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Cell Transplant Track Abstracts from the Cell Transplant Society - International Xenotransplantation Association 2011 Joint International Congress

Parallel Session 1
Islets

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Effects of immunosuppression on proliferation in transplanted islets

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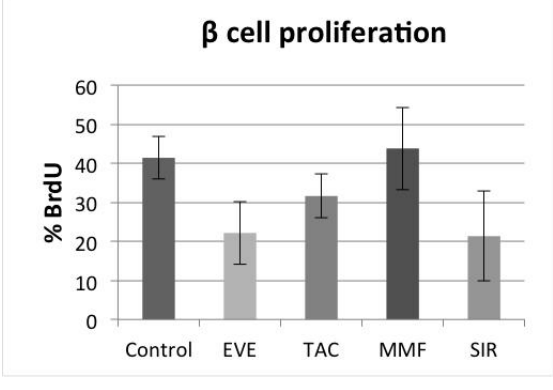
Background: The antiproliferative effects of immunosuppressive drugs such as sirolimus and tacrolimus used in human islet transplantation interfere with the capacity of β cells to balance cell renewal and cell loss. This feature may be an important contributor to progressive graft dysfunction in islet transplant recipients over time. We analyzed the influence of different immunosuppressants on α and β cell proliferation and transplant outcome following syngeneic β cell transplantation in mice.
Methods: Syngeneic islets (300 IP) were injected into the right liver lobes of C57BL/6 diabetic recipients. Osmotic pumps filled with bromodeoxyuridine (BrdU) (control) or BrdU and an immunosuppressant [tacrolimus, sirolimus, everolimus, or mycophenolate mofetil (MMF)] were implanted. Glycemic control was assessed using glucose tolerance tests. After four weeks, proliferation of α and β cells was detected by BrdU incorporation. In addition, fractional β cell area and average β cell size was determined by morphometric analysis.
Results: The average blood glucose levels were significantly higher in all treatment groups compared to controls. Glucose tolerance was improved only in control animals (P = 0.009). The fractional β cell area and β cell proliferation in MMF-treated mice were comparable to control mice (P = 0.66). In contrast, treatment with everolimus and sirolimus led to a significant reduction in β cell proliferation and fractional β cell area. While transplanted β cells from animals treated with tacrolimus also presented a reduced replication rate (P = 0.023), the fractional β cell area was not affected compared to untreated controls (P = 0.72).
Conclusions: Our results demonstrate that the β cells of transplanted islets have a strong capacity for self-renewal when not affected by immunosuppression or immune assault. In contrast to other immunosuppressants, MMF does not affect β cell replication and fractional β cell area; therefore, its use may lead to improved long-term results in islet transplantation.



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Vascularised small intestinal segment as an alternative site for pancreatic islet transplantation

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Background: Islet transplantation into the liver via the portal vein is a potential treatment for patients with type 1 diabetes mellitus that can restore normal blood sugar without the need for insulin injections and can improve quality of life. However, complications such as hemorrhage, thrombosis and an immediate loss of islets through the 'instant blood-mediated inflammatory reaction' (IBMIR) represent main disadvantages of this modality.
Methods: Animal groups were established to determine engraftment, survival and function of islets transplanted into either intestinal segments or portal vein. Islets were isolated from adult male Lewis rats and transplanted into the small intestinal segments, preliminary ablated from mucosa of diabetic syngeneic recipients. Blood glucose levels were monitored and intraperitoneal glucose tolerance tests carried out. Histological assessment for insulin, glucagon, von Willebrand factor (vWF) was performed. The reverse transcription-polymerase chain reaction was performed to analize the expression of insulin, glucagon, somatostatin, Glut1, PDX1, PAX6, TGFα, TGFß, bFGF and VEGF.
Results: Syngeneic islets transplanted into the intestinal segment ablated from mucosa restored euglycemia within 20 days and sustained function for over a year. Animals treated with islet transplants showed normal responses to glucose challenges. Removal of graft-bearing segments resulted in hyperglycemia. Transplanted islets demonstrated expression of insulin and glucagon. The PCR analysis showed that expression of multiple growth factors was generally retained in the transplanted islets, including beta cell transcription factors and hormones.
Conclusions: These encouraging features of the isolated intestinal segment open new research avenues for addressing biological mechanisms and clinical applications. We assume that clinical islet transplantation outcome can be significantly improved utilizing extrahepatic sites for islet implantation avoiding detrimental IBMIR that is associated with intraportal islet transplantation.

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Improving islet transplantation outcome by CXCR1/CXCR2 inhibition

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Objective: The aim of our work is to determine whether the CXCR1/CXCR2 inhibition improves islet transplantation outcome.
Methods and Results: Liver inflammatory status was studied before and after intrahepatic transplantation (Tx) of 500 syngeneic islets in diabetic C57BL/6 mice. Cytokine and chemokine transcripts 4h-24h-48h after Tx were determined using RNAse protection assays. Intrahepatic leucocyte (IHL) infiltration 1, 3, 5, 7, 10, 14 days after Tx was determined by FACS. The intrahepatic mRNA for CXCL1/KC was strongly induced immediately after islet infusion (100-fold increase after 4h). Polimorfonuclear cells (Gr1+/CD11b+/Ly6C-; PMN, 100% CXCR2+) was the first leucocytes subpopulation infiltrating the liver. To evaluate whether the block of CXCL1-CXCR2 axis improve islet engraftment, 400 syngeneic islets were alternatively transplanted in diabetic CXCR2-/- or CXCR2+/+ Balb/C mice. The absence of CXCR2 led to a significant improvement of transplant function. On this basis we tested whether Reparixin, a CXCR1/CXCR2 allosteric inhibitor, is able to improve islet transplantation outcome. In syngeneic marginal mass model of 250 islet Tx in diabetic C57BL/6 mice the probability and median time to reach euglycaemia were 100% and 2 days for Reparixin treated mice (n=29) as compared to 58% and 50 days for vehicle treated mice (n=34) ($p<0.001$). In an allogeneic full mismatched model of islet Tx (400 Balb/c islets infused in diabetic C57BL/6 mice) Reparixin significantly prolonged the time to rejection: median survival time 12+0.6 days (n=13) and 8+1.3 days (n=7) respectively for Reparixin and vehicle treated mice ($p<0.007$). Islet survival time was further improved using Reparixin in combination with Rapamycin+FK-506 or MMF+FK-506. In both models Reparixin treatment was associated to a decrease PMN and NKT cells (NK1.1+/CD3+) liver infiltration.

Conclusion: Inhibition of CXCR1/CXCR2 is crucial for improving islet engraftment and survival. On this basis a clinical trial (NCT01220856) is ongoing testing Reparixin in association with the conventional immunosuppressive therapy.

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Long term survival of allogeneic, nonhuman primate islets in non-biodegradable scaffolds within an omental pouch

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Previously, we have demonstrated that the omental pouch (OP) site supports survival of islets loaded onto biodegradable scaffolds in a nonhuman primate model. In this study, we tested whether a macroporous non-biodegradable scaffold can support function of islets alone (n=1) or together with immunomodulatory mesenchymal stem cells (MSC) (n=2) in an OP site. Recipient MSC (4.5 million/scaffold) were seeded onto 5-7 scaffolds (30 mm diameter-2 mm thick), and cultured 5 days before loading 15-25K allogeneic islet equivalents (IEQ)/kg on post-operative day (POD 0). Baboons were treated with 20 mg/kg anti-CD154 (5c8) on POD -1, 0, 3, 10, 18, 28 (induction) and every 10-28 days for maintenance. Insulin, administered to achieve normoglycemia, was tapered beginning on POD 40-60. Occurrence of nontherapeutic anti-CD154 levels between monthly maintenance doses resulted in rejection episodes and partial loss of function, whereby maintenance dosing was switched to every 10 days. A recipient of islets alone (25K IEQ/kg) was insulin independent from POD 130-374, minor graft destabilization occurred and daily treatment with the GLP-1 analogue Liraglutide started on POD 403. Removal of scaffolds on POD 445 was followed by a return to full diabetes. The first recipient of islets+MSC (15K IEQ/kg+MSC) was insulin independent from POD 96-200. Minor graft destabilization occurred and the implants were removed for analysis on POD 222, followed by a return to full diabetes (c-peptide negative, increased exogenous insulin requirements, EIR). Immunofluorescence revealed viable, vascularized, insulin-positive islets within the scaffolds. The second recipient of islet+MSC (25K IEQ/kg+MSC) experienced significant reduction of EIR, but rejection episodes precluded insulin independence. Removal of scaffolds at POD 188 resulted in full loss of c-peptide; insulin positive islets were observed. This non-biodegradable scaffold provides a novel means to locally deliver MSC and islets in a 3-D configuration, with the potential for local immunomodulation and growth factor delivery.

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Stimulation of VEGF secretion in rat pancreatic islets using Liraglutide

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Introduction: The formation of microvascularization by capillary sprouting at the site of islet transplantation is crucial for survival of the graft. Vascular Endothelial Growth Factor (VEGF), a major angiogenic factor, may be a key protein in modulating the angiogenesis of islets after transplantation. Development of a pharmacological approach enhancing VEGF synthesis could improve islet graft survival.
Liraglutide has been shown to decrease islets apoptosis and increase survival in cultured or transplanted islets. The mechanisms of its role in islets viability in culture and during transplantation have to be identified. The aim of this work was to study *in vitro* the effects of liraglutide on islet viability and its relation to angiogenesis *via* VEGF secretion.
Materials and methods: Previous studies have determined a protocol allowing the systematic evaluation of pharmacological molecules. Following this protocol, cultures of rats islets were incubated in presence of 1 and 10 µM of liraglutide (50 fold higher than pharmacological concentrations used) during 12, 24 and 48h. The islet viability was evaluated using fluorescein diacetate/propidium iodure dying and functionality was determined by glucose test stimulation. Islets insulin-secretion was expressed as an index of stimulation (IS). VEGF secretion was determined by ELISA assay.

Results: Islets viability was 100% in controls and with liraglutide. Ten µM of liraglutide induced a significant stimulation of VEGF secretion as early as 24h with 10.57 ± 3.55 *vs* control with 4.42 ± 1.09 pg of VEGF/µg of protein ($p< 0.05$, n=4), and was maintained after 48h. It only appeared after 48h with 1 µM liraglutide. Levels of secretion were respectively: 39.28 ± 14.81 with 1 µM, 53.60 ± 25.03 with 10 µM*;* 13.63 ± 4.48 pg of VEGF/µg of protein with controls, $p< 0.05$, n=4). At the same time, a significant stimulation of the insulin-secretion was observed at 24h of culture with 1 and 10 µM of liraglutide and controls with respectively 7.86 ± 1.78 and 8.17 ± 2.43 *vs* 4.27 ± 1.46 µg of insulin/g of protein (n=4, $p< 0.05$). The effect was maintained after 48h with 1 µM: 2.63 ± 1.64 and 10 µM: 6.28 ± 4.74 for liraglutide *vs* controls: 2.29 ± 1.05 µg insulin/g of protein, n=4).
Conclusion: *In vitro*, suprapharmacological concentrations of liraglutide had no toxicity and significantly stimulated VEGF secretion in islets. Also, insulin-secretion increased during the first 24h of culture. VEGF secretion could be one of the mechanisms involved in the improvement of islet viability during transplantation. Increased angiogenesis remains to be assessed.

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Comparing the intrahepatic and renal subcapsular sites for human islet engraftment into diabetic immunodeficient mice

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We evaluated the success of human islet engraftment into two sites of implantation: the liver and the renal subcapsular space. Athymic nu/nu (nude) mice were induced diabetic by a single injection of the beta-cell toxin streptozotocin and maintained on exogenous insulin given subcutaneously (either glargine insulin injections or implantation of slow release insulin pellets) until research human islet preparations become available. We compared the islet mass that was used in recent years for the kidney subcapsular space as either 'full' and 'suboptimal' mass (namely, 2,000 and 1,000 IEQ/recipient, respectively). Using the same human islet preparations each time, we performed subcapsular kidney space transplants in parallel to the intrahepatic ones (done in heparinized saline) in order to compare side-by-side the rate of engraftment in the two sites using eight individual human islet preparations.
Transplantation of 2,000 IEQ per recipient mouse resulted in diabetes reversal in 21.2±2.3 days in all recipients (12/12, 7 preparations). Transplantation of 1,000 IEQ per mouse also resulted in diabetes reversal in all recipients (15/15, 8 preparations) in a tempo of 5.2±8.5 days. Transplantation of comparable islet masses in the intrahepatic site resulted in lower engraftment rates. In particular, intrahepatic transplantation of 2,000 IEQ resulted in diabetes reversal in 78% of the recipients (7/9, 7 preparations) within 11.9±21.3 days, while transplantation of 1,000 IEQ led to normoglycemia in 44% (4/7, 6 preparations) within 30.4±49.5 days.
Collectively, our data indicates that the intrahepatic site may lead to higher degrees of injury resulting in reduced viability and engraftment of human islets in our model. Indeed, primary non-function was observed in 22% and 56% of the recipients of 2,000 and 1,000 IEQ intrahepatic grafts, respectively. Conversely, all animals receiving the grafts under the kidney subcapsular space achieved normoglycemia after transplantation.
Supported by JDRF (4-2004-361) and Diabetes Research Institute Foundation.

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Evaluation of vascularization and immunomodulation potential of islet/MSC scaffolds implanted in a nonhuman primate model

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Mesenchymal stem cells (MSC) have been demonstrated to modulate immunity, limit fibrosis, and enhance angiogenesis and tissue repair. Co-infusion of MSCs and islets into the liver has been shown to enhance islet engraftment and function in a nonhuman primate (NHP) model. To explore the potential of the co-localization of

NHP MSCs with islets in improving islet engraftment, biomaterial scaffolds were employed. We pre-cultured MSCs onto macroporous PDMS scaffolds 4-5 days prior to islet loading, evaluated growth, viability, and phenotype of MSCs during pre-culture, and the effect of MSCs on islet viability (MTT assay) and function (glucose-stimulated-insulin-release). Finally, implantation of scaffolds with MSC + islets into baboons evaluated the effects of islet source (autologous or allogeneic), MSC source (autologous or 3rd party), and site (intramuscular, subcutaneous, or omental pouch) on the graft response. Scaffolds (islets + 3rd party MSCs, islets + recipient MSCs, islets only, 3rd party MSCs only, recipient MSCs only, and material only) were implanted into the three sites (n=3 each) for both an autologous and allogeneic (immunosuppressed) non-diabetic baboon. On day 21, scaffolds were explanted, sectioned, and stained. Particular focus was paid to extracellular matrix deposition (Masson's Trichrome staining), local inflammation (CD3 and CD69 staining), and intra-implant vascularization (vWF and α-SMA staining). Preliminary assessment of engraftment at these three sites indicates a greater degree of cellular infiltration, fibrosis, and inflammation at the subcutaneous site. Overall, the presence of MSCs within the scaffold appears to dampen inflammation and fibrotic tissue deposition, when compared to scaffold only implants. Vascularization analysis for all found over a 7-fold increase in the 3rd party MSC+islets group vs empty silicone scaffold control. These results suggest a correlation between implantation site and positive engraftment and vascularization. Furthermore, localization of MSC to the site may enhance vascular tissue growth and engraftment. Authors acknowledge JDRF support.

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Naturally pure islets for autotransplantation after total pancreatectomy for chronic pancreatitis

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The enzymatic digestion step for islet isolation from a chronic pancreatitis (CP) pancreas separates the intact pancreas into acinar cell and islet components. In general, the purity of islets at the digest level will be <10%. In some cases of diseased pancreases, however, after digestion there is a low to very low amount of exocrine tissue, presumably because of the destruction of exocrine tissue by the fibrotic process that occurs from the disease. In these cases, islet purity is naturally greater (>30% islet purity) and high islet purity (up to 98%) is achieved without the need for traditional density gradient purification. In this study we present isolation and clinical outcomes from 34 cases in which islet isolation resulted in naturally pure islets (NPI) with >30% islet purity. Islets were isolated using standard protocols throughout the study period. Patient characteristics, islet isolation, and clinical outcome are summarized in the table. Interestingly, 10 (29%) patients were pediatric, and the genetic mutations of cystic fibrosis transmembrane conductance regulator (CFTR) or cationic trypsinogen genes (PRSS1) were associated with the CP in 14 (41%). Although the average pancreas weight from these CP organs was 50.3±22.8 gms as compared to 94.0±28.5 gms in normal deceased donor (DD) pancreases (n=139), the tissue volumes after digestion were disproportionally small (6.9±5.9 vs 28.6±13.8 mL from DD pancreases), indicating a low acinar cell mass. Mean transplanted islet mass of NPI was 4,082±2,383 IEQ/kg with a purity of 30-98% (mean, 49±21%), and average portal pressures were elevated to 13.2±11.0 mm Hg. The gross morphology (score of 8.7±0.6) and viability (89.6±5.8) of islets isolated from the CP organs were normal. Insulin independence and partial function was achieved in 56% of patients receiving >2000 IEQ/kg. Unpurified autologous islet preparations are routinely transplanted after total pancreatectomy for CP. When the islets are naturally pure, the tissue volume is low and islet loss from gradient purification can be avoided, allowing as many islets as possible to be transplanted without elevating portal pressure to unacceptable levels.

Table : Naturally Purified Islets Auto-transplantation (>30% purity)	
Patient Characteristics	
Number of Isolations (n)	34
Age (yr)	29.5±13.9
Gender (M/F)	7/27
BMI (kg/m²)	23.0±5.2
Pancreatitis Etiology	Cystic Fibrosis=7; Fibrosis Carrier=1; Idiopathic=9; Hereditary=7; Sphincter of Oddi Dysfunction=5; Pancreas divisum=4; Mohr Goldston Syndrome=1
Previous Surgery	Cholecystectomy=2; Distal Pancreatectomy=1; Drainage=2; Frey Procedure=1; None=11; Puestow=1; Sphincterotomy=1; Sphincterectomy=1; Unknown=7; Whipple=2; Multiple=5
Type of Pancreatectomy	Completion=6; Partial=1; Total pancreatectomy=27
Islet Isolation Characteristics	
Pancreas Weight (g)	50.3±22.8
Severity of Fibrosis	Mild/Moderate=3; Moderate=7; Moderate/Severe=5; Severe=11; Extreme=8
Distention Method	Interstitial=1; Perfusion=25; Perfusion and Interstitial=7; Syringe=1
Isolation Enzyme	SERVA=16; NEM*=18
Phase I Digestion Time (min)	22.5±6.1
Undigested Tissue (g)	9.1±9.3
% of Embedded Islets	28±25.3
Digest IEQ	250,654±170,599
Digest IEQ/g	5,551±3,552
Transplantation Characteristics	
Total islet number	283,232±170,401
Total IEQ	239,310±154,069
IEQ/kg	4,082±2,383
Islet Purity (%)	49±21
Tissue Volume (mL)	6.9±5.9
Islet Score	8.7±0.6
Viability (% by FDA/PI)	89.6±5.8
Transplantation outcome	Independent=12; Low-dose insulin=4; Multiple insulin injection/day; No F/U=1; Excluded=1
**NEM, new enzyme mixture, composed of VitaCyte Clzyme™ collagenase and SERVA NB neutral protease*	

Parallel Session 2
Stem Cells

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Pharmacologically active microcarriers enhance the therapeutic effects of MIAMI cell transplanted in hemi-parkinsonian rats

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L-DOPA, currently the most efficient therapeutic treatment for Parkinson's disease (PD), aims at replenishing the amount of dopamine decreased in the degenerated patient's striatum. However, long-term treatment with L-DOPA slowly becomes less effective and shows numerous undesirable side effects. In the present study, we assessed the effectiveness of a tissue engineering approach which combined marrow-isolated adult multilineage inducible (MIAMI) cells, a subpopulation of human MSCs, and biomimetic drug releasing microcarrier scaffolds, prior to their transplantation in a rat model of PD. The scaffolds, named pharmacologically active microcarriers (PAMs), are biodegradable and biocompatible poly(lactic-co-glycolic acid) microspheres, coated by a biomimetic surface and releasing a therapeutic protein, which acts on the cells conveyed on their surface and on their microenvironment. In this study, PAMs were coated with laminin and engineered to release neurotrophin 3 (NT3), which stimulates the neuronal-like differentiation of MIAMI cells and promotes neuronal survival. MIAMI cells were subjected to a dopaminergic induction protocol and the dopaminergic-induced (DI)-MIAMI cells were adhered to PAMs in vitro.

