (‘don’t-eat-me’ signal) and complement system (CD55/CD59). We measured immunogenicity of hAEC on purified immune effector cells involved in disease pathogenesis (with particular attention to Th1/Th2 and M1/M2 switch and Treg/Breg induction). We measured amnion-derived cells to actively interact and crosstalk with innate and adaptive immune cells not only by cell-to-cell interactions but also through soluble mediators and vesicles with characteristic surface profile.

Conclusions: High level expression of ecto-enzymatic axis and non-canonical HLA molecules likely play a key role in immunological tolerance and long-term acceptance of the human xeno-cell graft in immunocompetent mice. The ability to treat the most common (liver) diseases with one stem cell therapy without the administration of immunosuppressive drugs could be a “game changer” and will greatly expand the number of patients who could receive cellular therapy. Based on their safety and the successful preclinical studies, approval was granted to begin banking of hAEC under cGMP condition at Karolinska Institutet, and to perform hAEC transplants on 10 patients with liver disease without immunosuppression.
Use of Nonhuman Primates as a Preclinical Model to Test In Vitro Treatments That Were Reported to Improve the Outcome of Muscle Precursor Cell Transplantation in Mice
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Introduction
Transplantation of muscle precursor cells (MPCs) is a major strategy for the potential future treatment of several skeletal muscle diseases. The protocol of MPC transplantation that we developed in non-human primates (NHPs) allowed the restoration of the genetically missing protein (dystrophin) with better efficacy than previously in muscle fibers of patients with Duchenne muscular dystrophy. However, our protocol needs improvements to obtain a broader cell engraftment and thus increase dystrophin restoration. Studies in mice reported methods that substantially increased the MPC engraftment, based on in vitro treatments and sometimes co-injection of the cells with growth factors or other molecules. Since these methods would be easily applicable in the clinic, we wanted to verify them in NHPs, given that this animal model is crucial in preclinical transplantation research before translating protocols to humans.

Methods
We allotransplanted CD56+ MPCs labeled with β-galactosidase (β-Gal) to muscle regions of 1 cm³ (around 25 x 10⁶ viable cells per site) in four cynomolgus monkeys. Approximately 20 x 10⁶ cells were injected per site, always following the same pattern: matrices of 25 injections per cm², delivering 25 µl of cell suspension per injection. Tacrolimus, combined to dexamethasone, was administered daily to control acute rejection. The strategies to improve the MPC engraftment consisted in the in vitro treatment of the cells (and sometimes co-injection of them) with the following molecules: insulin growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), IGF-1 + bFGF + plasmin, concanavalin A and SB203580 (a p38 MAP kinase inhibitor). One of two cell-grafted regions was treated by electroporation to induce broad muscle fiber regeneration concomitant to the graft. Cell grafted regions were sampled one to two months after cell transplantation, snap frozen in liquid nitrogen and sectioned in a cryostat. Muscle cross-sections were stained with hematoxylin and eosin, and for histochemical demonstration of β-Gal. β-Gal+ muscle fibers were quantified as an indication of engraftment success.

Results
The only factor that substantially increased the number of β-Gal+ muscle fibers was the extensive muscle fiber regeneration produced by the muscle electroporation. Otherwise, for the same cell transplantation conditions (injection with or without electroporation) none of the cell treatments increased the amount of β-Gal+ muscle fibers and therefore the success of the cell transplantation in terms of genetic complementation.

Conclusion
Cell treatments reported to increase the MPC engraftment in mice had no incidence in NHPs. The only strategy capable of increasing the MPC engraftment in NHPs remained the induction of extensive muscle regeneration concomitant with the cell transplantation. As in other areas of preclinical research, this study also questions the clinical validity of results reported only in mice. This work was supported by a grant of the Jesse’s Journey Foundation for Gene and Cell Therapy of Canada to D.S. and a grant of the Canadian Institutes of Health Research to J.P.T.

Successful Transplantation of Human Muscle Precursor Cells in Nonhuman Primates Using Tacrolimus Immunosuppression: A New Model for the Preclinical Test of Other Potentially Myogenic Human Cells
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Background
Different cells isolated from human tissues were proposed for cell transplantation in the skeletal muscle as a treatment of myopathies, essentially muscular dystrophies. However, the myogenic properties of most of these cells were found only after xenotransplantation in immunodeficient mice. Given that cell transplantation protocols developed in nonhuman primates (NHPs) were better extrapolated to humans than those that were not verified in this model, and considering that NHPs are crucial in preclinical transplantation research, we wanted to test the feasibility of xenotransplantation of human myogenic cells in NHP muscles.

Methods
Human CD56+ muscle precursor cells (MPCs) were transduced with the LacZ gene using a replication defective retroviral vector. They were injected into muscle regions of 1 cm³ (around 25 x 10⁶ viable cells per site) in four cynomolgus macaques immunosuppressed with oral tacrolimus (Advagraf) and dexamethasone. Allogeneic LacZ-labeled MPCs were transplanted similarly in other muscles as a positive control for cell engraftment. Cell-grafted regions were sampled 1 month later and analyzed by histology in cryostat serial sections using β-galactosidase (β-Gal) histochemical detection, hematoxylin and eosin stain, and CD8 immunodetection. Blood was taken before transplantation and before muscle sampling to detect antibodies against the grafted cells. Tacrolimus blood levels were quantified by liquid chromatography tandem mass spectrometry.