These complexes were grafted in the partially dopaminergic-deafferented striatum of rats. This treatment led to a strong reduction of the amphetamine-induced rotational behavior together with a protection/repair of the nigrostriatal pathway. These effects were assumed to result from the observed enhanced survival of DI-MIAMI cells when combined to PAMs, as well as from their ability to secrete a wide range of growth factors and chemokines in situ. In addition, overexpression of Tyrosine Hydroxylase by DI-MIAMI cells transplanted in combination with PAMs may also have contributed to the observed functional recovery. To our knowledge, this is the first study that successfully combines adult stem cells and tissue engineering to protect and repair dopaminergic neurons in a rat model of PD, and we expect to evaluate this strategy in larger animal models of PD in the future.

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Encapsulated human multipotent mesenchymal stromal cells maintain differentiation capacity and have anti-fibrotic effects

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Introduction: Human multipotent mesenchymal stromal cells (MSC) secrete anti-inflammatory cytokines such IL1ra. We have tested the viability, proliferation and differentiation capacity of human MSC after microencapsulation *in vitro*. To test the anti-inflammatory effect in vivo, we transplanted encapsulated human MSC into mice with liver fibrosis.
Materials and methods: Human bone marrow-derived MSC were isolated from femoral heads of patients undergoing total hip replacement. MSC (at passage 2) were microencapsulated using a novel alginate-poly(ethylene glycol) hybrid hydrogel (0.4 mm). *In vitro*, we analyzed viability, proliferation, and differentiation capacity of encapsulated MSC. *In vivo,* we transplanted encapsulated MSC or empty capsules into the peritoneum of DBA-1 mice with liver fibrosis induced by bile duct ligation. Liver fibrosis was evaluated after 5 days by quantification of collagen deposition on liver sections using the MetaMorph software.
Results: The viability and proliferation of encapsulated MSC was similar when compared to non-encapsulated MSC. When exposed to specific medium, MSC maintained their capacity to differentiate into adipocytes within the capsules (14.8% ± 3.7 of MSC after two weeks of culture versus 18.02 ± 7.3% among non-encapsulated cells, p= 0.7). *In vivo,* encapsulated MSC were viable and vimentin positive at 26 days after intraperitoneal transplantation in DBA-1 mice. When transplanted in mice with bile duct ligation, encapsulated MSC significantly delayed the development of liver fibrosis compared to empty capsules, i.e. the collagen deposition per hepatocyte was reduced from 3.6% ± 0.4% to 2.4% ± 0.2% (p = 0.029).
Conclusion: Transplantation of encapsulated MSC represents a promising strategy for local and systemic delivery of anti-inflammatory and immuomodulatory molecules secreted by MSC.

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Pancreatic islets–derived Mesenchymal stromal cells can enter the pancreatic endocrine commitment but do not achieve consistent maturation

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Mesenchymal Stromal Cells (MSC) can be derived from various organs, display a wide differentiation potential and appear to have trophic effects. They have been shown to promote islet survival and function and are thus attractive for diabetes treatments that may include composite transplants. Furthermore, MSC have been shown to give rise to

islet cell-like clusters. Pancreatic islet-derived mesenchymal stromal cells (PI-MSC) may be more prone to commit to islet specific phenotypes due to their tissue derivation.
We isolated and expanded MSC from human pancreatic islets. Cell populations were characterized by flow cytometry and immunofluorescence. The differentiation potential toward mesenchymal and pancreatic endocrine cells was tested *in vitro* and *in vivo* after transplantation in streptozotocin-induced diabetic rats.
After expansion in Chang medium, pancreatic islet-derived stromal cells expressed MSCs markers: CD44+, CD73+, CD90+, CD105+, CD146 low+, CD14-, CD19-, CD34-, CD45-, HLA-DR-. The cells showed multidifferentiation potential toward adipocytes and endothelial cells; moreover, they differentiated towards endocrine lineages both *in vitro* and *in vivo*, increasing insulin levels and, to a higher extent, glucagon levels. Under our induction conditions, PI-MSC gave rise to cells with a mixed phenotype and incomplete differentiation. After transplantation in diabetic rats, a transient effect was evidenced by a lowering of glycemia levels and of water intake. Two weeks after xenotransplantation in the absence of immune suppression, glucagon- and insulin-expressing cells were detected in grafts. Cells coexpressing the pericytic marker CD146 and endocrine markers were observed.
PI-MSC seem to have the potential to enter the pancreatic endocrine commitment resulting in *in vivo* amelioration of blood glucose control in transplanted animals. Improvements in expansion and differentiation protocols will result in additional efficacy.

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Inhibition of miR-7 in developing pancreas and in cultured pancreatic buds affects beta cell differentiation

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miRNAs are small non-coding gene products that negatively regulate gene expression post-transcriptionally. MiRNAs control key biological events such as embryonic development and organ differentiation, including pancreatic specification and islet function. miR-7 is highly expressed in human fetal and adult pancreatic endocrine cells. We studied the expression profile of miR-7 in the mouse-developing pancreas by RT-PCR and *in situ* hybridization. MiR-7 expression was low between embryonic day e10.5 and e11.5, then began to increase at e13.5 through e14.5 and eventually decreased by e18. Endocrine marker Isl1 expression co localizes with miR-7 suggesting that miR-7 is expressed preferentially in endocrine cells. Whole mount *in situ* hybridization shows miR-7 highly expressed in the embryonic neural tube as well. To investigate the role of miR-7 in development of the mouse endocrine pancreas, antisense miR-7 morpholinos (MO) were delivered to the embryo at an early developmental stage (e10.5 days) via intrauterine fetal heart injection. Inhibition of miR-7 during early embryonic life results in an overall down-regulation of insulin production, decreased beta cell numbers and glucose intolerance in the post natal period. The cell death assessment by TUNEL assay of *in vivo* treated samples showed extensive cell death throughout the pancreatic tissue in MO anti-miR-7 injected animals but not in the MO controls. Because of the high miR-7 expression in the embryonic brain and the fact that all cell types were affected equally, we assume that a systemic effect is the major contributor.
On the other hand, the *in vitro* inhibition of miR-7 in explanted pancreatic buds led to beta cell death and generation of beta cells expressing less insulin than those in MO control. Therefore, besides the potential indirect effect on pancreatic differentiation derived from miR-7 systemic downregulation, its knockdown *in vitro* proves to have a beta cell-specific effect as well.

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In utero intracardial injection of a transducible MafA protein accelerates pancreatic islet maturation

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In concert with Pdx1 and Beta2/NeuroD, the nuclear protein MafA is essential for the maintenance of the adult beta cell phenotype by contributing to the proper regulation of the insulin promoter. When ectopically expressed together, MafA, Pdx1 and Ngn3 (an upstream regulator of Beta2/NeuroD) were recently shown to reprogram acinar

exocrine cells into functional, insulin-producing beta cells. This novel approach has opened the door to potential clinical interventions aimed at restoring beta cell mass in the context of pancreatic disease. For this to happen, however, alternatives to the viral delivery of these factors need to be defined. Protein transduction is one such possible alternative. Using this technology, any protein of interest can be made cell-permeable by the mere addition of a short membrane-penetrating peptide. Recombinant purified proteins can then be added *in vitro* to the culture medium or delivered locally or systemically *in vivo*, where they are expected to exert their function in the same manner as their native counterparts. This DNA-free system is deemed to be safer than viral-based approaches for future medical use. We have already described transducible versions of Ngn3 and Pdx1, and here we present a recombinant transducible version of the MafA protein (TAT-MafA) that penetrates across cell membranes with an extremely high efficiency and binds to the insulin promoter *in vitro*. When injected *in utero* into the heart of live murine embryos, TAT-MafA reaches the developing pancreas and increases the expression of key target genes, enhances insulin production and causes cytoarchitectural changes that are consistent with faster islet maturation. The purification and characterization of a functional TAT-MafA protein sets the stage for prospective therapeutic applications that circumvent the use of viruses. To our knowledge, this is also the first report on the use of protein transduction *in utero*.

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Human amnion epithelial (hAE) stem cell transplant significantly improves phenotype and survival in a murine model of intermediate maple syrup urine disease (iMSUD)

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MSUD (OMIM 248600) is a rare disorder of branched chain amino acid (BCAA; leucine, isoleucine, valine) catabolism caused by mutation of the branched-chain keto-acid dehydrogenase (BCKDH) enzyme complex. Treatment requires lifelong dietary restriction and compliance is variable, often resulting in catabolic crisis. Liver transplantation has greatly improved patient outcome. Previous studies verified that hepatocyte transplantation partially corrects a transgenic murine model of iMSUD, establishing that a small number of proficient cells transplanted into the liver significantly improves disease phenotype and survival (Mol. Ther. 2009, 17(7):126; Biochim Biophys Acta 2009, 1792(10):1004). Applying this rationale, human placental amnion-derived stem cells, which share many characteristics with pluripotent embryonic stem cells, were explored as an alternative to hepatocytes for use in cell transplant. During the first 10 days of life (DOL), neonates were given two direct hepatic injections of $1x10^6$cells. After 21 DOL, bi-weekly injections (2x10^6 cells) were administered until 35 DOL. Growth of transplanted iMSUD mice mimicked wildtype, and survival was significantly lengthened compared to untreated iMSUD. BCKDH enzyme activity was significantly improved, as well as serum and brain amino acids, at 35 and 100 DOL. A ratio of BCAA to alanine, a more representative indicator of disease status than BCAAs alone, was decreased >50% at both time points while alloisoleucine was not statistically different from unaffected controls. Neurotransmitter alterations and brain injury is characteristic of MSUD resulting from toxic accumulation of BCAAs. Importantly, brain monoamines showed improvements in hAE transplanted animals at both time points, and dopamine and serotonin turnover was normalized at 100 days. Similar to mouse hepatocytes, transplants of human AE stem cells partially corrected this mouse model of iMSUD. We propose that these placental stem cells may be an alternate to hepatocytes as therapy for MSUD, and possibly other liver-based inborn errors of metabolism.

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Generation of induced pluripotent stem cells from Amnion Epithelial cells

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Placenta is a readily available and non-controversial source of cells that could be used in regenerative medicine. Human Amniotic Epithelial (AE) cells and induced pluripotent stem (iPS) cell technologies would seem to be devoid of most if not all of the ethical concerns associated with ES cells. We generated of iPS cell lines from primary human AE (AE-iPS). After exposure to lentiviral constructs carrying the reprogramming factors, cells with appropriate morphology were collected and were analyzed for markers of pluripotency including Oct4, Nanog, Sox2 and alkaline phosphatase. A profile of AE was conducted pre- and post-reprogramming by Flow Cytometry for surface markers including: SSEA-3, SSEA-4, TRA1-60, TRA1-81, HLA-ABC, CD24, CD29, CD34, CD44, CD49f, CD73, CD105, CD90, CD117, CD133/2, CD146, CD166, EpCAM and ABCG2. FACS analysis revealed a morphological difference in forward scatter characterized by a compartment with a higher side scatter in AE, not present in AE-iPS thought to indicate a different level of cellular complexity and granularity. The percentage of SSEA-3, SSEA-4, TRA1-60 and TRA1-81 positive cells after reprogramming was dramatically increased in AE-iPS cells (30-90%). We also observed that stem cell marker such as CD133/2 was highly expressed only after the reprogramming procedure. Colonies generated have morphological features and the surface marker and gene expression profile of fully reprogrammed cells and also form teratomas upon transplantation. These studies confirm that iPS lines can be generated from primary human amnion. Protocols to differentiate these cells as previously published by our group on AE cells toward a hepatic phenotype are in progress

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Contact-dependent modulation of the human immune response to pig cells by adipose-derived mesenchymal stem cells (AdMSC) from GTKO/CD46 pigs

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Introduction: The immunomodulatory and anti-inflammatory effects of MSC could prove to be a potential therapeutic approach for prolongation of survival of cell xenotransplantation. Genetically-modified pigs could be an abundant source of organs and cells, but also of donor-specific MSC.
Methods: pMSC were isolated from adipose tissue of 1, 3-galactosyltransferase gene knock-out pigs transgenic for human (h) CD46 [GTKO/hCD46]). pMSC were identified by differentiation and by surface phenotype by flowcytometry (FCM). Naïve human and sensitized baboon IgM/IgG binding to GTKO/hCD46 pAdMSC and GTKO pig aortic endothelial cells (pAEC) was measured by FCM. The immunomodulation of human peripheral blood mononuclear cell (PBMC) responses to GTKO pAEC by GTKO/hCD46 pAdMSC was compared with commercially-available hAdMSC by measuring 3H-thymidine uptake. The supernatants from the MSC cultures were used to determine the role of soluble factors.
Results: GTKO/hCD46 pAdMSC (i) did not express Gal 1, 3Gal (Gal), but expressed hCD46, (ii) differentiated into chondroblasts, osteoblasts and adipocytes, (iii) expressed CD29, CD44, CD90, and CD105, but did not express CD45 or CD31, (iv) expressed lower levels of SLA I and II than pAEC before and after pIFN- stimulation ($p<0.001$), and (v) expressed costimulatory molecules (CD80, CD86) constitutively. Naïve human and sensitized baboon antibody binding to GTKO pAEC was greater than to GTKO/hCD46 pAdMSC. The proliferation of human PBMC to GTKO/hCD46 pAdMSC and hAdMSC stimulators was similar, and both were significantly lower than to GTKO pAEC ($p<0.05$). Human PBMC proliferation to GTKO pAEC was equally suppressed by GTKO/hCD46 pAdMSC and hAdMSC ($p<0.01$). The supernatant from GTKO/hCD46 pAdMSC did not suppress the human xenoresponse to GTKO pAEC.
Conclusions: (1) Genetically-modified pAdMSC are less immunogenic than GTKO pAEC, and no more immunogenic than hAdMSC. (2) The immunomodulatory function of pAdMSC is comparable to that of hAdMSC and is contact-dependent. (3) Genetically-modified pMSC may provide a potential therapy in cell xenotransplantation.

Parallel Session 7
Bioengineering & Biomaterials

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An engineered three-dimensional composite islet graft: a possible solution to two major obstacles in islet transplantation

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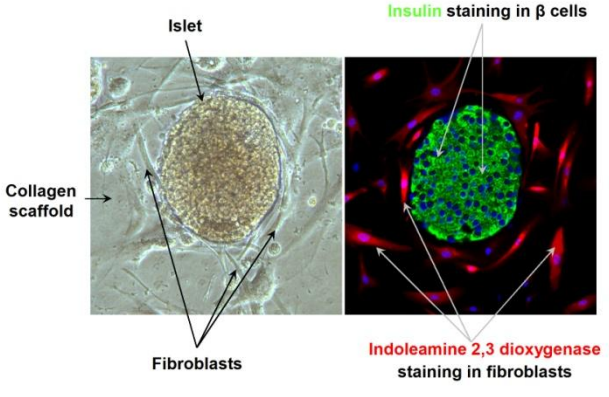
Requirement of systemic immunosuppression after islet transplantation is a major drawback to clinical islet transplantation. Moreover, due to loss of substantial mass of islets early after transplantation, islets from two or more donors are required to achieve insulin independence.

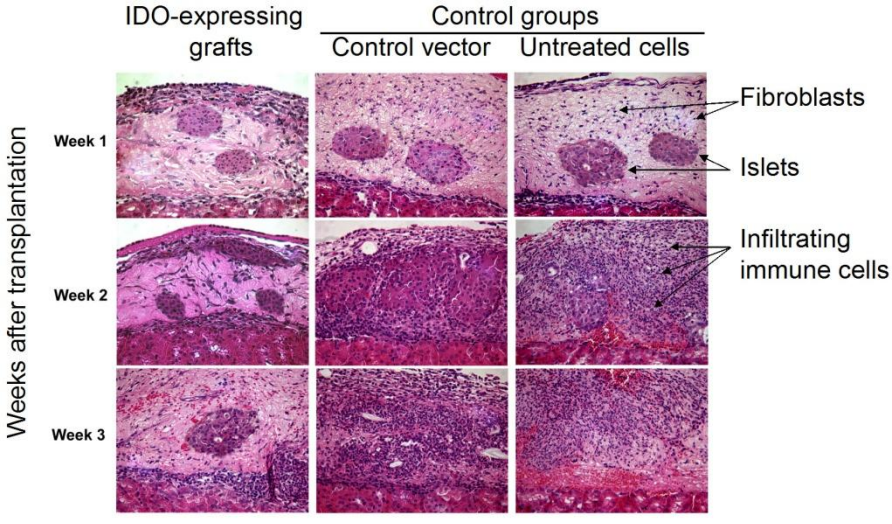
To address these concerns we developed a novel composite three-dimensional (3D) islet graft equipped with 1)a fibroblast populated collagen matrix (FPCM) as a novel scaffold to improve islet cell viability and function post-transplantation and 2) a local immunosuppressive system that prevents islet allograft rejection without systemic anti-rejection agents. in this composite, collagen matrix improves islet cell viability and expression of indoleamine-2-3-dioxygenase (IDO), a tryptophan degrading enzyme, in fibroblasts provides a low tryptophan microenvironment within which T-cells cannot proliferate and infiltrate islets.

Composite 3D islet grafts were engineered by embedding mouse islets and adenoviral transduced IDO-expressing fibroblasts within collagen matrix (fig1). Viability and function of islets in FPCM was evaluated *in vitro* and in a syngeneic murine islet transplantation model.Composite grafts were then transplanted to streptozotocin-induced diabetic immunocompetent mice. The viability, function and criteria for graft take were then determined in these mice.

The composite scaffold significantly improved islet cell viability and function, promoted islet graft survival and reduced the critical islet mass required for diabetes reversal by half (from 200 to 100 islets per recipient). IDO-expressing grafts survived significantly longer than controls (41.2±1.64 vs. 12.9±0.73 days, $p<0.001$) without administrating systemic immunesuppressive agents. Local expression of IDO suppressed effector T-cells at the graft site (fig2), induced a Th2 immune shift, generated an anti-inflammatory cytokine profile, delayed alloantibody production, and increased the number of regulatory T-cells in draining lymph nodes which resulted in antigen specific impairment of T-cell priming.

These results confirm that 1)fibroblast populated collagen matrix significantly promotes islet viability and functionality, enhances engraftment of islet grafts and decreases the critical islet mass needed to reverse hyperglycemia and 2) local IDO expression prevents cellular and humoral alloimmune responses against islets and significantly prolongs islet allograft survival without systemic anti-rejection treatments.





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In situ oxygen delivery to cellular transplants via hydrolytically activated biomaterials

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The implantation of cellular transplants within avascaular sites is hampered by inadequate oxygen delivery. The inevitable delay in angiogenesis following implantation results in substantial cell loss. Islets transplantation is particularly susceptible to functional impairment at moderate oxygen tensions. Herein, we developed an oxygen generating biomaterial to supplement oxygen, to bridge the lag between cellular implantation and development of an adequate vascular network.

Oxygen generating disks (OXY-SIL) were fabricated by mixing calcium peroxide with PDMS polymer and curing within molds to form disks, rods, or sponges. Oxygen production was assessed via sealed chambers/sensors. Cell studies co-incubated MIN6 or islets (human or rat) with OXY-SIL, cultured at either normal (20%) or hypoxic (5%) oxygen. Cell viability and insulin secretion were assessed.

We found that the oxygen generative capacity of the biomaterial could be modulated via the peroxide concentration and total polymer volume or surface area. For example, OXY-SIL sponges produced oxygen for ~2 weeks (minimum of 100 mmHg/day), while OXY-SIL disks produced oxygen for over 40 days (minimum of 100 mmHg/day). Co-culture of MIN6 cells or islets under hypoxic conditions with OXY-SIL resulted in ~2-fold increase in viability over hypoxic controls, with viability comparable to normoxic controls ($p<0.05$). Islets cultured in hypoxic conditions demonstrated significant insulin dysfunction, while islets incubated with OXY-SIL exhibited insignificant changes.

We have established the ability to fabricate a biomaterial capable of providing optimal, sustainable, and controllable delivery of oxygen. Encapsulation of solid peroxide within hydrophobic PDMS shielded the surrounding milieu from the peroxide and any detrimental degradation by-products. Enhanced cell viability and function for beta cell lines and islets was observed when incubated with OXY-SIL at hypoxic conditions. Given the ubiquitous need for optimal oxygen delivery within implants, we believe this material can provide benefit to numerous cell transplants.