Results
Tacrolimus blood levels and Advagraf doses in the monkeys of the study are shown in figure 1. In monkeys #1 and #2 we targeted tacrolimus blood levels over 40 µg/L, while in monkeys #3 and #4 we targeted levels closer to those used in humans (around 20 µg/L). Abundant β-Gal+ myofibers were found in all regions grafted with human MPCs (an average of around 25/mm²) distributed in bands aligned according to the injection trajectories (figure 2, red arrows indicate the original sense of the cell injections). The histological analyzes showed absence of specific cellular immune responses in three monkeys and minimal focal lymphocytic infiltrates in the monkey that had the lowest tacrolimus blood levels. Similar patterns of β-Gal+ myofibers were observed in all regions grafted with cynomolgus MPCs (figure 2), in the absence of specific immune responses. Anti-donor antibodies were not detected in the sera.

Conclusions
We demonstrated that human MPCs can form hybrid myofibers in NHP muscles and that a conventional tacrolimus-based immunosuppression is sufficient to control rejection in this case. This opens the door to NHP studies with other human cells in which myogenic properties were found by xenotransplantation in immunodeficient mice, validating the myogenicity of these cells in a more appropriate model than mice for clinical translation and investigating the administration parameters necessary in humans.

This work was supported by a grant of the Jesse’s Journey Foundation for Gene and Cell Therapy of Canada to D.S. and a grant of the Canadian Institutes of Health Research to J.P.T.
The Effect of Transplantation of Undifferentiated Mesenchymal Stem Cells in Experimental Model of Myocardial Infarction: Preliminary Results

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Introduction: Cardiovascular diseases like ischemic myocardial infarction remain worldwide with high lethality and mortality beside the extended researches for pharmaceutical therapies. Isolation of mesenchymal stem cells (ADSCs) from adipose tissue created a new spectrum of research. ADSCs are now known to be able to differentiate to myocardial cells and create cardiac tissue. In our protocol we use the genetic factors GATA-4 and nkx2,5 which are associated with the regeneration and differentiation of the myocardium respectively.

Methods: We used 17 adult Wistar rats of conventional microbiological status (15 females – receivers and 2 males - donors) weighting 200-250gr. The adipose tissue was collected from the male donors and was immediately transferred to the laboratory at 4°C. The female-receivers were randomly allocated in 5 groups (3 animals per group). On Day -7 (7 days pre-operatively) we performed a SPECT–CT with injection of 99mTc-sestamibi to all female animals. On Day 0 (operation day) all groups underwent left thora-coctomy and - besides sham operated - ligation of LAD for 45min with ECG monitoring confirming ischemia. Control 1: injection of N/S intramyocardially and removal of ligation. Control 2: injection of N/S intramyocardially without removal of ligation. Sham: no ligation. Experimental 1: injection of ADSCs intramyocardially and removal of ligation. Experimental 2: injection of ADSCs intramyocardially without removal of ligation. On Day +7 and Day +14 (7 and 14 days post-operatively) the animals that survived underwent new SPECT-CT followed by euthanasia on Day +16 (16 days postoperatively) together with blood sampling and heart harvesting for histological and immunochemical evaluation.

Results: ADSCs were successfully engrafted into the myocardium and had beneficial effect on the ischemic myocardial area of the experimental groups with regeneration and increase of contractility especially in the 14th post-operative day compared with the control groups. The expression of GATA-4, nkx2,5 and IL-6 was significantly upregulated compared with the control groups.

Conclusion: ADSCs are definitely the therapeutic approach of the future and GATA-4 as nkx2,5 are the genetic factors that are leading to an extended regeneration of the ischemic myocardium. The promising results so far are leading us to increase the number of animals in our protocol to further evaluate the clinical significance of ADSCs transplantation in cases of acute myocardial infarction.

Introduction: Cardiovascular diseases like ischemic myocardial infarction remain worldwide with high lethality and mortality beside the extended researches for pharmaceutical therapies. Isolation of mesenchymal stem cells (ADSCs) from adipose tissue created a new spectrum of research. ADSCs are now known to be able to differentiate to myocardial cells and create cardiac tissue. In our protocol we use the genetic factors GATA-4 and nkx2,5 which are associated with the regeneration and differentiation of the myocardium respectively.

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Results: ADSCs were successfully engrafted into the myocardium and had beneficial effect on the ischemic myocardial area of the experimental groups with regeneration and increase of contractility especially in the 14th post-operative day compared with the control groups. Specifically imaging with SPECT-CT revealed significant absorption of 99Tc and viability of the myocardium on postoperative Day 14 in the experimental groups. The expression of GATA-4, nkx2,5 and IL-6 was significantly upregulated compared with the control groups.

Conclusion: ADSCs are definitely the therapeutic approach of the future and GATA-4 as nkx2,5 are the genetic factors that are leading to an extended regeneration of the ischemic myocardium. The promising results so far are leading us to increase the number of animals in our protocol to further evaluate the clinical significance of ADSCs transplantation in cases of acute myocardial infarction.
Bioartificial Endocrine Pancreas Generated from Decellularized Human Placenta for Type-1 Diabetes Treatment

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3Institute of Medical Research, Ilia State University, Tbilisi, Georgia.

Background
Development of vascularized biological scaffolds providing mechanical protection for islets is a challenging objective of modern regenerative medicine. The aim of our study was to develop vascularized endocrine pancreas using decellularized placental cotyledons.

Methods/ Materials
Human placentas were decellularized by perfusion, using 0.5% sodium dodecyl sulfate. Decellularization was assessed by histological analyses, scanning electron microscopy (SEM) and residual DNA quantification. Glucosaminoglycans (GAG) and hydroxyproline were analyzed. Placental cotyledons were recellularized by intravascular perfusion of human umbilical vein endothelial cells (HUVECs) during the 24h, followed by intraparenchimal injection of INS-1E cells. Recellularization was assessed by histological and immunohistochemical methods, and endocrine function was confirmed by glucose stimulated insulin secretion tests performed at different time points.

Results
Histological staining and SEM showed complete decellularization and well preserved ECM structure. This was confirmed by the absence of residual DNA. Immunostainings of recellularized cotyledons showed insulin expressing islet like structures with intense vascularization confirmed by CD31 staining. Adequate insulin secretion in response to high glucose stimulation was also observed, confirming the functional activity of INS-1E cells.

Conclusion
These data demonstrate that acellular placental cotyledons seeded with endocrine pancreatic tissue and endothelial cells could be used for functional pancreas bioengineering.
Islets Loaded in Hydrogel Derived from Human Amniotic Membrane Reverse Diabetes in Mice

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1Department of Surgery, Cell Isolation and Transplantation Center, Geneva, Switzerland.
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Background
Development of macroencapsulation, neovascularized devices and biopolymer scaffolds that could be easily loaded with islets and implanted, with the aim of constructing a bioartificial pancreas may help to reduce the risks and improve the success rate of islet transplantation. Human amniotic membrane (HAM) is inexpensive and attractive as a biomaterial due to its structural similarities to islet extracellular matrix (ECM), and its immunomodulatory, anti-inflammatory and antifibrotic properties. The aim of our study was to develop hydrogel derived from HAM and assess whether it could support islet function in vitro and in vivo.

Methods/ Materials
The hydrogels were generated from HAM and accessed for porosity and ECM content. The protein content in HAM derived hydrogels and native HAM lysates were measured. To assess hydrogel impact on islet viability and function isolated rat islets were incorporated into the hydrogels and cultured for one week. The cell viability was evaluated by FDA/PI staining. To demonstrate islet function the glucose stimulated insulin secretion (GSIS) tests were performed using standard ELISA. Next, we assessed whether incorporation of islets into hydrogel could enhance engraftment and lead to better glycemic control in diabetic SCID mice. For this purpose 350 rat islets (IEQ) loaded into the hydrogels or islets alone (control) were transplanted into the epididymal fat of diabetic SCID mice. Blood glucose levels were monitored daily and intraperitoneal glucose tolerance tests (IPGTTs) were carried out. Grafts and serum were harvested at 1, 2, 6 and 12 weeks after transplantation to assess outcome.

Results
The ECM concentration in the hydrogel affected the pore size. Insulin and glucagon expression and viability of islets incorporated into hydrogel was significantly higher than that of islets in free-floating culture. In addition, significant enhancement of GSIS was observed from islets embedded in hydrogel as compared to controls. In vivo experiments showed that, transplantation of 350 IEQ embedded in hydrogel lead to enhanced engraftment, vascularization, viability and better glycemic control compared to control mice transplanted with islets alone.

Conclusions
Incorporation of pancreatic islet into amnion-derived hydrogels enhances islet engraftment and is a valuable approach to improve islet transplantation outcomes.

This work was supported by a grant from the Swiss National Science Foundation (Grant #310030_173138 to TB, EB, DB) and a grant from the European Foundation for Study of Diabetes (to EB and DB).
The Westmead Islet Transplant Program Evaluation of Factors Influencing Islet Isolation Outcomes Over 20 Years

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Aims
Successful islet cell isolation to treat type 1 diabetes is influenced by multiple factors including donor selection, organ procurement and isolation parameters. This study aimed to identify the key contributing factors that affected the outcome of islet preparations in order to proceed to transplantation.

Methods
Islets were isolated from DBD donor pancreata using collagenase and neutral protease (SERVA). Donor characteristics, procurement data, isolation yield and outcomes were analysed to determine variables associated with transplantable yields. Data was further divided into Transplanted (Tx) and Non-transplanted (NTx) to identify factors significantly related to successful outcomes.

Results
Data collected from 250 islet isolations between July 2000 and February 2019 were evaluated. On average, 28% of islet preparations were transplantable, with 54% of isolation outcomes, resulting in significant improvements to transplantable yields of islets for treatment of our patients with Type 1 diabetes.

Conclusion
Our focus has been to improve our overall outcomes and minimize our complications. Data was further divided into Transplanted (Tx) and Non-transplanted (NTx) to identify factors significantly related to successful outcomes.

Compared to NTx (n=180), Tx (n=70) had significantly higher total IEQ (573,977±29,352 VS 325,563±14,625 IEQ) and IEQ/g pancreas (7,018±388 VS 2,798±45 IEQ/g), with higher donor weight, BMI, pancreas weight, and significantly lower CIT (10hrs) compared to NTx. Tx islets also exhibited significantly higher viability, purity, beta cell viability and stimulation indices compared to Non-tx (p<0.05).

Bioresorbable scaffolds have been used to improve islet yields and minimize complications. Data was further divided into Transplanted (Tx) and Non-transplanted (NTx) to identify factors significantly related to successful outcomes.

Introduction:
During the last decade, stem cell therapy has rapidly progressed and is considered one of the most promising therapeutic strategies for a broad range of fatal diseases. Regardless of the intense research and the continuous advances on this topic, the current therapeutic approaches are still lacking on the efficient non-invasive optimization of the treatment. To overcome these barriers, the design of tools, which could provide real-time tracking of transplanted cells, allowing the early monitoring of their biodistribution and viability, is of utmost importance. Gold nanoparticles (GNPs) are established as contrast agents in Computed Tomography (CT) and they can be easily radiolabelled and imaged through Single Photon Emission Tomography (SPECT). Following these methods, non-invasive imaging can be used to perform cell tracking, in a stem cell therapy scheme regarding muscle regeneration.