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Sustained function of syngeneic and allogeneic islets in a macroporous scaffold implanted in the rat omentum

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Currently, islet transplantation consists of intrahepatic embolization into the porto-venous system, which makes islets irretrievable and prone to mechanical, chemical, and inflammatory stress. We designed a macroporous polydimethylsiloxane (PDMS)-based scaffold, co-delivered with a polymer-bound angiogenic factor, to serve as a support structure for transplanted islets and competent vascular network development. PDMS scaffolds (10mm diameter/2mm height) were fabricated via solvent casting and particulate leaching. Fibrin matrix was functionalized with a fibronectin fragment to promote cell adhesion and cell-demanded platelet-derived growth factor BB (PDGF-BB) release. Lewis (1800 IEQ, syngeneic) or Wistar-Furth (3000 IEQ, allogeneic) rats islets were transplanted into streptozotocin-induced diabetic Lewis rats. Islets were loaded onto scaffolds, sealed with PDGF-fibrin matrix, and wrapped in an omental pouch. Control islets were implanted under the kidney capsule.
Syngeneic implants corrected diabetes with a median time to normoglycemia (MTN) of 2.5 days (range 1-6; n=6) in the omental scaffolds, compared to 3 days (range 1-6; n=3) for kidney capsule controls. Removal of the scaffolds-bearing grafts (>100 days) invariably resulted in prompt return to hyperglycemia. Histological assessment showed high material compatibility, viable islets, and strong vascular infiltration. Immunosuppression of allograft recipients consisted of anti-lymphocyte serum (day -3), 3-week course mycophenolic acid (MPA, 10mg/ml), and chronic fingolimod treatment (FTY720, 1mg/kg starting on day 0). Allografts implants corrected diabetes with a MTN of 2 days for omental scaffolds (n=4) and kidney capsule grafts (n=2). All grafts maintained long-term (>60 days) function under systemic immunosuppression.
Our data indicates that the three-dimensional bioengineered scaffold is highly biocompatible and efficacious in supporting islet engraftment and function in both syngeneic and immunosuppressed, allogeneic diabetic rat models. These characteristics, along with its ease of retrieval, make the platform an excellent support structure permitting implantation of insulin-producing cells in alternate sites for the treatment of type 1 diabetes. Supported by JDRF and DRIF.

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Localized drug delivery in a islet transplant site via engineered biomaterials

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Inflammation during engraftment is a prominent issue in cellular implantation. For islet transplantation, it contributes to low graft survival rates and the necessity for high islet loadings. The local, sustained delivery of anti-inflammatory agents at the site of transplantation might alleviate this problem, while avoiding side-effects caused by systemic administration. As a model system, we evaluated the local delivery of dexamethasone (Dex) from polydimethylsiloxane (PDMS) implants.
Dex-PDMS materials were fabricated by incorporating dexamethasone into PDMS prior to curing. Geometries tested include: disks, rods, and cages. Drug release was characterized in-vitro. Suppression of activation of human monocytes and macrophages by Dex-PDMS was assessed via surface marker expression and IL-6 release, respectively. Drug release and effects on islet function of Dex-PDMS implants in-vivo were assessed in murine syngeneic transplants.
With a constant surface area-to-volume ratio (SA/V) among geometries, dexamethasone release profiles were comparable. Release studies exhibited an initial burst (1-3 d), followed by sustained release levels that linearly correlate with percent drug loading of the construct, with plateau values from 0.5-0.1 μg/day/construct. Computational models predict sustained drug release from 1-12 months, depending on the drug load and construct volume. Urine and blood measurements of mice implanted with 5–20% Dex-PDMS disks support in-vitro data, with similar burst (1-7 days) and plateau profiles (>90 days). In-vitro activity studies of PDMS-Dex disks (5-20%) with THP-1 monocytes or macrophages resulted in substantial suppression of inflammatory activation, indicating a therapeutic level of release. Preliminary studies in diabetic mice recipients of syngeneic islets using Dex-PDMS rods showed reversal of diabetes and sustained graft function.
Sustained drug release from Dex-PDMS constructs was confirmed at therapeutic levels with the ability to release long-term (>1 year). The rate and duration of the release can be modified by varying the surface/volume, total volume, and drug loading parameters. Supported by JDRFand DRIF.

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Silicone rubber membrane devices allow islet culture at 20 times the standard surface density with no adverse effects on viability, recovery, or potency

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Introduction: Islet culture in T-flasks requires seeding the islets at surface densities below 200 IE/cm^2 to provide sufficient oxygenation through the media. More than 20 T-175 flasks are needed for large porcine or human islet preparations, especially when impure. Culturing islets on top of gas-permeable (GP) silicone rubber membranes increases proximity of islets to ambient oxygen and allows entire preparations to be cultured in 1-2 flasks. In this work we summarize the characterization of 2-day porcine islet culture in GP silicone rubber devices (Wilson Wolf, New Brighton, MN).
Methods: Porcine islets from 30 donors were cultured for 2 days along with standard culture (T-flask, 200 IE/cm^2, 1000 IE/mL) paired controls. Conditions were seeded at 100-4000 IE/cm^2 measured by DNA (1 IE = 10.4 ng DNA). The primary outcomes were islet viability, measured by oxygen consumption rate to DNA ratio (OCR/DNA), DNA recovery, viable tissue (OCR) recovery, and islet potency, assessed with the diabetic nude mouse bioassay (2000 IE under the kidney capsule).
Results: For GP devices, OCR/DNA, DNA recovery, and OCR recovery were similar to paired controls, even at 4000 IE/cm^2. In contrast, all these outcomes deteriorated with increasing surface density for non-GP devices. For surface densities over 1000 IE/cm^2 and relative to paired controls, OCR/DNA was 58 ± 6% (mean ± SEM, n = 13, P = $1.1\text{x}10^{-5}$) for non-GP devices and 95 ± 4% (n = 25, P = 0.27) for GP devices; DNA recovery was 67 ± 6% (n = 8, P = $2.2\text{x}10^{-4}$) for non-GP devices and 114 ± 10% (n = 12, P = 0.21) for GP devices; and OCR recovery was 34 ± 4% (n = 8, P = $8.2\text{x}10^{-7}$) for non-GP devices and 113 ± 14% (n = 12, P = 0.36) for GP devices. Islets from 5 preparations cultured at 1000 or 4000 IE/cm^2 in non-GP and GP devices were transplanted into nude diabetic mice. Diabetes reversal rates were similar for islets cultured at high densities in GP devices (14/16) as for paired control islets (14/15). In sharp contrast, only 1 of 13 mice (P=$1.0\text{x}10^{-5}$) exhibited delayed diabetes reversal after being transplanted with islets cultured at high densities in non-GP devices.
Conclusion: Culturing islets in GP devices allows the increase of islet seeding density from 200 to 4000 IE/cm^2 while maintaining islet viability, recovery, and potency relative to paired standard controls. Islets cultured at similar high surface densities in non-GP devices exhibit decreased viability and recovery and fail to reverse diabetes in nude mice.

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Development and in vitro evaluation of Silica-based iron oxide particles as intracellular contrast agent for cellular imaging with MRI

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Objectives: Magnet Resonance Imaging (MRI) can be used for non-invasive monitoring during and following cell transplantation. Cellular imaging with MRI requires labeling of cells with intracellular contrast agents. Micrometer-sized iron oxide particles have a stronger effect on the magnetic field compared to nanometer-sized particles, but clinical applicable micron-sized particles not available. The aim of this study was to develop a new class of micron-sized particles for cellular imaging based on clinical applicable materials.
Methods: We developed silica-based particles (1,18µm) with different surface modifications: positively charged Poly-L-Lysin, neutral Streptavidin, and negatively COOH-groups. Huh7-cells, primary rat hepatocytes, and primary human hepatocytes were used for *in vitro* evaluation. Uptake mechanisms were investigated by inhibiting endocytosis using hypothermia (4°C.) or culture medium supplemented with NaN_3. Phantom studies were performed using 3.0 MRI and a clinical whole body coil. Transaminase leakage was measured to investigate possible adverse effects of particles on labeled cells.
Results: Labeling with Poly-L-Lysin particles produced iron load of approximately 35pg iron/cell. COOH-coupled particles lead to about 22pg iron/cell and Streptavidin-particles achieved 6pg iron/cell. Treatment with endocytosis

inhibitors caused no significant differences in particle uptake, indicating passive particle uptake. Cells labeled with Poly-L-Lysin-particles were detectable from a cluster of at least 5000 cells, whereas detection threshold for COOH- and Streptavidin-labeled cells were 10.000 and 25.000 cells, respectively. Effects on transaminase leakage were similarly low for the three particles.
Conclusions: We showed that the new Silica-based particles enabled in vitro detection of cell cluster under conditions of clinical MRI without negative effects on labeled cells. The different surface modifications offer the opportunity of creation of multifunctional theranostic agents enabling diagnostic imaging, drug delivery and therapeutic monitoring.

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Supporting islet transplant using polymer nanofiber scaffolds loaded with proangiogenic and immune suppressive compounds

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Alternate islet transplant sites must be pre-vascularized or very quickly vascularized following transplant in order to prevent hypoxia induced islet necrosis. The local release of the S1P analog pro-drug FTY720 (fingolimod), can induce microvascular remodeling. We are investigating the ability of nanofiber scaffolds loaded with FTY720 to modulate the process of angiogenesis and inflammation as well as provide physical cues that mimic the extracellular matrix. Nanofibers are electrospun from polymer solutions of PLAGA or PLAGA/PCL with and without FTY720. Islets are assessed for viability (Propidium Iodide and Fluorescein Diacetate staining) and function (response to 28mM and 2.8mM glucose). Microvascular remodeling is assessed by automated image analysis of intravital light microscopy images obtained from dorsal skinfold window chambers.
Image analysis of repeated measures of microvessel metrics show significant differences between diabetic and non-diabetic animals within 7 days of implant in response to FTY720 local release ($p<0.05$). Islets cultured with nanofibers for 48 hours show a reduced viability (45% fibers verses 70% for controls), however the addition of FTY720 improves the viability to at least that of untreated islets (76%). No significant differences in Stimulation Index have been found (1.85 controls verses 2.0 fibers). Preliminary data from *in vivo* studies suggest that local release of FTY720 may reduce the rejection process in allotransplant models and that the pocket does not interfere with insulin secretion.
A polymer nanofiber pocket provides a device in which islets can be transplanted in alternate sites. The porous nature of the nanofibers allow blood vessels to penetrate the pocket to reform the important intraislet vasculature. The local release of factors, such as FTY720, from the biodegradable polymers provides a way to guide the healing and inflammatory process where a strict immune barrier may not be required. Further animal studies, including xenogenic transplants are planned.

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Oxygen modulation in the culture of islets of Langerhans: the use of PFC-PDMS gas permeable membranes

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Research into the metabolic demands of islets of Langerhans has highlighted their exquisite sensitivity to external oxygen partial pressures. Exposure to oxygen levels that are non-physiological results in islet loss and dysfunction, either the result of hypoxic cell necrosis/apoptosis or hyperoxic free radical mediated damage. There is a need for static culture platforms that allow for the culture of islets at oxygen partial pressures that better approximate their *in vitro* niche without the sacrifice of culture practicality or islet viability and function.
In this study, we examined the use of novel platforms in the culture of human islets of Langerhans compared to standard methodologies. These perfluorocarbon impregnated polydimthylsiloxane platforms allowed us to tailor environmental oxygen levels to better match the physiological niche of isolated islets and we theorized that doing so would improve islet viability and function. We investigated the effect of physiological oxygenation by comparing overnight loss, oxygen consumption rate, glucose stimulated insulin release, and in vivo efficacy via marginal mass (1,000 IEQ) sub-renal capsular islet transplants in immunodeficient mice.
Our results demonstrated significantly better outcome in all *in vitro* assessments for islets cultured at physiological pO_2 (8-12%) on PFC-PDMS membranes relative to control conditions. Overnight loss in the PFC-PDMS group was

40% that of control conditions (p = 0.011), oxygen consumption rate index was 1.3 fold that of control conditions (p << 0.01) and the "delta insulin" (stimulated insulin produced – basal insulin produced) was 1.6 fold that of control conditions (p << 0.01). In diabetic mouse transplantation experiments, we observed a non-significant difference in reversal rates (p = 0.12) by log rank (Mantel-Cox) test, although significant (p = 0.04) by the Gehan-Breslow-Wilcoxon test. There was a promising trend in the median reversal time demonstrating a more rapid return to normoglycemia in the PFC-PDMS group (9 vs 14.5 days).

Parallel Session 8
Immunology (Immunoisolation)

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High resolution, non-invasive longitudinal live imaging of immune responses

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In vivo imaging has emerged as an indispensible tool in biological research. Intravital studies have revealed the significance of immune cell dynamics in the lymph nodes during immune responses. However, little is currently known about the *in vivo* behavior of T-lymphocytes in peripheral organs, including grafted tissues such as pancreatic islets. This has been primarily due to the combination of the limited noninvasive access to the same tissue in the living animal longitudinally and the relatively low spatial resolution of existing intravital imaging modalities. Here, we noninvasively studied the *in vivo* movement dynamics of T-lymphocytes that infiltrate pancreatic islets after transplantation. MHC-mismatched DBA/2 mouse islets were transplanted without immunosuppression into the anterior chamber of the eye (ACE) of C57BL/6(B6.129P2-Cxcr6tm1Litt/J) recipients expressing GFP in effector and memory T-lymphocytes. Rejection was characterized by progressive accumulation of GFP-positive lymphocytes paralleled by loss of volume and function of allogeneic islets in the ACE. *In vivo* analysis of morphology and dynamic behavior of islet-infiltrating effector T-lymphocytes revealed a "ruffled" phenotype of cytotoxic T-lymphocytes that predominated during the effector phase of rejection. Ruffled cells engaged in contacts with other surrounding T-lymphocytes and target islet cells simultaneously. The ruffled phenotype was characterized by a significantly increased but constrained dynamic behavior within the islet allografts. In summary, our results demonstrate that intraocular transplantation enables: **(1)** longitudinal, non-invasive monitoring of transplanted tissues*in vivo*; **(2)** *in vivo* cytolabeling to assess cellular phenotype and viability*in situ*; **(3)** local intervention by topical application or intraocular injection; and **(4)** real-time tracking of infiltrating immune cells in the target tissue.

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Antibodies: barriers to islet cell transplantation

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Background: HLA antibodies have been shown to have a deleterious effect on most organ transplants. Based on this it is also possible that donor specific HLA antibodies (DSA) are a major cause of function loss in islet cell transplant (ICT) recipients. In addition anti-GAD (GADA), anti ZnT8 (ZnT8A) and anti-IA2 (IA2A) autoantibodies (AutoAb) may also lead to poor outcomes in islet recipients. Herein we describe the association between antibodies (allo- and auto-) and outcomes in ICT patients.

Methods: ICT patients (n=44, 37 Islet Transplant Alone and 7 Islet after Kidney Transplant) between 2001 and 2010 were studied. Sera (n=385) were serially collected from the date of transplant and tested for DSA (via single antigen beads), GADA, ZNT8A and IA2A.
Results: The median survival of transplant from first islet cell infusion (Tx) was 441±216 days. Insulin independence, partial function and early graft loss were achieved in 48% (21/44), 32% (14/44) and 20% (9/44) of recipients, respectively. Regarding DSA, 27% (12/44) of the patients became positive post-transplant (isotype: 4/12 IgG, 3/12 IgM, 5/12 IgG and IgM). Regarding AutoAb, 36% (16/44) showed a significant rise post-transplant (8/16 GADA; 5/16 ZnT8A; 2/16 GADA and IA2A; 1/16 GADA, IA2A and ZnT8A). The median time of Ab appearance was 14±3, 16±1, 28±2, 90±73 and 189±59 days post Tx for GADA,IA2-A, DSA IgM, ZnTA and DSA IgG, respectively. The probability to develop DSA was associated with the number of donor and mismatches while the probability to develop AutoAb was associated with the absence of rapamycin treatment. Of major importance it was found that development of DSA and/or AutoAb was significantly associated with loss of islet function ($p < 0.005$).
Conclusion: The development of DSA and AutoAb after Tx is highly associated with failure. This data suggests that monitoring these antibodies in ICT patients is important and that treatment to remove these antibodies may benefit outcomes.

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CD39 over-expression prevents autoimmune diabetes through adenosine 2 receptor (A2R) dependent mechanisms

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Background: Type 1 diabetes results from autoimmune mediated destruction of islet cells. Although the transplantation of islet cells offers a potential cure, graft survival is compromised by a number of factors including recurrent autoimmune disease.
Aim: To examine the effect of CD39 over-expression on the development of autoimmune diabetes.
Methods: C57BL/6 male mice over-expressing CD39 (CD39tg), CD39 knock-out (KO), A2ARKO, A2BRKO and wild type (WT) were treated with multiple low dose streptozotocin (MLDS - 50mg/kg ip for 5 days). Some mice were treated with A2BR inhibitor (A2BRi; 0.5mg/kg ip bd). A blood glucose level (BGL) >20mM was deemed diabetic. Adoptive transfer experiments were performed to isolate the effect of CD39 over-expression.
Results: WT mice became diabetic 42 days following MLDS (BGL 21.2±2.4mM). The onset of diabetes was faster in CD39KO, A2ARKO (days 12 & 10 respectively), and A2BRKO mice (day 30). CD39tg mice remained normoglycemic throughout the 90 day follow-up period (BGL 8.1±0.2mM). The protective effect of CD39 over-expression was lost by crossing CD39tg with A2ARKO mice (BGL 21.2±1.2mM at day 30) or by treating the CD39tg with A2BRi (BGL 17.6±1.5mM at day 22). The over-expression of CD39 on tissues alone prevented diabetes (BGL 10.3±1.1mM at day 30).
Conclusion: The over-expression of CD39 mitigates MLDS induced autoimmune diabetes through A2A and A2BR mechanisms. CD39 over-expression on the tissues alone was sufficient to confer this protection. Modification of human islets to over-express CD39 may improve islet graft survival by inhibiting recurrent autoimmune disease.

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Influence of islet culture on angiogenic and inflammatory mechanisms

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Introduction: The early events hampering islet engraftment may be related to IBMIR and to the insufficient revascularisationof islets inducing β cells death. However, the influence of time of culture of islets on cellular mechanisms involved in islet revascularization and IBMIR are not well understood and must be elucidated. The aim of this work was to study the influence of islet culture on angiogenesis and inflammatory reactions *in vitro*.

Materials and methods: Rat pancreatic islets were cultured for 0, 12, 24 and 48h. The identification of signaling pathways involved in angiogenesis and IBMIR was performed by PCR array. 84 genes were analyzed using the RT ² Profiler PCR Array Data Analysis Template v3.2. Insulin expression was evaluated using qPCR. The comparative study of gene expression has been made toward t=0h (n= 3).
Results: After 12h of culture, islets exhibit a significant decrease in gene expression of growth factors and their receptors such as IGF1, the kdr… with respectively -18.42 and -5.65 fold (p <0, 001) which is maintained for 48 hours. No significant modulation of the expression of VEGF A, B and C is observed during the study. Then, the metallopeptidase 9 (MMP9) is 16.1 times overexpressed at 12h while its inhibitor TIMP1, was 55.01 times overexpressed (p <0.001). This unfavorable environment for angiogenesis is confirmed since 48 h of culture, MMP9 is not overexpressed in contrast to TIMP1 (20.3 times, p <0.01). Then, only after 48 hours of culture, we observed a significant increase of the expression of extracellular matrix genes such as procollagen 18α1 (5.61 times, p <0.05). Moreover, after 12h of culture, the islets have a significant overexpression of proinflammatory cytokine such as IL-6 and CXCL1 with respectively 597.9 (p <0.001) and 429.2 fold (p <0, 05). However, this overexpression of proinflammatory cytokines decreased after 12h. Finally, in association to these results, the insulin expression decrease after 12h of culture with 0.68 ± 0.323 fold overexpression after 24h of culture *versus* 2.059 ± 0.597 fold after 12h (p< 0,05).
Conclusion: This study showed that current conditions of culture are deleterious to a good implantation of islet after transplantation. These results may help finding different ways of islets protection, if they are confirmed by proteomic data.