Methods & Materials:
Biocompatible and functional nano-based multimodal imaging agents are developed in order to enable non-invasive monitoring of living stem cells in small animal models through SPECT and CT imaging. Following these methods, non-invasive imaging can be used to perform cell tracking, in a stem cell therapy scheme regarding muscle regeneration.

Conclusion:
The purpose of this study is to present the workflow and first results of monitoring living stem cells on a muscle regeneration mouse model, through SPECT/CT imaging. The stem cells are labelled with [111In] In DTPA and imaged through Single Photon Emission Tomography (SPECT). The number of cells remaining in the region under treatment is also quantified, giving a preliminary estimation of the success of a cell therapy scheme.

Using these methods, non-invasive imaging can be used to perform cell tracking, in a stem cell therapy scheme regarding muscle regeneration.

Results & Discussion:
The purpose of this study is to present the workflow and first results of monitoring living stem cells on a muscle regeneration mouse model, through SPECT/CT imaging. The stem cells are labelled with [111In] In DTPA and GNPs, through an established protocol and kinetics are monitored non-invasively through CT and SPECT. The number of cells remaining in the region under treatment is also quantified, giving a preliminary estimation of the success of a cell therapy scheme.

Conclusion:
An in vivo platform and a standardised methodology to perform stem cell tracking on a muscle regeneration model, through non-invasive SPECT/CT imaging, were established and are being presented. The first results, showing the fate of the GNPs and of labelled cells is being presented.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 761031. This study was co-supported through the Programme of Industrial Scholarships of Stavros Niarchos Foundation.

Figure 1. The concept of the nTrack project: non-invasive imaging to perform cell tracking, in a stem cell therapy scheme regarding muscle regeneration.

Figure 2. GNPs as a CT contrast agent, to label and track stem cells after intramuscular injection on a muscle regeneration model.
Bone Regeneration Assessment Through SPECT/CT Imaging in a Mouse Calvarial Defect Model

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Introduction

The evaluation of novel synthetic bone implants relies mostly on histological examination, but as bone repair takes several weeks, non-invasive imaging techniques have gained interest in the recent past. In the frame of this study, we establish a SPECT/CT imaging protocol for the longitudinal assessment of bone healing of a calvarial mouse defect model using mineralized collagen-based scaffolds.

Materials and Methods

Two symmetrical calvarial bone defects, 1.5 mm in diameter each, were created in female Swiss mice. Gaps were monitored weekly through SPECT/CT imaging (γ-CUBE, X-CUBE, Molecubes, providing a resolution of 0.6 mm and 0.05 mm, respectively), using [99mTc]-MDP for 5 weeks. Defects created on the left part of the mouse skull were left unfilled (control group), whereas those on the right part were filled with mineralized horse collagen scaffold materials (HA/Coll).

Results and Discussion

The monitoring of bone healing was successfully and accurately performed via SPECT/CT imaging. CT imaging showed the actual bone filling process and the formation of new bone tissue, whereas SPECT imaging provided the increased metabolic activity. For the HA/Coll treated defect, a peak in metabolic activity was noted at week 1 post-surgery and the defect was filled by newly developed bone tissue by week 3 post-surgery (Figure 1). In contrast, the metabolic activity at the control defect area was stable but significantly lower and a complete healing was not observed even up to week 5 post-surgery. These results show that combined SPECT/CT imaging can provide an accurate tool for bone healing monitoring. CT is a high resolution tool for new bone formation control and SPECT is a high sensitivity tool for early metabolic activity confirmation, also having a high prognostic value for bone healing.

Conclusions

SPECT revealed the earliest signs of new bone tissue formation due to the increased metabolic activity and CT provided a clear visualization and quantification ability of the healing process over time.

This study is part of the EU Horizon 2020 research and innovation VIVOIMAG project under the Marie Skłodowska-Curie grant agreement No 645757. This study was co-supported through the Programme of Industrial Scholarships of Stavros Niarchos Foundation.

Figure 1. Indicative axial CT (a), sagittal SPECT/CT (b) and axial SPECT/CT (c) at 3 h [99mTc]-MDP post-injection (~2 mCi) of a bilateral calvarial mouse defect treated (HA/Col) and non-treated (control), at week 1 (first row) and at week 3 (second row) post-surgery.
Enhanced In Vitro Hepatocyte-like Clusters Differentiation From Human-induced

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2Chemical and Life Science Engineering, Virginia Commonwealth University, Richmond, VA, United States.

Introduction: Engineered tissues represent a great promise for the treatment of liver diseases. Differentiated hepatocyte-like clusters (HLC) have shown to be a potential alternative to native liver-derived hepatocytes. In this study, we optimized our differentiation protocol by enhancing specific signaling pathways in order to improve the maturation and function of our differentiated hiPSCs into HLC.

Material and Methods: Generation of human embryoid bodies (hEBs) using hiPSCs interlaced with endothelial cells (hiPSC+EC-EB), was achieved using our ROCK/Spin free technology. Our modified four-stage hepatocyte differentiation protocol was applied to both experimental conditions. We performed clinical relevant assays for hepatic function and markers in vitro and we are performing an induced acute liver failure rat model for the in vivo studies.

Results and Discussion: Enhancement of differentiated HLCs was determined by performing real-time for the gene expression of several hepatocyte markers such as Albumin, A1AT, OTC, HNF-6, CYP1A2 and CYP2B6, UGT1A1, UGT1A4, as well as several coagulation factors (Figure 1). By flow cytometry, the HLCs showed the presence of an increased percentage of double positive cells for albumin and A1AT, as well as albumin and HNF-4 α when compared with our control. Ammonium metabolism showed a better clearance of the NH4CL in the medium after 6 hours. Urea concentration showed a similar trend with our control indicating stability in its generation process followed by the ammonium metabolism. Functional analysis of coagulation factors X and thrombin showed a marked improvement in the generation of both factors, indicating that the modification of our differentiation protocol led to a more functional interaction between human endothelial cells and our differentiated HLCs (Figure 2).