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Longitudinal, live imaging of islet autoimmune destruction in NOD mice

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NOD mice spontaneously develop Type-1 diabetes (T1D). Islet cells are difficult to access *in vivo,* because they are scattered within the exocrine tissue of the pancreas. Multiphoton/confocal fluorescent microscopy allows performing live imaging with cellular resolution. However, surgical exteriorization of the pancreas is required to study islet immunobiology, precluding longitudinal studies. We hypothesized that the anterior chamber of the eye (ACE) represents a useful in vivo tool to study islet immunobiology. The progression of autoimmunity was assessed by transplanting NOD.SCID islets into either spontaneously diabetic female NOD (recurrence model) or NOD.SCID mice (autoimmunity adoptive transfer model).
Recurrence of diabetes occurred with a median of 10 (range 5-12) and 8 (5-13) days in the ACE (n=6) and kidney capsule (KDN; n=12), respectively. Adoptive transfer of splenocytes from newly diabetic NOD mice induced diabetes within 30-35days. Longitudinal assessment of individual islet volume and granularity in the ACE of reconstituted NOD.SCID mice demonstrated islet swelling starting one week before onset of overt hyperglycemia followed by relatively quick volume reduction within a week. Live time-lapse studies performed after direct cytolabeling and cell viability dye injection in the ACE in selected animals allowed assessing the behavior of infiltrating B and T cells and cell death in the target tissue with single-cell resolution prior, during and after the onset of diabetes. Preliminary assessment of islet grafts explanted after diabetes onset from ACE and KDN showed infiltrating T and B lymphocyte populations similar to native pancreas by immunofluorescence staining.
In conclusion, islet transplantation into the ACE represents a valuable model to study islet immunity and offers unprecedented advantages compared to other transplantation sites, particularly the possibility to perform longitudinal in vivo studies on the very same islets with cellular resolution to characterize the effector phase kinetics of the infiltrating cells on the site of immune attack.
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Antioxidant therapy maximizes tolerance induction to islet allografts

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We evaluated the effect of the combination of antioxidant drugs with blockade of Lymphocyte Function Associated Antigen-1 (LFA-1) on the survival of fully MHC-mismatched islet allografts.
Streptozotocin-induced diabetic C56BL/6 (H-2^b) mice received DBA/2 (H-2^d) islets under the kidney capsule. Anti-LFA-1 antibody (KBA clone; 100ug daily for 1 week) and/or 15-day antioxidant treatment with 15mg/kg/day of Decanedioic-acid-bis(1-hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)diester-dihydrochloride (Iacvita, IAC, Medestea) were given intraperitoneally. Nonfasting glycemic values were monitored after transplant.
Untreated mice rejected with a median survival time (MST) of 15 (range 7-24) days (n=12). IAC treatment alone resulted in a MST of 17 (12-19) days (n=4; p=N.S.). Anti-LFA-1 alone resulted in long-term (>100 days) survival in 62.5% of the recipients (n=8; p=0.03 vs. controls). Combinatorial anti-LFA-1+IAC resulted in 100% long-term (>100 days) graft survival (n=3; $p<0.014$ vs. control; $p<0.03$ vs. IAC). Re-challenge with donor-specific islets without further treatment after nephrectomy of graft-bearing kidney showed that 50% of anti-LFA-1 treated mice (n=2) rejected on 60 days, while all anti-LFA-1+IAC treated animals (n=3) accepted donor-specific islets (>100 days). Administration of anti-CD25 antibody associated with a reduction of Treg cell numbers without resulting in islet rejection. Challenge with donor-specific and third-party (C3H, H2^k) skins resulted in rejection of the grafts without loss of islet function.
Our data indicates that peri-transplant LFA-1 blockade results in long-term survival of islet allografts in only a proportion of recipients. A synergistic effect of the combinatorial treatment with LFA-1 blockade and IAC, an antioxidant drug, was observed in this model with all animals maintaining a functional graft long-term and accepting indefinitely donor-specific islets, while able to reject donor-specific and third-party skins. This data suggests the achievement of operational tolerance in this model. The use of antioxidants, such as IAC, may represent a viable strategy to enhance the success of tolerance inducing protocols.

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Mesenchymal stem cells prolong the survival of xenogenic neurons in the brain

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Cell transplantation is of great interest for the treatment of neurodegenerative diseases. Transplantation of human fetal neuroblasts in the brain of Parkinsonian's patients has provided interesting results but tissue availability and ethical concerns limit this approach. An interesting alternative would be to use fetal pig neuroblasts (pNB) but xenogenic neurons are systematically rejected, even in the brain. Since mesenchymal stem cells (MSC) have potent immunosuppressive functions, we ask whether MSC could protect xenotransplanted neurons from rejection. To solve this issue, pNB or pNB+MSC were transplanted in the striatum of immunocompetent rats. In the pNb group, the graft was systematically rejected before 63 days, and the rejection process was associated with a local induction of proinflammatory molecules followed by a peak of anti-inflammatory cytokines. In the pNB+MSC group, 50% of the rats exhibited at 120 days, healthy grafts with porcine $NF70^+$ neurons and dopaminergic cells (TH^+). This long survival observed in the presence of MSC was associated with an inhibition of cytokine and chemokine induction and a prolonged upregulation of neurotrophic factors such as BDNF and GDNF. An active role of MSC in the long-term survival of pNB in the rat brain was supported by the fact that MSC protected pNB from cell death in culture and blocked T cell proliferation in vitro. To check that co-grafting strategy did not affect function of the transplant, MSC and pNB were co-transplanted into the striatum of 6-OHDA lesioned rats (model of Parkinson's disease). Behavioral analyses showed motor recovery in the rats that had received MSC and pNB. Taken together, these data indicate that MSC are of great interest as a local immunosuppressive and neurotrophic agent in case of intracerebral transplantation.

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Transplanted donor Treg cells can stably engraft leading to tolerance to allogeneic bone marrow cells in NOD mice

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There is increasing interest in the use of naturally occurring $CD4^+Foxp3^+$ regulatory T cells (T_{reg}) in their application for adoptive immunotherapy in the treatment of autoimmune diseases, including type 1 diabetes (T1D). Studies have shown T_{reg} cells have therapeutic effects on the course of autoimmunity in NOD mouse, a model of human T1D. However, T_{reg} immunotherapy is hindered by the inability to generate sufficient cell number to inhibit the desired immune response(s) and achieve stable donor T_{reg} engraftment. In the present study, we examined the in vivo biological environment necessary to promote stable donor T_{reg} engraftment and induce tolerance to allogeneic (allo) BM cells. We describe that not only is peripheral space required, but competition from endogenous T_{reg} cells needs to be overcome for successful engraftment of transplanted donor T_{reg} cells together with IL-2, which is critically important for driving their proliferation and survival. We found that conditioning prediabetic NOD mice with varying amounts of total body irradiation resulted in a dose-dependent level of donor $Thy1.1^+$ NOD T_{reg} engraftment. Furthermore, treatment with busulfan and cyclophosphamide (Bus+CyP) leads to significant engraftment of transplanted donor T_{reg} cells compared to unmanipulated mice or mice treated with Bus or CyP alone. Importantly, adoptive transfer of syngeneic or allo-T_{reg} cells together with short course of anti-IL-2 (clone JES6)/IL-2 complex and rapamycin, which favors T_{reg} expansion, induces tolerance to small numbers ($10x10^6$) of allo-BM cells resulting in mixed chimerism and prevention of diabetes in these recipients. Importantly, donor T_{reg} cells were readily detected 48 weeks post-transfer. Collectively, our findings indicate that some level of manipulation of the recipient's immune system greatly facilitated long-term donor T_{reg} cell engraftment and therapeutic efficacy. Thus, T_{reg} immunotherapy can lead to alloantigen tolerance even in NOD mice, which are highly resistant to tolerance induction (Supported by ADA Junior Faculty Award 7-09-JF-06 and Diabetes Research Institute Foundation)

Parallel Session 9
Hepatocytes and Myoblasts

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Assessment of cold stored human hepatocytes for transplantation: Storage of liver tissue vs. isolated hepatocytes

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Background: Hepatocyte transplantation is a useful treatment for patients with acute liver failure or metabolic disease. Repeated infusions over 1-2 days improve engraftment and clincial outcome and isolated hepatocytes are usually cold-stored during this time. This study aimed to determine the best way to cold preserve human hepatocytes for repeated transplantations.
Methods: Hepatocytes were isolated from tissue obtained from resections or organ donors by collagenase digestion. Hepatocytes were analysed directly after isolation (fresh) or stored for 48 hours at 4° C in University of Wisconsin Solution (UW cells). Liver tissue from the same donor was stored at 4° C in UW and hepatocytes were isolated after 48 hours (UW tissue cells). Hepatocyte functions were evaluated by trypan blue exclusion, plating efficiency, ammonia metabolism and drug-mediated metabolic activities including CYP1A1/2, 2C9, 3A7, 3A4 and conjugation, caspase and TUNEL assays.
Results: Mean viability of freshly isolated hepatocytes was 79%. Viability decreased after 48 hours of UW cold storage to 55% which was significantly less than UW tissue cells (71%). Plating efficiency decreased by 50% in UW cells whereas UW tissue cells retained 70% of the original plating efficiency. Fresh and UW tissue cells showed a similiar ammonia and drug metabolism activity which was significantly higher than in UW cells. Hepatocytes stored in 48 hrs in UW showed a strong increase of TUNEL positive cells whereas TUNEL staining in cold-stored liver tissue and hepatocytes isolated after 48 hrs was nearly unchanged, indicating an increased rate of apoptosis during cold storage of isolated hepatocytes.
Conclusions: Cold storage of liver tissue and hepatocyte isolation after 48 hrs is often superior to cold storage of isolated hepatocytes as measured by hepatocyte viability and function.

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Effects of pro-inflammatory cytokines on human hepatocyte drug and ammonia metabolism

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Hepatocyte transplantation (HTx) has shown benefit as a treatment for metabolic liver disease and acute liver failure. Patients with urea cycle defects are common HTx recipients. Because tracking the cells after transplantation is difficult, continued function of the donor cells is inferred from the ammonia levels in recipients. However, in cases of sepsis or viral infection, ammonia levels can significantly and abruptly increase in patients with urea cycle defects. If this occurs in a HTx recipient, one might suspect that the cell graft was being rejected. Pro-inflammatory cytokines associated with viral or bacterial infections are known to suppress many liver functions, including the drug metabolizing enzymes and hepatic transport activities. We examined the influence of mediators of the immune response such as tumor necrosis factor-alpha (TNFa), interleukin-1beta (IL-1b) and interleukin-6 (IL-6) on drug metabolizing activity and ammonia metabolism in primary cultures of human hepatocytes. Human hepatocytes were isolated from normal donors and patients with different metabolic diseases. Ten different measures of hepatocyte viability and function including, plating efficiency, ammonia metabolism, conjugation reactions and cytochrome (CYP) P450-mediated metabolism were examined. Ammonia metabolism and other CYP isoform activities were analyzed immediately after isolation and after three days of exposure to concentrations of 0.1 to 10 ng/ml of the three cytokines, alone and in combination.
Both CYP-mediated drug metabolism and ammonia metabolism were profoundly suppressed in hepatocytes exposed to IL-6 and/or IL-1 b. Metabolic activities were less affected by TNF-alpha exposure. These data indicate that like CYP-mediated drug metabolism, suppression of ammonia metabolism occurs in hepatocytes exposed to pro-inflammatory cytokines in the absence of cell death. These observations have significance for urea cycle patients following HTx procedures in that abrupt spikes in ammonia levels concurrent with bacterial or viral infections do not necessarily indicate a rejection response or loss of the cell graft.

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Hepatocytes from metabolic disease patients: a potential cell source for domino transplant

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Most inborn errors of metabolism are caused by single defects in an enzyme or transport protein that alters a metabolic pathway. Although individually rare, considered together, liver-based metabolic diseases represent approximately 10% of pediatric liver transplants and, in many centers, are the second most common indication for liver transplant after biliary atresia. Hepatocyte transplantation has been proposed as an alternative to liver transplantation for certain metabolic liver diseases. A major obstacle is the limited supply of donor tissue for cell isolation. Many tissues available for hepatocyte isolation are from organ donors (OD) rejected for organ transplant and are of marginal quality. We analyzed hepatocytes from 11 different metabolic diseases in term of recovery, viability, plating efficiency, ammonia and drug metabolizing capacity. Long-term hepatocyte quality was accessed by the expression and metabolic activity of Cytochrome P450 (CYP) 3A4, 3A7, 2C9 and 1A1/2 both on freshly isolated cells and *in vitro* after exposure to prototypical inducing agents. We obtained cells with acceptable viability and function from most of the 26 metabolic cases analyzed (viability 79±20% compared to 77±13% from 33 OD) and the mean plating efficiency of these cells was higher than those from ODs. Organs with urea cycle defects provided cells with an expectedly low (or null) capacity to metabolize ammonia, however they showed robust CYP activity immediately after isolation and surprisingly high activity after induction (up to 5 times the best OD). Cells isolated from diseases such as Oxalosis, Crigler-Najjar or Maple Syrup Urine Disease provided cells with robust phase I and II activities. Cirrhotic or cholestatic tissues such as Biliary Atresia provided cells which varied in quality

with each donor. These results suggest it is reasonable to investigate hepatocyte domino transplant with cells obtained from donors with metabolic diseases with specifically matched recipients.

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Neohybrid Liver Graft - a novel concept of in vivo tissue-engineering

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Introduction: Liver transplantation is an effective therapy for end-stage liver disease, but life-long immunosuppression may lead to serious complications. Hepatocyte transplantation alone still has not reached long-lasting effects, mainly due to hepatocyte failure over time. Here, we investigated whether transplantation of syngeneic hepatocytes into allogeneic liver grafts may be used as a novel concept to improve tolerance induction.
Methods: Male Lewis rats were pretreated with 2-acetaminophen and partial hepatectomy to induce proliferation of hepatocytes and progenitor cells, especially oval cells. Hepatocytes and progenitor cells were isolated in a modified two-step procedure according to Seglen with density gradient separation of progenitor cells. Cells were transplanted into female Lewis rats that had undergone allogeneic liver transplantation. Livers were retrieved from female Dark Agouti rats pretreated with Retrorsin to facilitate cell engraftment. 5 x 10_6 Hepatocytes and progenitor cells were transplanted via the spleen, either immediately following organ transplantation, or up to 24hrs after organ transplantation after full recovery of recipient rats. Transplanted Lewis rats received continuous immunosuppression with Cyclosporin A and were sacrificed at days 8, 15, 30 and 90. Liver cell integrity was evaluated measuring transaminase levels. Detection of transplanted male cells was carried out by FiSH-typing of y-chromosomes and additional immunhistochemical staining of OV-6, CK18, CK19 and BrdU.
Results: Animals surviving the first 3 days after combined hepatocyte and liver transplantation showed stable liver function until they were sacrificed. FiSH-typing revealed moderate to good engraftment of transplanted male cells at all time-points mainly close to portal tracts. Counting of male cells/vision field (200x) showed a proportion between 13% and 38% of male cells per total cell count. So far no statistically significant difference between time-points could be detected (n=4/time-point).
Conclusion: In order to establish the new concept of a *neohybrid liver graft* we could proof a relevant proportion of engrafted cells up to 90 days after combined liver and hepatocyte transplantation. Cell transplantation via the spleen seems to be a safe and reliable technique, even after major surgery like liver transplantation. Influence of progenitor cells on engraftment and proliferation of transplanted hepatocytes are currently under evaluation, especially with respect to minimization of immunosuppression and induction of tolerance.

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Autologous transplantation of bone marrow mononuclear cells in patients with decompensated cirrhosis

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Background: Liver cirrhosis is characterized by distortion of the hepatic architecture and the formation of regenerative nodules. Liver transplantation is one of the few available therapies for such patients. It has been shown recently, that hat bone marrow cell infusion repairs liver fibrosis in the cirrhotic liver.
Methods: This study was performed to determine the safety and tolerability of intrahepatic transplantation of autologous bone marrow mononuclear cells into ten patients with liver insufficiency. The bone marrow mononuclear cells were isolated and infused into liver via hepatic artery. At different time points after the transplantation, the liver function and prothrombin time (PT) were evaluated, and the survival rate and symptoms of the patients were recorded.
Results: No complications or specific side effects related to the procedure were observed; six patients showed improvements in serum albumin, bilirubin and ALT after one month from the cell infusion.

Conclusion: Our study has shown both the safety and feasibility of this type of liver cell therapy and may be a bridge to liver transplantation

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Generation of induced pluripotent stem cells from fetal human hepatocytes in feeder-free conditions

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Hepatocyte transplantation has been used as cellular therapy for liver disease. If iPS cells could be produced and induced to differentiate to hepatocytes, they might be useful for this cellular therapy. We report the generation of iPS cell lines from primary human fetal hepatocytes in feeder-free conditions following exposure to lentiviral constructs carrying the reprogramming factors OCT4, SOX2, KLF4, c-MYC or OCT4, SOX2, NANOG, LIN28 or OCT4, SOX2, NANOG. A profile was conducted pre- and post-reprogramming by Flow Cytometry for 18 surface markers commonly found on different types of stem cells. The percentage of SSEA-3, SSEA-4, TRA1-60 and TRA1-81 positive cells after reprogramming was dramatically increased in iPS cells (up to 90%). The putative liver stem cell marker, EpCAM, was expressed in approximately 50% of the cells prior to, and in nearly all cells following reprogramming. Similar observations were made with three additional stem cell markers, CD24, CD133/2 and c-Kit, which increased from near zero to 95%, 35% and 70%, respectively after reprogramming. Reprogrammed colonies have morphological features and the surface marker and gene expression profile typical of fully reprogrammed cells and they also form teratomas upon transplantation into NOD/SCID mice. Fetal human hepatocytes are more easily reprogrammed than adult hepatocytes. Reprogramming of adult hepatocytes was quite inefficient ($<1/10^6$ cells) while, with four factors, reprogramming efficiency of fetal hepatocytes was approximately $1/5x10^4$ cells. Moreover, iPS cells were also created following exposure to only 3 reprogramming factors (OCT4, SOX, and NANOG). Protocols to differentiate these cells back to a hepatic phenotype are in progress. These studies demonstrate that iPS cell lines can be efficiently generated from primary human fetal hepatoblasts/hepatocytes and suggest that this source of stem cells might be useful for regenerative medicine.

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Autologous transplantation of porcine muscle-derived cells into uninjured urethral sphincter enhance urethral closure pressure

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Myogenic cell therapy is considered as an alternative method to treat urinary incontinence. Cell transfer into urethral sphincter of rodents was reported to be successful. However, larger animal models usually allow for more adequate testing of new potential therapies. The present study analyses the fate of muscle-derived cells (MDCs) autotransplanted into uninjured porcine external urethral sphincter (EUS) and the effect on maximal urethral closure pressure (MUCP).

The experiment was performed on immature female pigs. Cells isolated for transplantation were identified by immunoblotting and their ability to differentiate. After the third passage, 60x10(6) of MDCs were labeled with fluorochrome PKH26 and injected in three depots into the EUS using urethrocystoscopy (n=5). Urodynamic assessments were performed before transplantation (day 1) and 4 weeks after treatment (day 28). Pigs were euthanized at day 28 and urethras were collected. In control, untreated group (n=5) urodynamic evaluations were performed in the same time points to assess the effect of growth on MUCP changes. Additionally, two pigs in which the vehicle was injected only served as negative control for histological studies.

Isolated MDCs expressed desmin and were able to fuse into myotubes in vitro. Transplanted cells were present at the sites of injections. The areas of fluorescence corresponded to three depots what indicated that injected cells did not migrate. Donor cells were localized within muscle layer, but also in other parts of urethral sections what suggested that the technique of injection was not precise enough. Nevertheless, the mean MUCP value in

transplanted group increased from 23.4 at day 1 to 34.5 mmH2O at day 28 ($p<0.05$). Moreover, the difference between MUCP in transplanted and control groups at day 28 was also significant (increase by 32%, $p=0.01$).
In conclusion, porcine model is suitable for further optimization of procedures associated with cell therapy in cases of urinary incontinence.

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Whole organ engineering for liver replacement: a regenerative medicine approach

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Liver transplantation is the only effective treatment for end-stage liver disease and the treatment is limited by the critical donor organ shortage. A promising regenerative medicine approach to address this issue is the construction of organs in vitro for transplantation. In this study, we aimed to regenerate liver tissue using adult hepatocytes, endothelial cells and biliary cells using decellularized rat livers as scaffolds in an organ perfusion system. Rat livers were decellularized using a new, minimally disruptive method based on trypsin/triton X. Native liver matrices were recellularized with adult primary rat hepatocytes for the parenchymal space, rat biliary cells for the bile duct and microvascular endothelial cells for the vascular system of the liver. The grafts were perfusion cultured for 5 d in vitro. The perfusate was sampled daily to assess hepatic secretory and synthesis function. Decellularized livers were examined by morphologic, biochemical, and immunolabeling techniques for preservation of the native matrix architecture and composition. The decellularization process preserves the three-dimensional macrostructure, the ultrastructure, the composition of the extracellular matrix components, the native microvascular network of the liver, and the bile drainage system, and up to 50% of growth factor content (HGF, bFGF). The three-dimensional liver matrix reseeded with the multistep infusion of hepatocytes generated ~90% of cell engraftment and supported liver-specific functional capacities of the engrafted cells, including albumin production, urea metabolism, and cytochrome P450 induction. More than 60% of the bile duct was adequately recellularized and the endothelial cells preferably aligned approximately 50% of vascular spaces. Whole-organ liver decellularization is possible with maintenance of structure and composition suitable to support hepatic function.

Parallel Session 10
Islets II

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Evaluation of transplanted islet beta-cell proliferative response in an ectopic site during pregnancy

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Beta-cell proliferation is rare in adulthood, while during pregnancy lactogenic hormones and serotonin mediate selective beta-cell proliferation peaking mid-pregnancy (day 12.5-14.5) in mice. Islets represent ~1-2% of pancreatic tissue. Enrichment of endocrine cells allows performing molecular studies aimed at identifying the transcriptome pattern of proliferating beta-cells, but requires isolation of islet at different stages of pregnancy. Islet isolation induces cellular stress responses that may alter basal expression profile, thus interfering with the detection of most relevant molecular pathways involved in beta-cell replication. We developed a model of syngeneic islet transplantation under the kidney capsule of chemically-induced diabetic female mice. Following completion of engraftment (≥4 wks), to allow recovery from islet isolation and implantation-related stress, animals are mated. Using this approach, the large mass of endocrine pancreatic tissue (that is, purified islets) can be easily retrieved without harsh manipulation for analysis.
Immunohistopathology assessment of bromodeoxyuridine incorporation in transplanted beta-cells showed proliferation rates comparable to those of the native pancreas. The proportion of proliferating beta-cells in the

pancreas was 7.4±2.9% in control mice (n=5) and 22.8±7.2% in pregnant mice (n=5) on day 12.5 ($p<0.01$). A similar pattern was observed in transplanted islets, with 4.5±2.1% in control (n=6) and 30.8±6.8% in pregnant mice (n=6) on day 12.5 ($p<0.001$). Microarray analysis of mRNA expression performed on islet grafts obtained from control and pregnant mice (day 12.5) showed that 296 of the ~25,000 genes detected were significantly differentially expressed in islet grafts of pregnant vs. control mice (n=3 independent microarrays with a $p<0.08$). 68 genes resulted significantly differentially expressed both in our analysis and a previous report on isolated islets (Rieck and coll. 2009). Differential expression of multiple genes was confirmed by qRT-PCR.
Transplanted islets proliferate under physiological conditions (e.g., pregnancy) in an ectopic site. This may open new avenues for in depth molecular studies.

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Improved allogeneic islet graft survival in mice by oxidized Adenosine Tri-Phosphate treatment

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Lymphocyte Function-associated Antigen 1 Anti-(LFA-1) plays a key role in lymphocyte trafficking and co-stimulation. Short-course anti-LFA-1 blockade is partially successful in preventing murine allograft rejection in mice. Modulation of early post-transplant inflammation has been recognized to improve engraftment and survival of allografts. Oxidized Adenosine Tri-Phosphate (oATP) is largely utilized as a nonselective blocker of the purinergic (P2X7) receptor with recognized anti inflammatory and analgesic proprieties. Our study aimed at evaluating the effects on islet allograft survival of oATP therapy alone or combined with LFA-1 blockade.
DBA/2 (H2d) islets were transplanted under the kidney capsule of chemically-induced diabetic C57BL/6 mice (H2b). Single or combined treatments included: oATP (Medestea Research) 0.25mg/mouse intravenously daily on day 0-4, then biweekly for 4 weeks; 7-day course of anti LFA-1 antibody (KBA clone) 100ug/mouse/day intraperitoneally from 0.
Naïve splenocytes showed a dose-dependent suppression of proliferative response to mitogenic stimulation (both mixed lymphocyte reaction and anti-CD3) in the presence of increasing concentrations of oATP in vitro. Control animals rejected allogeneic islets with a median of 15 days (n=12). Long-term allograft survival (>100 days) was observed in 25% of animals receiving oATP treatment alone (median survival=63 days; n=8; $p=0.04$), and in 57% of animals treated with anti-LFA-1 (n=7). Combination therapy based on oATP and LFA-1 blockade yielded 100% long-term graft survival (n=6; $p=0.0007$ vs. control group).
Our preliminary data shows a direct immunomodulatory effect of oATP both in vitro and in vivo. Furthermore, administration of oATP enhanced the efficiency of LFA-1 blockade in inducing long-term islet allograft survival in a fully MHC-mismatch transplant model. Combinatorial strategies targeting purinergic receptors and co-stimulatory blockade may allow for the development of efficient tolerogenic clinical protocols in the near future.

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A novel platform for islet transplantation using silk hydrogels

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Current biomaterials for pancreatic islet encapsulation have had limited clinical success due to mechanical and chemical instability, limited permeability, and stimulation of fibroblast overgrowth, leading to islet cell death. Silk, despite being used as a biomaterial for centuries, has not been fully investigated as an islet encapsulation material. Maintenance of islet graft function depends, in part, on stimuli from the islet microenvironment that promote revascularization, survival and proliferation of the islet cells. To reestablish the islet microenvironment during the

peritransplant period, we are developing and testing *in vitro* a non-immunogenic silk-based hydrogel scaffold for islet encapsulation. The silk hydrogel scaffold includes extracellular matrix proteins (i.e. laminin and collagen IV) and mesenchymal stem cells (MSCs), known to have immunomodulatory properties and to enhance islet cell graft survival and function. Up to 7 days post encapsulation in silk, islets maintained greater than 95% viability *in vitro*, as determined by LIVE/DEAD fluorescent staining. Islet function was assessed by quantifying insulin secretion in response to high glucose stimulation *in vitro*. After 2 days of culture, islets encapsulated in silk with collagen IV had a 1.6 fold increase in insulin secretion compared to free islets ($P<0.05$). At day 7, islets encapsulated in silk with laminin had a 1.6 fold increase in insulin secretion compared to islets encapsulated in silk without extracellular matrix proteins ($P<0.05$). Co-encapsulation with MSCs increased islet insulin secretion *in vitro* by 1.4 fold. In addition, co-encapsulation with MSCs suppressed splenocyte proliferation of anti-CD3/CD28 activated- or alloreactive splenocytes by 3.3 and 4.6 fold, respectively. These results suggest that a silk hydrogel-based MSC bioconstruct may be a suitable platform for islet transplantation by enhancing beta-cell function and diminishing the immune response.

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Isolation of human islets induces islet-intrinsic NF-kappaB which predicts impaired graft function and accelerated rejection

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In the context of islet transplantation, experimental models show that induction of islet intrinsic NF-κB-dependent pro-inflammatory genes can contribute to islet graft rejection. Isolation of human islets triggers activation of the NF-κB and mitogen-activated kinase (MAPK) stress response pathways. However, the down stream NF-κB-target genes induced in human islets during the isolation process are poorly described. Therefore in this study, using microarray, bioinformatic, and RTqPCR approaches, we determined the pattern of genes expressed by a set of 14 human islet preparations. We found that isolated human islets express a panel of genes reminiscent of cells undergoing a marked NF-κB-dependent pro-inflammatory response. Expressed genes included; matrix metallopeptidase 1 (MMP1) and Fibronectin 1 (FN1), factors involved in tissue remodelling, adhesion and cell migration; cytokines, including IL-1band IL-8; A20 and ATF3, genes regulating cell survival; and notably high expression of a set of chemokines that would favour the recruitment of neutrophils and monocytes, including CXCL2, CCL2, CXCL12, CXCL1, CXCL6, CCL28. Of note, the inflammatory profile of isolated human islets was maintained after transplantation into RAG-/- recipients. Thus human islets can provide a reservoir of NF-κB-dependent inflammatory factors that have the potential to contribute to the anti-islet-graft immune response. To test this hypothesis we extracted rodent islets under optimal conditions, forced activation of NF-κB, and transplanted them into allogenic recipients. These NF-κB activated islets not only expressed the same chemokine profile observed in human islets, but also struggled to maintain normoglycemia post transplantation. Further, NF-κB activated islets were rejected with a faster tempo as compared to non-NF-κB-activated rodent islets. Thus isolated human islets can make cell autonomous contributions to the ensuing allograft response by elaborating inflammatory factors that contribute to their own demise. These data highlight the potential importance of islet intrinsic pro-inflammatory responses as targets for therapeutic intervention.

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Prevention and rescue of diabetes by all-trans retinoid acid and exendin-4 in NOD mice with and without islet transplantation

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Type 1 diabetic patients have progressive decrease in pancreatic beta-cell mass. Eventually, they are insulin deficient and depend on exogenous insulin for life. Recent studies showed new beta-cell formation occurred even after long-standing type 1 diabetes. Therefore, type 1 diabetes may be treated by targeting inhibition of beta-cell destruction and promotion of beta-cell regeneration. It has been shown that all-trans retinoid acid (ATRA) hinders the development of autoimmune diabetes by inducing immune tolerance status. In addition, exendin-4, a glucagon-like peptide-1 receptor agonist, stimulates growth and differentiation of beta cells and exerts antiapoptotic effect on beta cells. Thus we hypothesized that the ATRA and exendin-4 therapy may prevent and rescue diabetes in NOD mice with and without islet transplantation. In the first experiment, NOD/scid mice were intravenously transferred with splenocytes isolated from 12 week-old female NOD mice. After adopted transfer, mice were treated with ATRA (0.5 mg/mouse intraperitoneally qd), exendin-4 (3 mg/kg subcutaneously bid) or ATRA+exendin-4 for 6 weeks. In either ATRA or exendin-4 treated groups, all of recipient mice developed diabetes before 8 weeks. Only the combination of ATRA and exendin-4 delayed the onset of diabetes till 10-12 weeks ($p<0.05$). In the second experiment, NOD mice were treated with ATRA or exendin-4 after the onset of diabetes. All but one treated with ATRA remained diabetic and the survival time was not different among control (52±31 days), ATRA (45±19 days) and exendin-4 (62±34 days) groups. One mouse achieved normoglycemia after ATRA treatment and is alive for 366 days with periodic hyperglycemia. In the third experiment, diabetic NOD mice were transplanted with 300-600 islets from NOD/scid mice and then treated with ATRA or exendin-4. All mice remained diabetic and the survival time was not different among control (52±51 days), ATRA (101±101 days) and exendin-4 (48±19 days) groups. In conclusion, the combination of ATRA and exendin-4 is an effective intervention to prevent autoimmune diabetes. However, ATRA and exendin-4 alone did not prevent or rescue diabetes in NOD mice with and without islet transplantation.

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Islet size affects engraftment but not functionality in pancreatic islet transplantation

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Background: Better results have been recently reported in clinical pancreatic islet transplantation (ITX) due mostly to improved isolation techniques and immunosuppression. However, optimization and standardization of protocols are still needed to improve success. Many areas have been investigated including morphologic characterization of the graft to determine best islet size for optimal engraftment and functionality.
Material and Methods: Pancreatic islets were isolated from 9-12 weeks old C57/BL10 male mice using standard and overdigestion techniques. Islets were separated by size in three different groups using stainless steel mesh filtrations (150μm and 300 μm). Islet size was then confirmed through observation at light microscopy and islets were counted and divided for ITX in 3 groups: small (<150 μm), medium (150-300 μm), and large (>300 μm). Streptozotocin induced diabetic syngeneic recipients received 600, 400, and 200 Islet Equivalent under the kidney capsule for each size. Controls received ITX for each isolation without dividing the islet per size. Animals were monitored for blood glucose level, and body weight. Time of diabetes reversal was reported as early, late (10 and 20 days post-TX) and no reversal. In reversed animals, islet functionality was assessed by multiple intra-peritoneal glucose tolerance tests (IPGTT).
Results: Small islets reversed diabetes in 100% of the animals regardless of the mass (most of them within 7 days post-ITX) and performed significantly better than medium and large islets (table). No significant difference was observed in IPGTT results from all animals that reversed diabetes (early or late).
Conclusion: This data suggest that small islets are superior in engraftment even when organs are slightly over digested. When islets are engrafted, functionality doesn't seem to be affected by size. This suggests that clinical isolations should be standardized on obtaining smaller islets.

Islet Mass (IEQ)	Reversal							
	Small (n=13)		Medium (n=25)		Large (n=18)		Control (n=10) (all sizes)	
	tot%	late%*	tot%	late%*	tot%	late%*	tot%	late%*
600	100	0	77	14	71	20	100	0
400	100	20	62	40	86	50	66	50
200	100	0	62	20	20	100	66	50

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Baboon as a model for the study of insulin sensitivity, beta cell function and subclinical inflammation following islet transplantation

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Obesity and insulin resistance occur naturally in baboons and may be associated to low grade systemic inflammation. We assessed β-cell function and insulin sensitivity (IS) in baboons, and the relationship between circulating cytokines with glucose levels and anthropometric measures of adiposity. We measured waist, BMI and percent body fat (%BF), and performed a two step hyperglycemic clamp (HC) with arginine stimulus measuring b-cell function and IS in 46 non-diabetic baboons (NDB). Partial correlations were performed between IS (M/I), acute insulin response (AIR_{0-12}), second phase insulin (AUC_{12-180}) secretion and arginine potentiated insulin secretion ($AUCArg_{180-210}$) in relation to BF composition. On a separate cohort of animals (n=80) representative of BF content in wild-type animals, we measured circulating sTNF-R1 and sIL6-R levels to correlate them with glucose metabolism and adiposity. Waist was strongly correlated with total %BF (r^2=0.72 p<0.0001) and trunk fat (r^2=0.41, p<0.01). Waist, BMI, and total body weight showed a markedly gender dimorphism although the strong relationship with %BF was maintained in both males and females. During the HC, M/I was negatively correlated with total insulin secretion (r^2=0.70, p<), AIR_{0-12} (r^2=0.12, p=), 2nd phase $ISEC_{0-180}$ and $ISEC_{ARG}$ (both r^2=0.66, p<0.0001) during each stage of clamp and with the insulin/glucagon ratio (r^2=0.30, p<0.0001). Finally, sTNF-R1 correlated with fasting plasma glucose (r^2=0.19, p<0.0001). Weight correlated with sIL6-R (r^2=0.09, p<0.004), sTNF-R1 levels correlated with sIL6-R (r^2=0.10, p=0.003). In the non diabetic range, insulin secretion during the HC correlated inversely with IS and fasting plasma glucose progressively increased parallel to an increase in circulating markers of subclinical inflammation strongly correlated to insulin resistance in humans by dysregulation of the TIMP-3/TACE dyad. This characterization of in-vivo baboon islet physiology and subclinical inflammation provide a useful tool for the study of metabolic events following islet transplantation.

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Simultaneous heart and intramuscolar islet transplantation in type 1 diabetes mellitus and acute heart failure

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Alternative site to liver for islet transplantation have been studied for long time, although few studies are reported in humans. Here we described the case report of the first intramuscle pancreatic islet allo transplantation. A 44 years old female affected by type 1 diabetes mellitus and by a severe post-myocardial infarction cardiopathy was admitted to the hospital for refractory heart failure and underwent heart transplantion from a multiorgan donor. Pre transplant insulin requirement was about 80u of insulin, c-peptide value was <0.1ng/ml. The day after heart transplantation

257000 equivalent islets (4283 EI/kgb.w.) by the same organ donor were transplanted into the brachioradial muscle. Liver was not suitable for islet implantation due to the severe intrahepatic blood stasis. The immunosuppression therapy was based on ATG induction, and high doses steroid, mycofenolate and cyclosporine as maintenance therapy. The post-transplant course was complicated by early heart graft dysfunction requiring high-dose inotropic support, two cardiac arrests that required resuscitation and defibrillation, sepsis, and coma due to drug-induced neurological toxicity. Then the patient stabilized. After transplantation glucose homeostasis remained acceptable with a reduced insulin requirement up to 35 units, HbA1c=7.2% and c-peptide 0.2-0.4 ng/ml (at 6 month 2.1 ng/ml after glucagon test). At two years follow up the patient is still in general good condition, with basal c-peptide 0.2ng/ml, 35u insulin requirement. Intramuscolar islet transplantation appears an interesting alternative site to the liver for islet implant in selected patients, and deserves evaluation in future studies.

SOTA 8
New Trends in Cell Therapy 2

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Radiofrequency energy in stem cell pluripotency and differentiation

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Radiofrequency (RF) waves from Wi-Fi (Wireless Fidelity) technologies have become ubiquitous, with Internet access spreading into homes, and public areas. The human body harbors multipotent stem cells with various grading of potentiality. Whether stem cells may be affected by Wi-Fi RF energy remains unknown.
We exposed mouse embryonic stem (ES) cells to a Radio Electric Asymmetric Conveyer (REAC), an innovative device delivering Wi-Fi RF of 2.4 GHz with its conveyer electrodes immersed into the culture medium. Cell responses were investigated by real-time PCR, Western blot and confocal microscopy. Single RF burst duration, Radiated power, Electric and Magnetic fields, Specific Absorption Rate, and current density in culture medium were monitored. REAC stimulation primed transcription of genes involved in cardiac (GATA4, Nkx-2.5 and prodynorphin), skeletal muscle (myoD) and neuronal (neurogenin1) commitment, while down-regulating the self renewal/pluripotency-associated genes Sox2, Oct4 and Nanog. REAC exposure enhanced the expression of cardiac, skeletal and neuronal lineage-restricted marker proteins. The number of spontaneously beating ES-derived myocardial cells was also increased.
Thus, REAC stimulation provided a “physical milieu” optimizing stem cell expression of pluripotentiality and the attainment of three major target lineages for regenerative medicine, without using chemical agonists or vector-mediated gene delivery.
We are currently investigating whether REAC treatment may also affect the differentiating potential of human mesenchymal stem cells isolated from the adipose tissue (ADhMSCs), a minimally invasive source for multi-pluripotent cells. To this end, we will use “Lipogems”, an innovative device operating through mild mechanical forces, without dissociating enzymes, to afford remarkable ADhMSC yields in few seconds from the patient’s subcutaneous tissue.

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Parallel Session 13
Hematopoietic, Neural Cells and Tissue Repair

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Genetic characterization of human autologous adipose mesenchymal stem cells differentiated in 3-dimensional osteogenic graft

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Introduction: Osteogenic differentiated autologous adipose mesenchymal stem cells (AMSCs) could be proposed for bone reconstruction. This work studied the safety of the procedure in term of human AMSCs isolation to differentiation and genetic stability.
Methods: AMSCs isolation (by collagenase digestion of subcutaneous adipose tissue) /differentiation into an original 3-dimensional « bone-like » structure were performed from 8 independent patients. Group 1: Five patients with bone tumour history (3 osteosarcomas, 1 chondroblastoma, 1 Ewing's sarcoma) characterized by several clonal cytogenetic alterations (Karyotyping and FISH study of tumor suppressor gene loci such as TP53/17p13, CDKN2/9p21, RB1/13q14) of the original tumour. Group 2: Three patients with pseudarthrosis (Neurofibromatosis and Diamond Blackfan syndrome). Graft characterization (Osteocalcin,Von Kossa staining) and genetic analysis were performed on AMSCs proliferation and differentiation phases. In addition, microarrays analysis studied the gene expression for osteogenic (RUNX2, BMP2, OPN3, FGF2, ALPL, SP7, FGF23, SMAD9, MEN1) and senescence/tumorogenic (c-Myc,TP53, NFKB,Cyclin D) development between un-/and osteogenic-differentiated states of AMSCs.
Results: A mean of 74 ± 30 days was required to achieve the autologous bone-like 3-D structure (osteocalcin expression, collagen deposition and mineralization). Microarrays demonstrated the up-regulation of osteogenic genes for differentiated cells ($p<0.05$) and no sign of upregulation for TP53,NFKB, Cyclin D, c-Myc for both un-/differentiated AMSCs. In Group 1, no native tumour anomalies was found prior and after osteogenic differentiation of AMSCs. However, AMSCs culture (up to Passage 4th) can induced, in both Group 1 and 2, tri-/tetraploidies (0,5-14% of cells), recurrent clonal alterations as trisomy 7 (in 6-20% of cells for 3 patients) and chromosomal breakage cht(3)(q13.3) (for 4 patients) for undifferentiated AMSCs in proliferation phase. Interestingly, the osteogenic differentiation reduced significantly anomalies found in proliferation state (trisomy 7: <2-5.5% of cells).
Conclusion: These preliminary results demonstrated, for the first time, the impact of proliferation and osteogenic differentiation on the genetic stability of human AMSCs.