Conclusions: The modification of our hepatic differentiation protocol using hiPSCs interlaced with human adipose tissue-derived endothelial cells, demonstrated improvement of critical hepatic markers and functions, necessary for progression to clinical translation.

Figure 1: Gene analysis showed an increased RNA expression several hepatic markers of our experimental condition compared with our experimental control.

Figure 2: Factor Xa generation showed an improved Vmax in our experimental condition compared to our control.
The Immune Regulatory Effect of CpG-ProB Cells on Allograft Survival

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2Institut Curie, Paris, France.
3Gastroenterology, Beth Israel Deaconess Medical Center, Brookline, MA, United States.

Introduction: Previous studies have identified a population of cells emerged when murine bone marrow cells were stimulated with CpG-B (c-kitlowSca-1lowCD19+ B220+CD1dhighIgM-CD5-). The CpG-ProBs were previously reported to be able to prevent diabetes in NOD mice and block progression of experimental autoimmune encephalomyelitis in C57BL/6 mice through immunoregulation. We therefore, hypothesize that CpG-ProBs might be able to suppress allo-immune response and prolong allograft survival in islet transplantation.

Materials and Methods: C57BL/6 (CD45.2) STZ diabetic mice were used as recipients, and transplanted with DBA/2 islets. The animals were divided into two groups, in which control group received PBS and the experimental group received C57BL/6 (CD45.1) CpG-ProBs the day of the transplantation. Graft survival was determined by blood glucose monitoring and all animals were sacrificed on day 10 post-transplantation (when all controls were rejected). The lymphoid organs and allografts were harvested and tracking studies were performed to evaluate the CpG-ProBs’ immune suppressive role on allograft survival.

Results and Discussion: 50-60,000 CpG-ProBs per transplant were corroborated to be the optimal number of cells that displayed immune suppressive effect. We followed the fate of the CpG-ProBs (CD45.1) into the C57BL/6 mice (CD45.2), and discovered that the progeny of injected cells expanded 2.5 - 3 fold by day 10. Of the recovered cells, 80–90% were TIM-1+, Ki67+, and had various stages of B cell differentiation. Moreover, CpG-ProBs were also recognized to express a certain level of TIM-1 which is a broad marker for Bregs. Ligation of TIM-1 with anti-TIM-1 triggers IL-10 expression, suggesting a link between CpG-ProBs and conventional Bregs. Tracking studies of the CpG-ProBs identified an increased population of CD4+FoxP3+ Treg as well as IL-10-producing cells in lymphoid organs. Proliferation (Ki67+) of CD4+FoxP3+ Tregs increased despite the fact that proliferation of both CD4+ T cells and CD8+ T cells decreased in the lymphoid organs. Histopathology analysis of the grafts in the experimental group showed well identified islets staining positive for insulin. In contrast, grafts from the control group showed no islet cells and polymorphic inflammatory cells at the graft site.

Conclusion: Syngeneic CpG-ProBs prolong islets graft survival by immune regulation. The underlying mechanisms possibly involve niche, B progenies, as well as T cell subsets. TIM-1 signaling might participate in CpG-ProBs regulatory effect.

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References:
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Derivation of Extracellular Vesicles In Vitro from Human Endometrial Mesenchymal Stem Cells with Potential for the Treatment of Neurodegenerative Diseases

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Introduction: Currently, human mesenchymal stem cells (hMSCs) are widely used in both research and cell-based therapy for various human diseases including neurological and neurodegenerative disorders. The biological unique characteristics of hMSCs include a relatively high proliferating-capacity and active secretion of both neurotrophic and anti-inflammatory factors contained in extracellular vesicles (EVs) when are cultured under animal-free conditions. The EVs secreted from hMSCs (EV-hMSC) contain in their cargo several mediators of biological functions attributed to hMSCs, which include tissue regeneration, immunomodulation and neuroprotection. In murine models of neurodegenerative, EV-hMSCs promote angiogenesis and neurovascular plasticity, neurogenesis; induce long-term neuroprotection and rodegenerative diseases. Currently, EV-hMSCs experiments are ongoing to study in non-human primates.

Materials and Methods: hMSC were derived and propagated in vitro from human endometrium obtained from a healthy voluntary donors who signed an informed consent. Then, we isolated EVs through ultracentrifugation from different culture conditions. The cargos of the resulting EVs were further characterized by using a transmission electron microscopy. The cargo proteins of the different EVs show differences between the different conditions. Presence of BDNF, NGF, NT-3, GDNF, VEGF and HGF in EVs correlates with the effects seen in preclinical studies of degenerative diseases and might act in synergy promoting neurogenesis in the neuronal stem cell niches: subgranular and subven- tricular zones.

Results: We were able to derive EVs and further characterized them in vitro from samples of human endometrium obtained from a healthy voluntary donors who signed an informed consent. Then, we isolated EVs through ultracentrifugation from different culture conditions. The cargos of the resulting EVs were studied and identified by using a combination of panels for growth and neurotrophic factors using a multiplex analyzing method. The correlation between neurogenesis and the EV-hMSC protein cargo was assessed for each culture condition.

Conclusion: Endometrial EV-hMSCs could be a potential treatment of neurodegenerative diseases. Currently, EV-hMSCs experiments are ongoing to assess the effects in vivo using a murine model of ischemia.

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Importance of the Timing Between the Induction of Muscle Regeneration and the Transplantation of Muscle Precursor Cells in the Outcome of the Graft: A Study in Nonhuman Primates

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Introduction

Transplantation of muscle precursor cells (MPCs) is a major strategy for a potential future treatment of genetic myopathies such as muscular dystrophies. The MPC transplantation protocol that we developed in non-human primates allowed the restoration of the genetically missing protein (dystro- phin) with better efficacy than previously in muscle fibers of patients with Duchenne muscular dystrophy, but needs improvements to obtain a broader cell engraftment and dystrophin restoration. Of the factors analyzed so far in non-human primates to increase the cell engraftment, the only one that favored a broader integration of the grafted cells in the muscle fibers of the recipient was the induction of diffuse muscle regeneration in parallel to the cell graft. Since muscle regeneration is a dynamic process, it is necessary to investigate the moment in which the grafted cells are more likely to integrate profusely into the recipient muscle fibers. To elucidate that, we carried out a study in non-human primates.

Methods

We transplanted allogeneic MPCs labeled with ß-galactosidase (ß-Gal) in muscle regions of 1 cm² of cynomolgus monkeys, using arrays of 25 parallel equidistant perpendicular injections. The sites were treated or not with intramuscular electroporation (3 pulses of 400 V/cm and 5 ms with a delay of 200 ms) to trigger massive muscle regeneration. MPC transplantsations were performed immediately after electroporation, as well as on days 1 to 7 after electroporation and days 1, 2, 3, 6 and 7 before electroporation. Tacrolimus was administrated daily to control acute rejection. Cell grafted regions were sampled one month after transplantation, snap frozen in liquid nitrogen and sectioned in a cryostat. Muscle cross-sections were stained with hematoxylin and eosin and for histochemical demonstration of ß-Gal. The amount of ß-Gal+ myofibers was quantified in the cross-sections as an indication of engraftment success.

Results

The amount of ß-Gal+ muscle fibers was significantly increased by a factor of 3.2 ± 1.2 in the muscles in which cell transplantation was done immediately after electroporation, with respect to regions grafted similarly but without electroporation (p < 0.0001 in a paired t test). The set of values from days +3 to +7 (cell transplantation post-electroporation) implied a significant reduction in the number of ß-Gal+ muscle fibers (p < 0.05 in a paired t test). There were no significant differences in the other timings, having in some cases a large variation of values.

Conclusion

According to these data, MPC transplantation must be concomitant with the induction of muscle regeneration to ensure an increase in the cell engraftment. If MPC transplantation is performed between days 3 and 7 after the induction of muscle regeneration, this can jeopardize the cell engraftment, while in the rest of the timings studied the results can be very variable.

This work was supported by a grant of the Jesse’s Journey Foundation for Gene and Cell Therapy of Canada to D.S. and a grant of the Canadian Institutes of Health Research to J.P.T.
CPS-1 Humanized Liver in FRGN Mouse as Model for Genetic Disorder

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Our laboratory has been pioneer in the use of cell therapy to treat patients with inborn errors of disease. However, for preclinical studies, many small animal models of monogenetic liver disease do not faithfully recreate the phenotype observed in human patients. Using special FAH and immune deficient mice (FRGN), we have created a chimeric model where the mouse liver can be highly repopulated with human hepatocytes. Hepatocytes were isolated from normal donors or from a patient who received a liver transplant for a severe urea cycle defect, carbamoyl phosphate synthase (CPS1) deficiency, an autosomal recessive urea cycle defect which causes hyperammonemia and central nervous system symptoms because of a greatly reduced capacity to convert the excess nitrogen from protein intake into urea for excretion. Isolated human hepatocytes were transplanted into the liver of FRGN mice, replacing 85–95% of the mouse hepatocytes with human hepatocytes as quantified by plasma human albumin levels.

Mice repopulated with normal hepatocytes (CPS1-proficient) displayed normal ammonia levels (<90 micromolar), while mice highly repopulated with CPS1-deficient hepatocytes displayed characteristic symptoms of human CPS1-deficiency including increased basal ammonia (150–300 micromolar), 80% reduction in CPS1 metabolic activity, delayed clearance of an ammonium chloride infusion, elevated glutamine and glutamate levels and impaired metabolism of [15N]ammonium chloride into urea, with no other obvious phenotypic differences.

Conclusions. Since most metabolic liver diseases result from mutations that alter critical pathways in hepatocytes, a model that incorporates actual disease-affected, mutant human hepatocytes is useful for the investigation of the molecular, biochemical and phenotypic differences induced by that mutation. The model is also expected to be useful for investigations of modified RNA, gene, cellular and small molecule therapies for CPS1-deficiency. Liver-humanized models for this and other monogenic liver diseases afford the ability to assess the therapy on actual disease-affected human hepatocytes, in vivo, for long periods of time and will provide data that is highly relevant for investigations of the safety and efficacy of gene editing technologies directed to human hepatocytes and the translation of gene editing technology to the clinic.