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Chronic wound healing by a bioengineered graft made of autologous adipose mesenchymal stem cells and allogeneic human acellular collagen matrix

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Background: Chronic wounds are difficult to heal. This work investigated the potential of a bioengineered graft made of autologous Adipose Mesenchymal Stem Cells (AMSCs) and allogeneic Human Acellular Collagen Matrix (HACM) to improve angiogenesis and dermal reconstruction.
Methods: Two patients developed untreatable chronic ulcerations consequently to (patient 1) a radiotherapy (50 Grays) as adjuvant therapy of a malignant sarcoma (one lesion of 12cm², proximal left tibia) and (patient 2) drepanocytosis (two bimalleolar hyperalgic ulcers of 95/127cm²). After one year of ulceration course, repeatitive unsuccessful skin grafts, infections and hospitalizations, autologous AMSCs were isolated by cGMP-collagenase digestion and proliferated up to Passage 4th before loading on the HACM. AMSCs (CD90 expression) were characterized by the capacity of osteogenic (Alizarin Red/Osteocalcin/VonKossa staining)/adipogenic (Oil red staining) differentiation and angiogenic properties (in vitro VEGF release at 0.1/5/21% Oxygen). Bioengineered grafts were implanted when the total HACM was colonized by AMSCs and were followed by inflammatory reaction (White blood cells/CRP/fibrinogen levels) and tissue reconstruction (CD3/CD68/Melanocyte/Proteoglycan and Mart-1 staining for lymphocytes/macrophages/ epithelialization, respectively).
Results: Prior tranplant, chronical ulcerations demonstrated a strong fibrotic tissue with a low vascular density. The composite graft was macroscopically incorporated into the scaring tissue at day 3 and completely integrated after 28 days post-implantation. Histological examination demonstrated a significant higher expression of VEGF,

angiogenesis (a capillar density over 600/mm², $p<0.005$) in the wound without any significant infiltration by CD3/CD68 cells. A skin-like tissue with a complete re-epithelialization and considerable glandular structure formation was also found for Patient 1. For patient 2, a superficial skin graft was secondly implanted (after 6 weeks post-AMSCs transplantation) and induced a complete wound healing without any wound recurrence at 4 months post-transplantation and complete pain relief.
Conclusions: This bioengineered graft made of autologous AMSCs potentiates angiogenesis and skin reconstruction to cure fibrotic area of chronical wound.

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Prevention of post-transplant proteinuria: possible role of rituximab

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Presence of proteinuria has been one of the exclusion criteria in clinical trials of islet transplantation, because of the risk for additional nephrotoxicity related to selected post-transplant immunosuppressive strategies. In addition, even in the absence of pre-transplant renal dysfunction, the development of post-transplant proteinuria has been a feared complication, as it could be associated with a progressive loss of kidney function and development of chronic kidney disease. As many of the current immunosuppressive strategies may contribute to the development of post-transplant proteinuria, identification of immunosuppressive agents that may be renoprotective is essential. Among them, rituximab recognizes CD20 on B lymphocytes, but it also bind sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) and preserves cellular function through modulation of sphingolipid. We studied a population of patients with focal segmental glomerulosclerosis (FSGS), a glomerular disease at high risk for post-transplant proteinuria. We hypothesized that rituximab prevents proteinuria and preserves podocyte SMPDL-3b expression. We studied 41 FSGS patients at high risk for post-transplant proteinuria, 27 of whom were treated with rituximab at time of kidney transplant. Rituximab treatment was associated with lower incidence of post-transplant proteinuria and stabilization of glomerular filtration rate. The number of SMPDL-3b$^+$ podocytes in post-reperfusion biopsies was reduced in patients who would ultimately develop proteinuria. Rituximab partially prevented SMPDL-3b downregulation observed in podocytes treated with the sera of proteinuric patients and preserved podocyte function through SMPDL-3b. Our study suggests that rituximab treatment at time of transplantation may be beneficial in the prevention of post-transplant proteinuria in high-risk patients.

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Improvement of bone allograft by mesenchymal stem cells: undifferentiated vs. osteogenic adipose / bone marrow stem cells

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Objectives: Lack of osteo-integration in bone allografting is a major limitation leading to progressive graft failure. Mesenchymal Stem Cells (MSCs) can improve the biological performance of acellular bone allografts. Our goal was to compare adipose (AMSCs) and bone marrow (BM-MSCs) MSCs at non-differentiated vs. osteogenic differentiated stages in view to improve the osteogenicity of bone allografts.
Material and Methods: Pig AMSCs and BM-MSCs at both non-/differentiated stages were investigated *in vitro* and *in vivo*. *In vitro* experiments compared the proliferation, differentiation, immunogenicity (Mixed Lymphocyte Reaction) and angiogenic (Vascular Endothelial Growth Factor (VEGF) secretion under 21%/5%/0.1% [O_2]) properties of stem cells. *In vivo* assays assessed the angiogenesis (histomorphometry for vessel development and VEGF expression) and osteogenesis (histomorphometry for osteocalcin expression and micro-CT scan) of cultured cells seeded on a acellular bone graft and implanted in the paravertebral musculature of nude rats up to Day 30 post-transplantation.
Results: *In vitro* experiments demonstrated a superiority of AMSCs in terms of proliferation (6.1 ± 2.3 days vs. 9.0 ± 1.9 days for BM-MSCs, respectively, $p<0.001$), immunomodulation (higher T-cell depression) and a higher secretion of VEGF (4839.01 ± 2840.40 pg/ml vs. 396.30 ± 206.30 pg/ml for non-differentiated AMSCs vs. non-differentiated BM-MSCs, respectively, $p<0.001$).

At day 30 post-transplantation, a significantly higher angiogenesis (19.6 ± 6.8 vs. 10.9 ± 4.9 vessels/0.016mm² for differentiated AMSCs and BMSCs, respectively, $p<0.005$), VEGF expression ($p<0.001$) and osteogenesis (osteocalcin expression, $p<0.001$, and amount of newly formed bone matrix on micro-CT scan, $p<0.05$) were found for osteogenic differentiated AMSCs. It was also demonstrated that both BM-/AMSCs keep their immunomodulatory properties after osteogenic differentiation.
Conclusions: Osteogenic AMSCs are an excellent option for bone allograft revitalization in view to promote (i) cellular engraftment by local immunomodulation / neo angiogenesis and (ii) induction of osteogenesis.

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Pig as a large animal model for bone tissue engineering development

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Introduction: Massive bone defects and non-unions remain major challenges in large animal models to demonstrate the potential of tissue engineering for clinical application. Our work investigated two surgical models developed in pigs: (i) a multi-level spinal fusion and (ii) a massive femoral bone defect to achieve a non-union at 6 months post-operation (defined as a sclerotic tissue without spontaneous bone formation for sham animals).
Material & Methods: (i) Spinal fusion (n=6): an Anterior Lumbar Interbody Fusion (ALIF) procedure was performed by lumbotomy. Empty interbody fusion cages of appropriate sizes were inserted laterally.
(ii) Femoral defect (n=2): a lateral approach provided an access to the femur in view to create a 15mm critical bone defect. Stabilization was realized by a 4.5mm titanium locking compression plate (LCP). A second LCP was positioned at 90° to the previous one, to obtain long term stability.
Animals were followed *in vivo* by imagery between 4 and 12 post-operative weeks (POW) with a CT scan performed at 4,8 and 12 weeks. Micro-CT-scan, histomorphometry (Hemalun-Eosin, Masson's trichrom) and immunohistochemistry for osteocalcin and Vascular Endothelial Growth Factor (VEGF) were also performed on explanted implants.
Results: (i) Spinal fusion: CT scan (at 4, 8 and 12 POW) demonstrated no spontaneous consolidation of empty cages. This was confirmed by micro-CT (no central calcification), histology (fibrosis, lack of angiogenesis) and immunohistochemistry (low osteocalcin expression and poor secretion of VEGF).
(ii) Femoral defect: osteosynthesis failure was found (<8 POW) due to implant disruption with only one LCP plate. A bone non-union (without osteoinduction) was obtained at 7 months post-operation with two plates as confirmed by CT-scan and macroscopic disruption between bone segments.
Conclusions: Two surgical pre-clinical pig models were developed to obtain a reproducible bone non-union model in view to test different cellular and/or biocompatible implants.

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Surface modified Schwann cells migrate, support corticospinal axon regeneration and promote functional restitution after implantation in an acute SCI model

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The implantation of Schwann cells (SCs) into injured spinal cord (SCI) promotes axonal regeneration, remyelination repair and functional. Reparative efficacy, however, may be limited due to the inability of implanted SCs to migrate outward from the graft across the host reactive astroglial scar. Genetic modification to alter SC cell-surface properties by over-expressing polysialic acid (PSA) on NCAM, a negative regulator of cell interactions, has been shown to promote migration and enhance functional outcome after SCI. In the current study a T8 thoracic spinal cord contusion was used to evaluate the migration, supraspinal axon growth support and functional recovery associated with PSA-over-expressing SCs (PST-GFP SCs) or controls (GFP SCs) when implanted sub-acutely one week post-SCI. Compared to GFP SCs, which remained confined to the injection site, PST-GFP SCs readily migrated from the lesion-implant site for distances up to 8 mm. In addition, with PST-GFP SCs, there was extensive serotonergic and corticospinal axon in-growth within the implants that was limited in the GFP SC controls. The enhanced migratory capacity of PST-GFP SCs was accompanied by enhanced growth of these axons caudal to

lesion. Animals receiving PST-GFP SCs exhibited a further enhancement in functional outcome, both in open-field locomotor performance and foot placement, over the modest improvements provided by GFP SC controls. The current study for the first time demonstrates that a lack of SC migration may be responsible for their inability to associate with and support the growth of corticospinal axons and that the extent of PST-GFP SC migration is highly correlated with their capacity to support enhanced functional recovery. These results provide further promise that PSA modified SCS will be a potent reparative approach for SCI.
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Umbilical cord blood as new source of myeloid derived suppressor cells

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By restraining T-cell activation and promoting T regulatory lymphocyte (Treg) expansion, myeloid-derived suppressor cells (MDSCs) could represent an important tool to control either self-reacting or anti-graft effectors T-cells. Since in a healthy donor suppressive MDSCs are extremely rare in the peripheral blood, we evaluated the possibility to differentiate them from hematopoietic precursors isolated from bone marrow (BM) or umbilical cord blood (UCB). We have found that, by incubating hematopoietic cells with a cocktail of cytokines already approved for clinical use (rh-GM-CSF and rh-G-CSF - Neupogen), we can generate a population with suppressive activity. By FACS analysis we were able to identify four major populations defined by the expression of the CD33 and IL4Rasurface markers: 1) $CD33^{high}/IL4R\alpha^{high}$; 2) $CD33^{int}/IL4R\alpha^{dim}$; 3) $CD33^{int}/IL4R\alpha^{-}$; 4) $CD33^{-}/IL4R\alpha^{-}$. The 4 populations were than sorted and tested in a suppressive assay using as effectors PHA stimulated, CFSE labeled autologous T-cells. Proliferation was analyzed 4 days later by FACS. While population 2, 3 and 4 did not reduce T-cell proliferation, population 1 ($CD33^{high}/IL4Ra^{high}$) completely abrogated $CD8^{+}$T-cell proliferation. This population is characterized by the expression of the following markers: $CD14^{+}$, $HLADR^{int}$, $CD15^{low}$, $CD13^{high}$ and variable level of CD34. Furthermore, the addition of Nor-NOHA, an arginase 1 inhibitor, and L-NMMA, a NOS inhibitor, abrogated their suppressive activity suggesting an important role of L-arginine metabolism in the inhibitory pathway of these cells. While $CD8^{+}$ T-cell proliferation was completely restored in the presence of the inhibitors, the proliferation of a small subset of $CD4^{+}$, $CD25^{high}$, $FoxP3^{high}$T-cells was still maintained; experiments characterizing the functionality of this putative Treg are currently being performed.
In summary, MDSC-like cells can be generated from the BM and the UCB and could represent an important tool for the treatment of autoimmune diseases and to prevent allograft rejection.

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Amniotic fluid cell therapy to relieve disc related low back pain and its efficacy comparison with long acting steroid injection

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Discogenic low back pain is often associated with varying degree of degeneration osteoporesis, spondylosis, spondyloarthrosis, intervertebral disc prolapse or even compression collapse apart from other medical problems.
42 patients participated and randomized in two equal groups. Group A(N=21, Male 10 and female11, Mean Age 56.4 ± 8.9yrs) was treated with 80mg Long acting Steroid (Methyl Prednisolone) in 10 ml water for injection under C-arm guidance in the Operation Theatre (O.T) after 1 percent infiltration with Xylocaine at the site of maximum tenderness in the back. Similarly Group B (N=21,Male 12 and female 9, Mean Age 59.4. ± 6.4yrs) was also treated in the O.T with similar protocol with 10 ml of freshly collected amniotic fluid from mothers undergoing hysterotomy and ligation. All the procedures passed through the donar and recipient's informed consent protocol and vetted by the Institute based Ethical committee.
Studying and comparing the clinically manifested effect of treatment it can be easily seen that both Steroid (Group A) and Cell therapy (Group B) patients showed improvement of pain and distress from the pretreatment value, however, Group B scoring are much better ($p,< .01$), as seen and assessed from the value of the VAS (Visual Analogue Pain Scale), WD (walking distance in meters), and HAQ (Health Assessment Questionnaire). Assessment of pain relief and patient's satisfaction in case of Group A it was 20/21 cases in first month which became 12/21 in

3rd months, 6/21 in 6th months, 4/21 in 12th months and 2/21 after 24 months followup. Similarly in Group B the identical values after the first month was 18/21, which became 21/21 in 3rd months, 21/21 in 6th months,14/21 in 12th months and 12/24 after 24 months followup.
Freshly collected amniotic fluid is a very important source of different stem cells and supportive cells in sterile condition.

Parallel Session 14
Immune Tolerance

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Successful induction of tolerance of both islets and kidneys and cure of diabetes following vascularized islet-kidney transplantation in non-human primates

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We have previously reported that transplantation (Tx) of prevascularized donor islets as composite Islet-Kidneys (IK) reversed diabetic hyperglycemia in miniature swine, while similar numbers of free islets did not. In order to enhance the IK strategy's clinical applicability, we attempted to induce tolerance of both islets and kidneys in non-human primates (NHP). Our tolerance strategy included hematopoietic cell transplantation (HCT), 100cGy-Total Body **I**rradiation, **T-**cell depletion and 45 days of **C**yA (ITC).
Methods: IKs were prepared by isolating islets from 70% partial pancreatectomies and injecting them beneath the autologous renal capsule of five rhesus monkey donors (Bwt 4.2-6kg) 3 weeks prior to HCT. Haploidentical HCTwas performed following GCSF mobilization and leukapheresis of the donors and ITCconditioning of the recipients. Three IKs were transplanted to HCTrecipients (Animal 1, 2, 3), one is awaiting Tx and one was used for histology. Animal 1, who was previously tolerant of it's HCT donor's kidney, received an IK from the same donor without immunosuppression 211 days post HCT. Animals 2 and 3 received IKs 3 weeks following HCT. IDDM was induced by STZat 80mg/kg IV before IK-Tx.
Results: IK preparation was successful in all monkeys (34000~45000IE). STZinduced IDDM and >15U/day of insulin was required to maintain BS <200mg/dl.Following IK-Tx, all recipients showed stable renal function without evidence of rejection. Insulin treatment (1-5U/day) maintained a BS less than 150mg/dl for 2-3 weeks post IK-Tx in Animals 1,2 and 3. Animals are currently days 139, 189 and 5 from IK-Txs, and FBS are 97, 120 and 77, respectively, without insulin treatment.
Conclusions: We successfully induced tolerance of allogeneic islets as a part of life-supporting IK grafts with HCT. These results demonstrate the feasibility of composite IK-Tx in NHP, with possible clinical applicability for the cure of diabetic nephropathy.

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Induction of donor specific tolerance in recipients of HLA disparate living donor kidney allografts by donor stem cell infusion

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Background: Renal transplantation is the preferred therapeutic approach for end stage renal disease. However, the chronic use of nonspecific immunosuppressive agents (IS) is costly and has significant toxicities including opportunistic infection, an increased rate of malignancy, nephrotoxicity, and other end organ damage. The induction of donor-specific tolerance would address these limitations. Bone marrow chimerism induces tolerance to transplanted organs and tissues. However, the toxicity associated with conventional hematopoietic stem cell transplants (HSCT), primarily graft versus-host disease (GVHD), and the need for aggressive ablative conditioning, has limited the therapeutic application of HSCT to tolerance induction. We have identified a novel tolerogenic bone marrow cell population of CD8+/TCR- facilitating cells (FC) that enhances engraftment of bone marrow in

mismatched recipients without causing GVHD. The discovery of FC is an important finding as it opens the door to employing HSCT as a viable cell-based approach for tolerance induction.
Methods: 8 HLA mismatched living donor renal transplant recipients have been entered into a tolerogenic protocol involving low intensity conditioning (fludarabine, cyclophosphamide, 200cGy TBI days -4 to -1). Patients received a living donor kidney transplant on day 0, followed by infusion of cryopreserved FC-enriched donor-derived CD34+ hematopoietic stem cells on Day +1 (0.49 - 4.48 X106 FC/kg recipient body weight). All subjects were discharged by post operative day 3 and managed as outpatients. Maintenance IS consisted of FK506 and MMF without steroids. Weaning of immunosuppression was designed to occur over a one year period.
Results: The first 8 patients are now 6,9,9,9,20,21,22,24 post-Tx. All pts demonstrated peripheral blood macrochimerism post-Tx, ranging from 6% to 100% at 1 month. Chimerism was lost in two evaluable patients at 3 and 6 months post-Tx. 7 evaluable pts demonstrated donor-specific hyporesponsiveness and are being weaned from IS, with one pt now off all IS for 10 months (22 months post-Tx)and three additional patients also weaned off of all immunosuppression. None of these pts have developed GVHD. None of the patients developed anti-donor antibody as assessed by flow crossmatch. Serious adverse events have included herpes zoster reactivation in 2 pts; no clinically significant CMV or polyoma viral infections have occurred. Renal allograft loss has occurred in one patient who developed aplastic anemia and sepsis following an atypical viral infection 2 months post-Tx, successful rescue with banked autologous HSCT. Subsequent re-transplantation with a living donor kidney was performed.
Conclusions: We conclude that low intensity conditioning in conjunction with FC enriched HSCT can safely achieve durable mixed chimerism in mismatched kidney transplant recipients, allowing for IS withdrawal.

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Immunosuppression free long-term islet allograft tolerance achieved by dual blockade of NF-kappaB and JNK/AP-1

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Islet allograft rejection is mediatedby host immune cells, but here we tested the hypothesis that graft-intrinsic inflammatory factors also contribute to allograft rejection. We targetedthe transcription factor nuclear factor-κB (NFκB) in the islet graft using three approaches: overexpression of the physiological NFκB inhibitors, A20(rAd.hA20) or IkBa (rAd.hIkBα); or by treatment with the pharmacological inhibitor PDTC. For our transplant model, islets from BALB/c H-2d donors weretransplanted under the renal capsule of full MHC-mismatched diabetic C57BL/6 H-2b recipients. NFκB, JNK and p38 activity was assed via western blot and RTqPCR. Blocking NFκB by A20, IkBα or PDTC effectively blocked the induction of CCL2, CXCL10, CXCL1, TNFα and ICAM1 at the level of transcription. However, neither hIκBα overexpression, nor PDTC treatment, improved transplantation outcome compared to control. In contrast, blocking islet graft NFκB activation by hA20 overexpression resulted in ~45% long-term(>100 days, n=10) acceptance. Graft pathology showed strong insulin-labelling, preserved islet architecture, and minimal infiltrate dominated by Foxp3+ mononuclear cells. Depletion of CD25+ CD4+ T cells reversed the graft protective effect of A20 - thus A20 induced functional graft tolerance mediated by Tregs. Interestingly, blocking NFκB with IkBα or PDTC impaired early graft function, whereas A20 co-expression restored graft function. We noted that hIκBα overexpression resulted in differential gene inhibition when compared to A20; this was particularly true for genes containing additional promoter elements such as AP1. Consequently, the effectof blocking NFκB on JNK-AP1 signalling pathway was investigated. Blocking NFκBwith IkBα or PDTC resulted in hyper-activation of JNK, whereas A20 suppressed both NFκB and JNK activation. These results show that islet graft intrinsic inflammatory pathways contribute to allograft rejection. Further, we demonstrate a novel regulatory circuit whereby NFκB controls islet-intrinsic JNK activation via the A20 gene. Enabling this circuit results in immunosuppression free islet allograft acceptance mediated by regulatory T cells.