Table 1. Patient Characteristics and Post Transplant Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>4.5 (0.4-12)</td>
<td>2.2 (0.2-13)</td>
<td>0.518</td>
</tr>
<tr>
<td>Female</td>
<td>21 (0.0)</td>
<td>15 (5.7-35.3)</td>
<td>0.946</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>1.2 (34.2)</td>
<td>1.2 (49)</td>
<td>0.431</td>
</tr>
<tr>
<td>Bi Ventriclel l CIED</td>
<td>3 (8.4)</td>
<td>5 (19)</td>
<td></td>
</tr>
<tr>
<td>Single-ventricle CIED</td>
<td>18 (51.4)</td>
<td>19 (77)</td>
<td></td>
</tr>
<tr>
<td>At Lesting</td>
<td>24 (99)</td>
<td>19 (70)</td>
<td>0.888</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>8 (27.0)</td>
<td>8 (25)</td>
<td>0.202</td>
</tr>
<tr>
<td>VAD</td>
<td>5 (17)</td>
<td>9 (36)</td>
<td>0.117</td>
</tr>
<tr>
<td>Isotopes</td>
<td>30 (69)</td>
<td>30 (89)</td>
<td>0.956</td>
</tr>
<tr>
<td>Post-Transplant</td>
<td>3 (1-8)</td>
<td>1 (1-6)</td>
<td>0.478</td>
</tr>
<tr>
<td>Length ICU stay (days)</td>
<td>13 (9-22)</td>
<td>10 (4-55)</td>
<td>0.460</td>
</tr>
<tr>
<td>Total (days)</td>
<td>25 (17-47)</td>
<td>26 (10-61)</td>
<td>0.946</td>
</tr>
<tr>
<td>Alive at 30 days</td>
<td>28 (90%)</td>
<td>25 (100%)</td>
<td>0.915</td>
</tr>
<tr>
<td>Accepted Donors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor: Recipient weight ratio</td>
<td>1.73 (1.37-1.93)</td>
<td>1.6 (1.24-2.26)</td>
<td>0.801</td>
</tr>
<tr>
<td>Donor sequence number</td>
<td>2 (1-3)</td>
<td>3 (1-4)</td>
<td>0.045</td>
</tr>
<tr>
<td>On Isotopes at time of offer</td>
<td>5 (17)</td>
<td>2 (9)</td>
<td>0.370</td>
</tr>
<tr>
<td>Reversed CPR</td>
<td>15 (45)</td>
<td>17 (45)</td>
<td>0.114</td>
</tr>
<tr>
<td>Rejected Donors n=382</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reversed due to donor quality</td>
<td>279 (60)</td>
<td>36 (72)</td>
<td>0.001</td>
</tr>
<tr>
<td>Denor refused per listed patient</td>
<td>4 (2-26)</td>
<td>1 (0-3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Donor on isotopes at time of offer</td>
<td>85 (29)</td>
<td>20 (55)</td>
<td>0.567</td>
</tr>
<tr>
<td>Rejected donors with LVEF &gt;59%</td>
<td>258 (54)</td>
<td>19 (31)</td>
<td>0.001</td>
</tr>
<tr>
<td>Rejected donors accepted at another institution</td>
<td>141 (35)</td>
<td>17 (47)</td>
<td>0.636</td>
</tr>
</tbody>
</table>

CIED: congenital heart disease, VAD: ventricular assist device, LVEF: length of stay (ICU); intensive care unit, CPR: cardiopulmonary resuscitation, LVEF: left ventricular ejection fraction. All continuous variables are median (IQR); categorical variables are n (%)
Effects of Wharton’s Jelly-derived Mesenchymal Stem Cells on Chronic Obstructive Pulmonary Disease

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Objectives: Chronic obstructive pulmonary disease (COPD) is a fatal disease that shortens one’s life expectancy, and reduces the quality of life of patients. The current known treatments for COPD can only act to alleviate the symptoms. Recently, stem cells have demonstrated efficacy in various medical areas. The aim of this study was to investigate the possibility of using human Wharton’s jelly-derived mesenchymal stem cells (MSCs) for lung recovery in a COPD mouse model.

Methods: Human Wharton’s jelly was obtained during natural delivery or caesarean section from healthy women. Wharton’s jelly-derived MSC was confirmed with expression of CD14, CD34, CD45, CD73, CD90, and CD105 using flow cytometry. Mice model (C57BL/6) of COPD were induced by injecting 10 µL elastase into the trachea and they were divided into three treatment groups (sham, vehicle, stem cell). The sham group was not induced COPD, nor provided any treatment; the vehicle group comprised of COPD-induced mice treated with normal saline; the stem cell group comprised of COPD-induced mice treated with Wharton’s jelly-derived MSCs. The vehicle and mesenchymal stem cells (5 × 10⁴ cells) were injected in tail vein 7 days following COPD induction. Mice were euthanized 7 days after vehicle and stem cell injection, and pathologic findings were confirmed. Mean Linear Intercept (MLI) was measured after emphysema-induced alveoli were identified.

Results: Cell surface markers were positive for CD105, CD90, and CD73 and negative for CD45, CD34, and CD14. Pathological tests showed that COPD-induced mice had significantly increased emphysema volume as compared with that in the sham group. The degree of emphysema in the stem cell group was reduced based on pathologic findings. The mean MLI of the sham group was measured as 38.85±6.45. The mean MLI of the vehicle and stem cell groups were 163.05±48.94 and 123.59±30.53, respectively, and there was a statistically significant difference between the two groups (p=0.008).

Conclusions: Though the number of mice in the experiment was not large, human Wharton’s jelly-derived MSCs showed pulmonary regenerative effects in the COPD mouse model. Although we cannot confirm the effects of Wharton’s jelly-derived MSCs in COPD through this experiment, it can be used as a basis for a larger clinical experiment.
67 Improved Human Islet Function and Survival When Cultured with a cGMP Human Growth Factor Product

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Beta cell replacement of allogeneic islet transplantation to T1D patients is an accepted, minimally invasive, process. However, stress due to islet processing and culture can reduce functional success. Conventional clinical islet culture media, CRML-1066 plus 0.2% Human Serum Albumin (HSA), is minimally effective at reducing islet stress or supporting islet recovery and function. In this study, we investigate a cGMP human growth factor product, Nucleus Biologics zIslet Factor (zIF) (San Diego, CA), on in vitro islet function and survival assays under the stressor of time. Our preliminary data demonstrates zIF supplemented media improved islet Glucose Stimulated Insulin Secretion (GSIS) after 7 and 14 days of culture, while stabilizing islet viability and islet loss.