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Immune-privileged Sertoli cells modulate the cellular immune response to survive as allografts

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Immune-privileged Sertoli cells (SC) survive long-term when transplanted across immunological barriers, as allografts or xenografts. On the other hand, MSC-1 cells (a mouse SC line) lack some of the immunoprotective

abilities associated with primary SC (pSC). The objective of this study was to compare the cell survival rate and immune response to these cells, to further understand the mechanism for SC immune-privilege. pSC or MSC-1 cells were transplanted as allografts underneath the kidney capsule of BALB/c mice and cell survival was analyzed by immunohistochemistry (IHC). pSC grafts survived throughout the study and were not rejected, whereas, very few MSC-1 cells were detected by day 11 and MSC-1 cells were completely rejected within 20 days. We then focused on the mechanisms behind allograft rejection, antibody-mediated and/or cell-mediated death. For antibody-mediated rejection, pSC or MSC-1 cell grafts and serum from the transplanted animals were collected at days 1-20, analyzed for antibody production and deposition by ELISA and IHC, respectively. Further, to look for complement deposition, grafts were immunostained for C4, C3 and MAC. No IgG production was detected whereas there was an IgM response against the grafted cells. Antibody deposition was not detected until day 14 post-transplantation and no complement deposition was observed in any of the grafts throughout the study. Therefore we concluded that antibody mediated cell death is not playing a major role in this allograft rejection model. We thus, hypothesized that SC were surviving as allografts by inhibiting cell-mediated killing. Significant apoptosis (TUNEL assay) was observed in MSC-1 cell grafts as compared to pSC grafts. Analysis of the grafts for immune cell infiltration revealed that CD4 T cells were present in both sets of grafts, while little to no CD8 T cells were detected in MSC-1 cell grafts as compared to pSC grafts. Macrophages (F4/80 marker) started infiltrating MSC-1 cell grafts at early time point (day 2) as compared to pSC grafts (day 5). Foxp3+ cells (T regulatory cells),were detected in pSC grafts a-t day 11, whereas, Foxp3+ cells were either absent or very few were detected in MSC-1 cell grafts. Overall, this led to the conclusion that SC delayed the migration of immune destructive macrophages and led to the production of immunoregulatory cells. Thus, by altering the immune cell response from immune destructive to immunoprotective, SC, enjoy long-term survival.

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Targeted Ccl21 promotes neogenesis of a paracortex-like environment and immunoprotects embryonic stem cell allografts from rejection in mice

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CCL21 expression in secondary lymphoid organs directs immune cell homing and orchestrates the immune response. In addition, it is becoming clear that CCL21-CCR7 signal transduction is an important determinant of effective immunity vs. self-tolerance. We previously showed that CCL21 expression by tumors triggers a cascade of events that ultimately promotes tumor immunological escape and growth. Here we aim at determining whether we could exploit tumor strategy based on targeted CCL21 delivery to invoke immune acceptance and survival of allografts as a novel therapeutic approach in transplantation. We chose embryonic stem cell allografts in mice as a model of indirect allograft antigen presentation by host immune cells because of its extensive applications in the field of regenerative medicine and transplantation and we overexpressed CCL21 in those cells. We were able to prove that CCL21 expression by the graft prolongs graft survival and allows teratoma formation in immunocompetent allogeneic recipients. This is associated with neogenesis of a lymph node-like paracortex with T cell compartmentalization within organized stroma and shift towards a regulatory phenotype with presence of myeloid derived suppressor cells that are functional in suppressing T cell activation and in generating a subtype of $CD25^{Hi}FoxP3^{Hi}$ regulatory T cells. This immune-privileged environment that is induced by targeted CCL21 expression at the graft site allows survival of donor specific alloantigens but not third party ones in the graft. Our findings have important implications for future novel transplantation therapies with targeted CCL21 delivery at the graft as an attractive alternative to current systemic immunosuppression regimes.

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Metabolic demand as an independent variable impacting tolerance induction to islet allografts

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Background: There is a growing appreciation for the connection between metabolic demand/distress and intra-islet inflammation, especially as found in Type 2 diabetes. Importantly, inflammation can also impair allograft tolerance induction. Therefore, we determined if these concepts could be linked by determining whether severe hyperglycemia can impact the capacity to induce allograft tolerance in mouse models of diabetes.
Methods: We compared conventional streptozotocin (SZ)-induced diabetic mice with non-autoimmune, spontaneously diabetic Ins-2^{akita}(akita) recipients on the same C57Bl/6 (B6) genetic background for their relative propensity for induced islet allograft tolerance. BALB/c islets were transplanted in these recipients with or without transient treatment with anti-CD154 therapy (hamster MR1, days -1, 2, 7,9 relative to transplant).
Results: B6 akita mice have an insulin secretory defect that results in a severe, irreversible hyperglycemia that was consistently higher relative to corresponding SZ-induced diabetic B6 mice. Diabetic B6 akita mice accepted wild-type syngeneic B6 islet grafts for >100 days (12/12), confirming that diabetes was not associated with detectable autoimmunity. Unmodified islet allograft rejection was significantly faster ($p < .01$) in diabetic B6 akita hosts (7.1 days) relative to SZ-induced diabetic B6 mice (13 days). Importantly, anti-CD154 therapy resulted in graft prolongation but not long term allograft acceptance in the majority of diabetic B6 akita recipients (only 1/14 allografts surviving >100 days). This was significantly different from results using SZ-induced diabetic B6 recipients in which 8/12 allografts survived >100 days ($p < .01$).
Conclusions: Results indicate that there can be significant differences in islet allograft survival depending on the model of diabetes examined (SZ-induced versus akita). Given the higher and persistent hyperglycemia found in akita recipients, we hypothesize that the degree of metabolic demand and/or tissue distress in islet transplants can be an independent variable in determining islet graft survival and can inhibit tolerance induction.

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Distinct allograft tolerance-promoting therapies do not require intrinsic inactivation of antigen-specific CD8+ T cells

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Background: CD8$^+$ T cells are primary mediators of acute islet allograft rejection and thus are potentially key targets of tolerance-inducing therapies. While monoclonal antibody therapy targeting LFA-1 (adhesion) or CD154 (costimulation) can independently induce allograft tolerance, their respective impact on graft-reactive CD8$^+$ T cells remains unclear. Therefore, we determined the direct impact of anti-LFA-1 or anti-CD154 on the fate of antigen-specific CD8$^+$ T cells *in vivo*.
Methods: As a model of monitoring CD8$^+$ T cell reactivity *in vivo*, OT-I T cell receptor transgenic CD8$^+$ T cells were stimulated with ovalbumin-expressing mAct-OVA transgenic antigen-presenting cells. Specifically, CD45.1 OT-I T cells were adoptively transferred into CD45.2 wild-type C57BL/6 (B6) hosts and challenged locally (footpad) with mAct-OVA APCs. Primary responses were assessed three days post challenge in the presence or absence of anti-LFA-1 (KBA) or anti-CD154 (MR1) therapy. For monitoring secondary responses, OT-I T cells were stimulated initially with mAct-OVA APCs *in vivo* as above, and then restimulated *in vitro* three weeks after primary challenge.
Results: Anti-LFA-1 dramatically restrained the overall number of OT-I T cells responding in the draining (popliteal) lymph node during primary stimulation. However, the actual proportion of OT-I T cells undergoing initial proliferation and converting to an antigen-experienced CD44^{hi}CD62L^{lo} phenotype was comparable between anti-LFA-1 treated and control animals. Thus, LFA-1 perturbation did not prevent initial CD8$^+$ T cell priming. Furthermore, OT-I T cells from challenged, anti-LFA-1 treated animals remained reactive in secondary responses *in vitro*, indicating that these cells were not functionally inactivated. In contrast, anti-CD154 treatment had very limited impact on either the quantitative or qualitative response of OT-I T cells *in vivo* relative to control animals.
Conclusion: Despite a prominent role for CD8$^+$ T cells in islet allograft rejection, neither LFA-1 nor CD154 directed therapies require the inactivation of antigen-specific CD8$^+$ T cells to achieve tolerance.

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Long term survival of PEGylated murine allogenic islets using short course immunomodulation

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Clinical islet transplantation has shown promise for Type 1 diabetes treatment. Nonetheless, inflammatory and immunological host responses to the implant lead to islet dysfunction and destruction, in spite of systemic immunosuppression. Cellular PEGylation, the addition of a single coating of poly(ethylene glycol) (PEG) to the cell/ tissue surface, has been shown to reduce inflammation and mitigate immune recognition via generation of a steric barrier. We sought to evaluate the potential of PEGylation, alone and in combination with a short-course immunomodulatory regimen, on the survival of fully-MHC mismatched islet allografts. Lymphocyte Function-associated Antigen 1 (LFA-1) plays a key role both in lymphocyte trafficking and co-stimulation and has demonstrated success in partially preventing murine allograft rejection.
PEGylated or unmanipulated DBA/2 islets (H2d) were transplanted under the renal subcapsular space of chemically-induced diabetic C57BL/6J mice (H2b). Control animals received untreated islets and saline (n=20) or anti-LFA-1 antibody (KBA, 100ug/day, i.p.) on days 0-6 (n=10). Islets were coated with a single layer of PEG (SVA-PEG, MW 5000) prior to transplantation into untreated (n=10) or anti-LFA-1 treated mice (n=9).
Ninety percent of the control islet transplants rejected within 60 days. Either the short course of LFA-1 blockade or PEGylation of islets resulted in long term (>100 days) function of the allograft in 50% or 60% of cases, respectively. Combination of islet PEGylation with LFA-1 blockade resulted in 78% of the transplants functioning long-term. Nephrectomy of the graft bearing kidney resulted in prompt return to hyperglycemia for all transplants.
Islet PEGylation represents a simple, highly cell compatible procedure to prevent allograft rejection. In combination with a short course immunotherapy, murine allograft rejection is prevented in a majority of the transplants. This study demonstrates the potency found with the combination of these two mild strategies, indicating a synergistic effect.
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Cell Special Symposium

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Purinergic modulation of tissue specific regulatory T cell

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The correct balance between distinct functional subsets of effector and regulatory CD4+ T cells is of fundamental importance to orchestrate protective and tolerogenic responses. Regulatory T cells (Tregs) down-regulate inflammation, avoid tissue destruction by excessive immune responses and help preventing autoimmunity. Increased oxidative synthesis of ATP in CD4+ T cells by TCR triggering is followed by release of ATP from stimulated cells through pannexin hemichannels. Autocrine purinergic stimulation by ATP through P2X receptors plays a crucial role in protracting TCR-initiated mitogen activated protein kinase (MAPK) activity and IL-2 secretion, thus determining productive T cell activation. Tregs produce significantly lower amounts of ATP than conventional CD4+ T cells following TCR stimulation. In addition, they are characterized by the combined expression of CD39 and CD73 ectonucleotidases, which rapidly degrade extracellular ATP to adenosine. However, the gene encoding P2X7 receptor is comprised in Tregs signature genes. We show that selective activation of P2X7 inhibits Tregs suppressive potential and lineage stability. The inflammatory cytokine IL-6 increased ATP synthesis and P2X7 mediated signaling, which in vivo determined Tregs lineage instability and conversion to IL-17 secreting T helper (TH17) effector cells. Moreover, pharmacological P2X antagonism promoted cell autonomous conversion of naïve CD4+ T cells into Tregs upon TCR stimulation. Thus, ATP acts as an autocrine factor that integrates stimuli from the microenvironment and cellular energetics to tune the developmental and immunosuppressive program of the T cell in adaptive immune responses.

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IL-6 disruption of tolerance: Changing the balance between TH17 and Treg

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We have developed a "color-coded" adoptive transfer model for T cell subset identification that enables serial analysis of islet allograft infiltrating yellow induced regulatory T cells (iTreg), green natural regulatory T cells (nTreg) and red effector T cells (Teff) in live animals using endoscopic confocal microscopy (Nat Med. 2010). We now report that delivery of IL-6 in this model via osmotic pumps promotes accelerated allograft rejection. The IL-6 triggered accelerated allograft rejection is associated with inhibition of conversion of naive $CD4^+$ T cells to iTreg as well as the loss of nTreg phenotype. Flow cytometry analysis based on incorporation of CD45.1 congenic marker into the model demonstrated that the majority of nTreg converted to either Th17 or Th1 phenotype post allograft transplant when IL-6 osmotic pump was present. Additionally, the accelerated rejection could not be prevented by anti-CD154 mAb plus Rapamycin, a strong tolerizing regimen that promotes iTreg conversion and nTreg stability in hosts not receiving IL-6 treatment. The data provides new texture as to the importance of the integrity of both iTregs and nTregs in achieving allograft tolerance. Furthermore, the data suggests the balance between Treg and aggressive Teff (Th17/Th1) may be an essential indicator prognosing allograft outcome. Ongoing experiment based on Foxp3 RFP-RorγT eGFP double indicator whole mice confirmed that the balance between Treg and TH17 cells was tilted upon IL-6 infusion, which was also associated with accelerated rejection. The data supports the the idea that selective anti-inflammatory treatment may be imperative for induction and maintenance of allograft tolerance, especially in complex clinical situations where inflammation plays a significant role.

Parallel Session 19
Clinical Islets

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Prolonged islet survival by filgrastim

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Objective: To evaluate the effect of treatment with filgrastim, a granulocyte-colony stimulating factor (G-CSF), on islet allograft survival and metabolic parameters.
Methods: Forty-four subjects with T1DM who received at least one islet transplant were analyzed. Demographic and metabolic variables were recorded. Patients who presented neutropenia received G-CSF (filgrastim). Mann-Whitney test was calculated to evaluate differences in metabolic parameters between the subjects who were treated with filgrastim and the patients who were not. A Kaplan-Meier analysis was performed to assess the graft survival between the 2 groups.
Results: Subjects were 41% male, 59% female, 50±8 y/o, with 37±11 years history of T1DM, 1.8±0.8 number of infusions and 1,663±1,133 average days of graft survival. Sixteen subjects received treatment with filgrastim (average dose: 1,727±1,858 mcg). The graft survival was increased in the group of subjects who received filgrastim (2003±964 vs 1469±1192 days). The Kaplan-Meier analysis showed that the percentage of patients with graft failure at 1,000 days of follow up was lower in the filgrastim group (12.5% vs 54%, $p<0.05$). There were no significant differences between the 2 groups in HbA1c, fasting C-Peptide, 90 minute mixed meal test C-Peptide and glucose levels at 6 months, 1 year, 2 years and 3 years after the first infusion.
Conclusions: G-CSF induces proliferation of myeloid-derived suppressor cells (MDSCs); these cells can inhibit the proliferation of either antigen- or mitogen-activated T lymphocytes, and might favor transplantation tolerance. In an animal model, the use of filgrastim was associated with an increased number of MDSCs in bone marrow, spleen and lymph nodes, and to a delayed skin allograft rejection. Therefore, G-CSF (filgrastim) treatment in islet allograft recipients may improve graft survival. Randomized studies are needed to evaluate this observation.

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Steatosis and islet graft survival

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This study aimed to evaluate the association between islet graft survival and the fat content of the liver in islet transplant (ITx) recipients. The liver fat content was evaluated by MRI [fat liver score = (signal intensity on in-phase - opposed-phase images)/signal intensity on in-phase images * 100 and divided by the average spleen signal intensity]in 33 type 1 diabetes mellitus (DM) ITx recipients. Variations in liver fat content according to islet function were evaluated by pared t test with Bonferroni correction. Kaplan Meier curves and Cox-regression analysis were performed with time-to-graft failure as the dependant variable and fat liver content ≥3% (which corresponds to histological steatosis grade 1) was an independent one, with adjustments to number of transplants, cold ischemia time, HLA mismatches and use of exenatide. The fat liver content did not change significantly overtime (P >0.05). A tendency to earlier islet failure in patients with some degree of liver fat was observed, but only in those with functional grafts after 40 months (overall period P = 0.52; after 40 months P = 0.057). The presence of fat liver was associated with earlier graft loss after adjustments to possible confounders (OR = 13.78; P = 0.024) In this sample the fat liver content did not change significantly overtime. However, the presence of fat liver (fat liver index corresponding to histological steatosis grade 1) was associated with earlier graft loss even after adjustments to variables known to be associated with ITx Prospective clinical trials may confirm the cause-effect association between liver fat and islet failure and evaluate if its reduction may prolong islet graft survival.

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Exenatide and long term islet survival

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Objective: To evaluate exenatide action on gastric emptying and metabolic control.
Methods: Ten islet allograft recipients treated with exenatide up to four years were evaluated. Data from Mixed Meal Test with (MMT+) or without (MMT-) administration of exenatide before boost ingestion were analyzed at 6, 12, 24, 36 and 48 months after exenatide initiation. In the MMT- two groups were identified, those with acetaminophen (ACT) level peak ≤ 120mins, were labeled good gastric emptying (n=4) and those with ACT level peak ≥ 180mins, delayed gastric emptying. The c-peptide, ACT absorption and glucose responses to MMT were analyzed with: T-test and one way ANOVA. Differences in outcomes (time-to-graft dysfunction or failure) in Miami center subjects (N=44) exenatide +or- were compared by Kaplan-Meier curves.
Results: Average exenatide dose was 12.75+/-9.46mcg/day and mean time on exenatide was 54.08+/-14.25 months (n=10). In the "MMT+" ACT absorption showed no difference between groups (P=0.27). Exenatide action remained present in both groups up to 48 months delaying time to peak of glucose, c-peptide, ACT and suppressing glucagon response to MMT mean peak 70.89+/-12.45pg/ml to 43.24+/-4.67pg/ml. No difference in the means of c-peptide (P=0.16) and glucose (P=0.62) was found when compared by study time points. Subjects treated with exenatide during study follow up showed longer graft survival (p=0.002); exenatide was tolerated by 37% of Miami subjects.
Conclusions: Exenatide administration up to 4 years is safe in islet transplant recipients, even in the presence of delayed gastric emptying. Effects of exenatide are acute and reversible when exenatide is stopped. The main difficulty with the use of exenatide is the poor tolerability, although the physiological effects are evident at lower doses. The use of GLP1 analogs might promote graft survival. New drugs with longer half life, less side effects, may help to attain higher GLP1 levels, therefore improve outcomes.

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Research design and statistical considerations in cell transplantation research-lessons from islet allograft transplantation

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Objective: To characterize the statistical challenges specific to clinical islet cell transplantation research, and explore possible solutions.
Background: Clinical islet transplantation research, as with most areas of cell transplantation, can be broken down into two broadly defined areas. First is determining factors that associated with improved islet viability and yield, resulting in transplantable islet preparations. Impelling factors arise from donor characteristics, or relate to the procurement and isolation processes. These basic science investigations are then extended to investigations of factors that affect graft survival and function *in vivo*. This includes evaluation of factors specific to the transplant procedure, recipient characteristics and immunosuppressive strategies including patient-wise modifications mid-course.
Statistical Problems: Many experimental design and statistical problems present themselves in evaluating factors related to successful clinical islet transplantation, including: determining the experimental unit (recipient, donor, islet preparation), handling multiple infusions, choice of primary endpoint (insulin independence, glucose, C-peptide, HbA1c, metabolic function, quality of life, etc), and the low number of subjects relative to factors.
Investigations of islet processing as a basic science may not relate to outcomes of clinical transplantation. Standardization of outcome measures presents problems, ie. insulin requirement is actually a patient-doctor decision. Additionally, many of impelling factors specific to donor, procurement and processing, islet quality, recipient, and therapeutic regimen, which may be associated with clinical outcomes, may be mutually related, complicating sorting out their individual effects. Moreover, few of these factors are actually experimentally controllable, complicating hypothesis driven research.
Conclusions: Preliminary results of allogeneic human islet transplantation investigations should be used to guide rigorously controlled Phase II/III trials to definitively isolate the best islet processing procedures and patient management strategies. Investigations in to the basic science issues should be resolved before combining those results with clinical investigations. Whether each factor can be experimentally isolated remains a major practical obstacle.

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Successful clinical islet isolations from donor pancreas under fifty years of age

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Pancreatic islet transplantation offers a specific, minimally-invasive approach to restore normoglycemia and insulin independence in patients with type 1 diabetes, but its clinical applicability is limited by the complexity of the islet isolation process and the lack of consensus regarding donor characteristics that can predict successful islet isolation outcomes. One characteristic previously thought to predict worse isolation outcomes is younger age of the donor (<50), but with advances in the enzymatic blends used to digest the tissue, this may no longer be true. Here we describe our experience with islet isolation from deceased donors less than 50 yrs. old.
Pancreata (n=10) were processed using a modified Ricordi method. Briefly, pancreata were perfused with a GMP grade Collagenase NB1 and Neutral Protease NB mixture (Serva) via the pancreatic duct followed by continuous chamber digestion. Islets were washed and then purified using a continuous density gradient. Eight of the ten processed pancreas were suitable for transplant with an average islet yield of 648,124 (±190,901) Islet Equivalents (IEQ). The Serva collagenase NB1 and neutral protease NB functioned within a tight range of donor ages: 33-48 years with a mean of 38 (±5) years and average BMI of 38 (±6). An average trimmed pancreas weight of 113 (±12) grams yielded 5,764 (±1,745) purified IEQ per gram of trimmed pancreas with a 90% post culture recovery. Five different lots of Serva enzyme were tested. Several factors or modifications were identified as contributing to these successful isolations. These included: 1) a brief average cold ischemia time of 8:10 (±2.0) hours; 2) Moderate average concentrations of Collagenase (1,976U) and Neutral Protease NB (238U) in the perfusate; and 3) addition of CaCl2 (11mM) per average pancreas weight of 113 (±12) grams. In addition, the digestion time was relatively brief, averaging 13 (±1) minutes at 37°C, and the digestate was diluted into cold RPMI containing 5.0% HSA, insulin, and heparin while the chamber temperature was maintained at 30°C. The resultant digest was then centrifuged and pooled into flasks containing 0.625% HSA and 2% Pentastarch solution at 4°C. Islets were then purified on a continuous density iodixanol (Optiprep) gradient after being washed in a solution containing 0.2% Pentastarch.
Using this approach, we have been able to demonstrate islet function (detectable c-peptide) in all 8 transplants 1 month after transplant, and have achieved insulin independence for at least 3 months in all but 2 patients. Current durations of independence in our patients are >12mos x 2, >10mos x 1, >3mos x 1; with 2 patients (<3 months psot-transplant) requiring half the amount of, pre-transplant, insulin and are listed for a second transplant. In summary, we provide evidence that with relatively minor modifications in the isolation protocol, high quality, clinically usable islets can be isolated from younger donors.