Human islets, obtained from the UCSF Islet Core, from brain-dead pancreas donors; mean age of 43 years (25–48 years); mean HbA1c of 5.5% (5.2–6.2%); mean BMI of 27 (19–34 kg/m2) with appropriate informed research consent were provided by Donor Network West (San Ramon, CA). Islet purities >90% were cultured free floating, 100 IEQ/mL, at 37OC, 5% CO2 in CRML 1066 (Corning, Manassas VA) containing heparin, DNAse, Ciprofloxacin; with either 0.2% HSA or 2% zIF in non-treated 60mm plates. Media was changed every two days up to Day 12 and islet count, viability, and GSIS was measured on Days 1, 7, and 14. The GSIS supernatants were assayed with a human insulin & glucagon ELISA kit (Mercodia AB, Uppsala, Sweden). GSIS index is a ratio of insulin in High-glucose to insulin in Low-glucose.

<table>
<thead>
<tr>
<th>Secreted Human Insulin (mU/L) (Average n=5)</th>
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</thead>
<tbody>
<tr>
<td>Conventional Clinical Media</td>
</tr>
<tr>
<td>2.8 mM Glucose</td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Average GSIS index</td>
</tr>
<tr>
<td>Day 7</td>
</tr>
<tr>
<td>Average GSIS index</td>
</tr>
<tr>
<td>Day 14</td>
</tr>
<tr>
<td>Average GSIS index</td>
</tr>
</tbody>
</table>

Islet counts and viability remained similar between clinical islet culture media and CRML-1066 plus 2% zIF until Day 7; clinical islet media did show the larger average islet loss of 25% compared to a <8% loss in zIF treated islets, while viability of surviving islets remained similar between both media each day. Interestingly, the zIF-cultured islets on day 7 and 14 show a highly improved average GSIS index and average level of secreted insulin compared to conventional clinical media (Table below). Average secreted insulin and GSIS index was similar on Day 1, but as time of culture increased, the average secreted insulin of the zIF-cultured islets high-glucose sample increased 2 fold on Day 7 and 3 fold on Day 14 compared to clinical islet media. The islet GSIS Index corresponds to secreted insulin results. zIF did not have a negative effect on islet alpha cells. Glucagon levels were similar in both media on Day 1 and Day 7 and on Day 14 glucagon levels doubled in both media; independent of glucose stimulus.

In conclusion, Nucleus Biologics zIF is a suitable supplement for research islet culture and possibly for clinical islet culture and transplant. zIF preserves islet survival, supports and restores beta cell function, and does not interfere with normal alpha cell function. The improvement in islet function prompted us to further study post-transplant islet graft survival and other possible islet survival pathways. We invite islet researchers to test zIF in their islet specific assays.
Rapid Viability and Potency Assessment of Encapsulated Islets and Insulin-producing Cells

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Introduction: Diabetes and its insidious complications sicken and kill millions of people worldwide. Our group has developed macroencapsulation devices capable of providing supplemental oxygen to macroencapsulated transplanted tissue. These devices house and protect islets and insulin-producing cells (IPCs) from the immune system, eliminating the need for immunosuppression of the transplant recipient. We predict these devices will facilitate transplant of small footprint devices containing high cell densities of islets or IPCs that can reverse diabetes in humans by fostering increased viability and functionality of transplanted islets and IPCs. However, in order to a) determine the number of cells encapsulation devices can support, b) assess the effects of encapsulation on islets and IPCs, c) meet FDA standards, and d) ensure the quality of a cellular transplant product, the viability and potency of islets and IPCs within encapsulation devices must be determined before clinical application.

Materials and Methods: We have developed 2 bioreactor systems integrated with BioRep perifusion machines capable of simultaneous measurement of cellular oxygen consumption rate (OCR) and hormone secretion. One bioreactor can be used to assay free cells and the other, macroencapsulated cells. Each bioreactor contains two integrated FOSPOR oxygen sensors (Ocean Optics) at the inlet and outlet of the bioreactor. Oxygen concentration is derived from the fluorescence lifetime of the sensor measured by fiber optic cables linked to a phase fluorimeter (Ocean Optics). Media flow, temperature and collection are controlled by the BioRep perifusion machine. Figure 1 depicts the general design of the integrated bioreactor systems.

Results and Discussion: We have determined that cellular density and encapsulation can affect the viability and function of islets. Islet density greater than 500 islet equivalents/cm² (without supplemental oxygen) was detrimental to islet viability during a 7-day transplant period. In vitro, densely encapsulated islets displayed delayed and diminished glucose-stimulated insulin secretion (GSIS) relative to free islets (Figure 2A), an important consideration for physiological control of glycemia with engrafted tissue. Furthermore, as expected in Krebs Ringer Bicarbonate Buffer, glucose-stimulated insulin secretion (GSIS) was correlated with an increased OCR (Figure 2B) highlighting the link between OCR and GSIS in this medium and the utility of OCR, an inexpensive real time assay as an islet potency indicator.

Conclusions: In concert, oxygen consumption and hormone secretion are excellent indicators of islet and IPC health and function. Our bioreactor will allow us to optimize encapsulation densities, cellular function, and supplemental oxygen delivery strategies ensuring a clinical high-grade cellular product and, together with our encapsulation devices, improve the treatment of diabetes.