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A new approach to contain costs for the establishment of a clinical islet transplant (ITX) program

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Background: Despite improvements in pancreatic islet auto- and allo-transplantation the diffusion of ITX programs has been limited. This is in part due to the high cost of building a specialized islet isolation facility and its operation. Facilities are built in areas requiring demolition and a complete refitting of large spaces to accommodate the isolation room, air filtration system, all ancillary services (pre-post clean area, scrubbing, changing, etc.) and new certifications that can cost $1-2 million. In addition these facilities require dedicated personnel for maintenance and operation (cleaning, monitoring, setting up etc.) 24/7. In our Institution, we developed a novel strategy for cost-containment of a clinical ITX program.
Methods and Results: We designed the islet isolation facility in the operating room (OR) area at our Institution. The facility consists of ~250 sq ft. that includes an ante-room (computer/monitor, storage, back-up refrigerator/freezer, CO2 tanks) and the fully equipped isolation facility. Floor, walls and ceiling were built of non porous surfaces for cleaning. Equipment included: 6 ft safety cabinets (2), COBE 2991 cell separator (2), mechanical shaker, organ perfusion apparatus, centrifuges (2), microscope with remotely connected camera and room screen, cabinets/storage, refrigerator/freezer, incubators, pass-through window, hands free intercom and door operation, room/equipment monitoring system (24/7) with alarm report for temperature, humidity, CO2, pressure. The cost of this conversion and equipping was approximately $200K, with an additional $100K to classify the room at 10.000 (ISO-7) particle level standards with HEPA filters. Air handler and power supply connected to emergency generator, sterilization equipment, changing and scrubbing area, storage, room monitoring system, ice maker, and certain certifications as well as personnel for cleaning, setting up and monitoring were already available in the OR reducing the cost of dedicated FTEs to 50%. Same location of cell preparation and recipient reduced risk of contamination and improved coordination between teams.
We performed 8 human islet isolations and 3 clinical auto-ITX with no complication. Positive pressure was maintained at all times and microbiologic cultures were negative in all samples.
Conclusions: Integration of an islet isolation facility in the OR area presents many functional advantages and a significant cost containment.

Parallel Session 20
Encapsulation

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Development of a new model of islet macroencapsulation - The MAILPAN

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Objective: MAILPAN's project aims to develop a macro-encapsulation device in order to treat type 1 diabetes patients. This medical device is devoted to encapsulate insulin-secretory cells after being implanted in the retroperitoneal cavity of patients. Yet, it has the functions of a bioartificiel pancreas.
Methods: The innovativeness is supported by the following aspects: 1) the use of semi-permeable membranes allowing the exchange of nutrients/glucose and Insulin, and inhibiting the killing of the cells by the immune system; 2) surface functionalization in order to induce the vascularization of the device and inhibit any inflammation; 3) Optimized dimensions in order to implant a sufficient number of islets/cells; 4) the use of a diffusion chamber allowing emptying the device from the dead cells and filling it with new fresh cells.
Results: MAILPAN® prototype has been developed during the past years. We have shown that: 1) we can implant a high number of cells sufficient to establish a normo-glycemia; 2) we are able to fill/empty the device which allows the replacement of dead cells; 3) pre-implantation of the device one month before cells' filling decreases by 50% the cells death; 4) the membrane functionnalization allows the cells to exchange rapidly with the surrounding tissues; 5) the biocompatibility of the device was validated on rats and pigs.

Conclusion: MAILPAN's project is a great opportunity for laboratories/Big Pharma in order to assess the function of their insulin-secretory cells from several origins (human, porcine and genetically modified) in clinical assays. Moreover, this medical device will be then developped for other therapeutic applications, such as kidney diseases, liver diseases and Alzheimer disease.

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Conformal coating with PEG-based hydrogels for pancreatic Islet immunoprotection

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Transplantation of pancreatic islets is a promising therapy for the Type-1 diabetes mellitus. Among current obstacles to the widespread clinical application of islet transplantation is the need for lifelong anti-rejection therapy. The encapsulation of islets within biocompatible hydrogels represents an attractive alternative to systemic immunosuppression. Being that islets are highly metabolically active and sensitive to low oxygen and pH variations, encapsulation of islets using methods that result in coatings on the order of hundreds of microns, such as typical alginate microencapsulation, results in diffusion impediments detrimental to islet viability and function. Microencapsulation also substantially increases graft volume, thereby restricting the transplant site to the poorly oxygenated site of the peritoneal cavity. To overcome these problems, we designed a novel method based on fluid dynamic principles to conformally coat islets within a very thin hydrogel layer (10-20 μm), which is 5 to 50-fold smaller than traditional encapsulation techniques. Through this method, coatings conform to the islet surface and are independent of islet diameter. We designed and built coating chambers capable of encapsulating 1,000 islets per minute in a highly reproducible manner. Efficacy of method was established for rodent (mouse and rat), non-human primate and human islets. While the chambers are adaptable to multiple hydrogel platforms, branched PEG and alginate combinations were used to permit modulation of perm-selectivity and mechanical properties. Coated islets demonstrated no detectable loss in viability following coating, per metabolic assays. Responsiveness of islets to glucose challenge in vitro was unaffected by the presence of the coating. Transplantation of Balb/c mice or Lewis rats (loading from 800-1500 IEQ) under the kidney capsule of chemically-induced diabetic C57BL/6J mice promptly reversed to normoglycemia, with tempos comparable to uncoated islets. Future work is focused on evaluating the capacity of these coatings to substantially delay or prevent allo- and xeno-graft rejection in rodent models.

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Islets xenotransplantation: New Zealand experience

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To meet regulatory requirements for xenotransplantation an elaborate program on xenovirology has been under development and implementation at LCT since 1997. This program allowed LCT to allocate one specific herd of pigs that was free from all conventional pathogens and was also qualified as "null" pigs or pigs that do not have a transmittable pig endogenous retrovirus (PERV). These pigs are the animal-founders for a DPF donor herd. A comprehensive program for patients' microbiology follow-up was also developed. This program includes assays developing for monitoring potential infection with PERV and other potentially xenotic pathogens.

Compliance with the requirements from New Zealand government allowed LCT to successfully complete its application for a clinical trial using porcine islets. In 2009 the New Zealand Government approved an open-label phase I/IIa safety/efficacy clinical trial using porcine islet cells. The primary objectives for the phase I/IIa clinical trial are the safety of xenotransplantation of DIABECELL® and improvement in blood glucose level reflected in a decrease of HbA1c (%) level. The secondary objectives include glucose lability, reduction of insulin dose and decrease in hypoglycaemia frequency and severity. A total of fourteen patients with severe unaware hypoglycaemia were enrolled into the trial and allocated to one of the four dosage groups, which range from 5,000 – 20,000 IEQ/kg delivered in a single dose. In October 2009 the first New Zealand patient was transplanted with DIABECELL®.

To date LCT reports that the trial is meeting objectives for safety. All fourteen patients were screened for the presence of pig viruses including PERV. There was no evidence of PERV infection during the 52 weeks of monitoring. Minimal reduction in insulin dose and HbA1c has been reported in transplant patients. However there

has been a marked improvement in hypoglycaemic episodes, with patients showing a decrease in the severity of these episodes as well as a reduction in unaware hypoglycaemia.

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Fabrication of nano-scale coatings for islet encapsulation

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The encapsulation/immunoisolation of cells has numerous applications in cellular transplantation, particularly for diabetes. Conventional encapsulation methods impose consequential mass transport limitations and transplant volumes. New approaches such as layer-by-layer coating generate thin coatings (0.1-50μm), but common challenges include incomplete encapsulation, coating stability, and method cytotoxity. As such, we developed novel, functionalized polymers capable of forming stable, covalent-linkages via Staudinger ligation, a spontaneous, chemoselective, and cell-compatible reaction. Herein, we used these complimentary PEG- and alginate-based polymers to fabricate the covalently linked, nano-thick coatings.

Following initial PEG coating, subsequent layers were formed on surfaces or islets via step-wise incubation with complimentary functionalized polymers (branched PEG and alginate in full media). Layer formation was characterized via ellipsometry, AFM, and confocal imaging. Islet viability and insulin release during glucose challenge was evaluated. Coated Lewis rat islets (600 IEQ) were transplanted into diabetic C57BL/6J mice to assess function.

Step-wise incubation of idealized surfaces or islets with polymers resulted in the formation of covalently linked nano-scale coatings. Ellipsometry data quantified the building of layers, with increasing thickness up to ~20 nm. Confocal microscopy images and AFM of surfaces illustrate uniformity of coating and specificity of binding to only complimentary polymers. Resulting coatings exhibited stability following washes with highly ionic solutions, indicating stable covalent linkages of layers. Co-incubation of polymer solutions with islets resulted in no decrease in viability or effects on insulin secretion. Transplantation of coated islets into the kidney capsule resulted in prompt return to normoglycemia, with reversal times identical to uncoated controls.

We have illustrated the capacity of these functionalized polymers to undergo Staudinger ligation-based covalent layer-by-layer assembly on islets. Stable layers formed in cell media with high specificity, with no detrimental effects on cell function/viability. Overall, these layers could serve as ideal platforms for cellular encapsulation.

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Optimization of nano-scale emulsions: perfluoro micellar solutions for enhanced oxygen transfer in biomedical applications

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Nano–scale emulsification has long been utilized by the food and cosmetics industry to maximize material delivery through increased surface area to volume ratios. More recently, these methods have been employed in the area of biomedical research to enhance and control the delivery of numerous pharmaceutical and cytoprotective compounds. Of particular interest is the use of perfluorocarbon (PFC) nano-scale emulsions to increase overall oxygen mass transfer within the surrounding milieu. In this work, we developed and implemented methods for optimizing the manufacture of stable emulsions focusing on component selection, emulsification time, emulsification pressure and accurate characterization of temporal emulsion stability and oxygen delivering capacity. Through careful analysis of these parameters we were able to design reproducible and well-defined oxygen-delivering PFC emulsions for use in cell-based applications.

Oxygen mass transfer, or diffusive permeability, is determined by the product of the effective oxygen diffusivity through and oxygen solubility in the emulsion. We found that particle size was the critical factor affecting oxygen mass transfer, as increased micelle size resulted in reduced oxygen diffusion, canceling the benefit of increased dissolved oxygen content in the perfluorocarbon phase of the emulsion. Particle size stability was directly related to the perfluorocarbon utilized, particularly the molecular weight, diffusivity through the liquid phase and interfacial tension between the hydrophobic and hydrophilic emulsion components. Overall, this work demonstrated the

importance of accurate characterization of emulsification parameters in order to generate stable, reproducible emulsions with the desired bio-delivery properties.

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A detailed insulin secretion model for encapsulated islets that incorporates oxygen dependence and spatial distribution information

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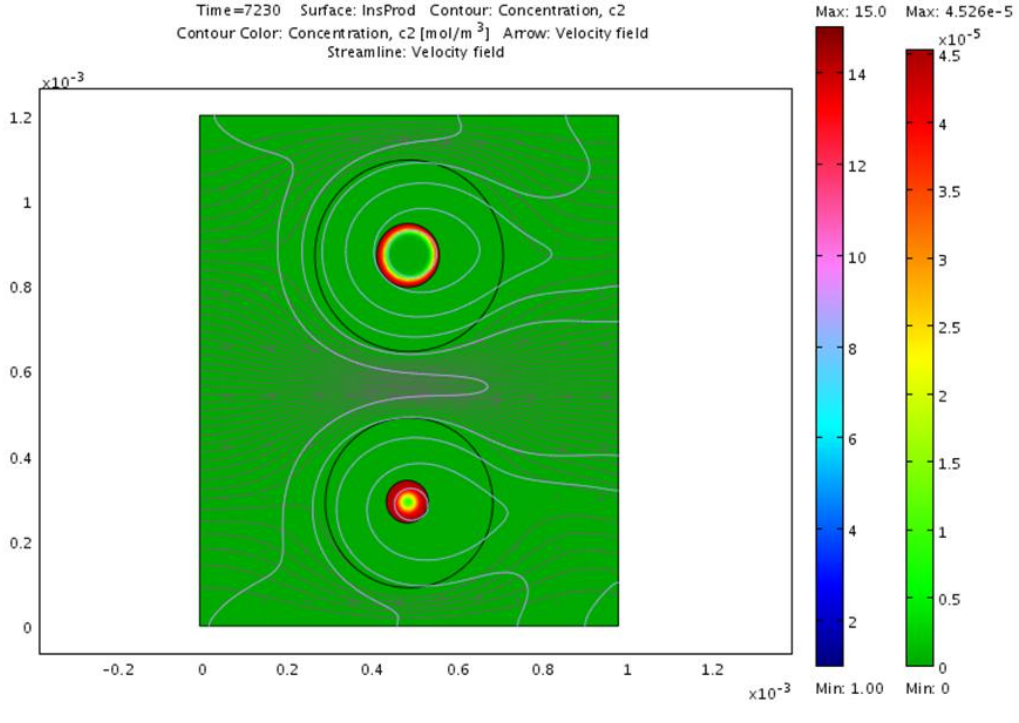
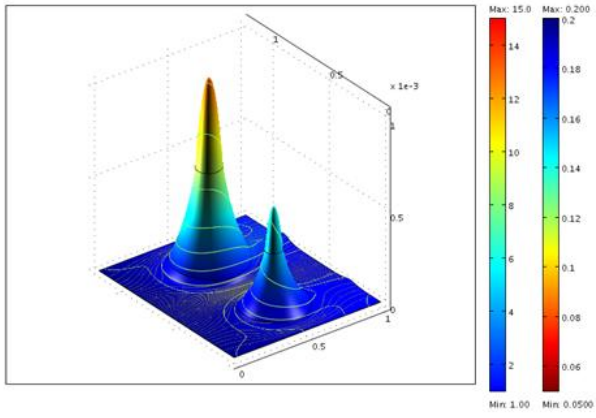
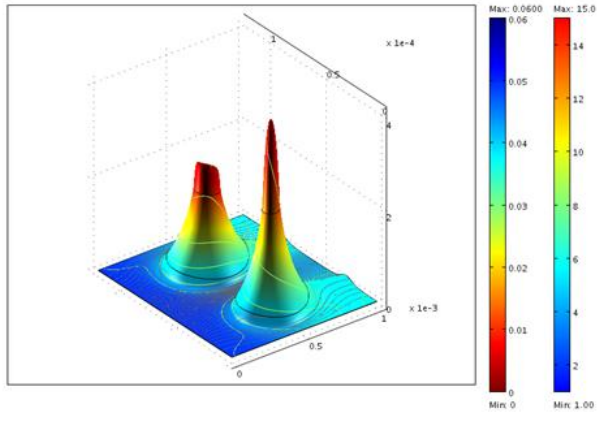
Accurate quantitative models of glucose-induced insulin secretion that also incorporate oxygen dependence are of critical value in the design of improved bioartificial pancreas systems, such as those using immune-isolated, encapsulated islets, because oxygen diffusion limitations are a major problem hindering their functionality. We have recently developed a detailed computational model of insulin secretion in avascular islets (*Theor. Biol. Med. Model* **2011**, in press) that couples local consumption and release rates to calculations of the spatial distributions of all species of interest by using a finite element method (FEM) framework (COMSOL Multiphysics). Insulin secretion rates were assumed to depend on both the local glucose concentration and its time-gradient, resulting in second- and first-phase responses, respectively, and the model was calibrated using experimental results from dynamic glucose-stimulated insulin release (GSIR) perifusion studies with isolated islets. Following parameterization, good fit could be obtained with experimental perifusion data of human islets (i.e., staircase experiment). With the model, it is now possible to obtain detailed estimates of the intraislet spatial distributions of insulin, glucose, and oxygen concentrations; and because of the general framework of the implementation, simulations can be carried out for arbitrary geometries of avascular islets including cultured, perifused, transplanted, as well as encapsulated islets. In agreement with recent observations, calculations suggest that smaller islets perform better when transplanted and/or encapsulated because of less severe hypoxia in their core regions. At lower oxygen concentrations, such as those that transplanted islets are likely to encounter even in well-vascularized tissues (p_{O2} = 35–45 mmHg; c_{oxy}= 0.05–0.065 mM), encapsulated islets loose more of their insulin secreting ability than free islets because they suffer more heavily from hypoxia as oxygen diffusion limitations severely restrict the hormone secreting ability of their core regions even with relatively thin microcapsule sizes.







Parallel Session 23
Cell & Molecular Biology

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Nephrin effect on glucose stimulated insulin release depends on dynamin-mediated nephrin phosphorylation

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Objective: We have previously demonstrated an important role of nephrin in the process of glucose stimulated insulin release (GSIR). We now want to test the hypothesis that nephrin phosphorylation by glucose is required for GSIR, and to determine if dynamin influences nephrin phosphorylation and function in pancreatic beta cells.
Research Design and Methods: MIN6 C3 glucose unresponsive nephrin deficient cells were transfected with either wild-type nephrin (WT-N) or with a mutated nephrin where the three tyrosines responsible for SH2 domains binding were mutated to phenylalanine (3YF-N). Cells were utilized for static incubation experiments, confocal live images of nephrin trafficking and vescicle formation, TIRF microscopy and confocal analysis of actin cytoskeleton remodeling. Regular MIN6 cells transfected with a different combination of dynamin, dynamin K44A dominant negative mutants, WT-N, 3YF-N and nephrin siRNA were utilized to study the functional interaction between nephrin and dynamin. HEK cells transfected with FLAG-Nephrin, GFP-podocin, GFP-Nck, FLAG-Pin were utilized

to study nephrin-dynamin interaction. Protamine sulfate (PS) and vanadate were tested for their ability to phosphorylate nephrin in MIN6 cells and to affect GSIR in human islets.
Results: Both glucose and PS induced WT-N phosphorylation and cytosolic internalization, which was instead prevented by the Src inhibitor PP2. On the contrary, 3YF-N had a cytosolic localization that was not affected by glucose or PS. 3YF-N abolished the positive effect of WT-N on GSIR and affected the morphology of MIN-6 cells to a less adhesive phenotype. Both vescicle formation and secretion were impaired in 3YF-N transfected cells. Nephrin siRNA abolished the positive effect of dynamin on GSIR, and K44A dynamin mutants prevented nephrin phosphorylation and abolished the positive effect of nephrin on GSIR. However, a direct interaction of nephrin and dynamin was not observed, and dynamin and nephrin mutants differently affected actin remodeling. Finally, not all stimuli resulting in increased nephrin phosphorylation facilitated GSIR in human islets, as both PS and vanadate increased nephrin phosphorylation but the had opposite effect on GSIR.
Conclusions: Nephrin phosphorylation occurs after glucose stimulation in a dynamin dependent manner and is necessary for nephrin modulation of GSIR. Pharmacological modulation of nephrin phosphorylation may represent a novel strategy to modulate GSIR in human islets.

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Low molecular weight dextran sulfate prevents human cytomegalovirus entry into porcine cells and is effective against clinical isolates

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Low molecular weight dextran sulfate (DXS) acts as an endothelial cell (EC) protectant and prevents human complement- and NK cell-mediated cytotoxicity *in vitro*. In combination with cyclosporin A, DXS induces long-term graft survival in a xenotransplantation model *in vivo* and is very effective in protecting vasculature and tissue from ischemia/reperfusion injury. Human cytomegalovirus (HCMV) infection following organ transplantation can initiate endothelial cell activation and vascular injury that may facilitate graft rejection. In this study, we further characterized DXS in order to define its influence on HCMV infection.
Human and porcine EC were treated with DXS and antiviral activity was quantified by measurements of virus titre and viral growth kinetics.
Dose response experiments performed in human EC indicated that pre-treatment 250 ng/ml DXS reduced HCMV infectivity by 75% and 2.5 µg/ml completely prevented the formation of infectious particles. Immunofluorescence as well as Western blot analysis demonstrated that viral replication was already blocked at the immediate early phase resulting in absence of pp65 nuclear accumulation and in limited expression of the immediate early proteins IE1/IE2. In addition, DXS had an inhibitory effect on a clinical isolate, as assessed by the reduction of HCMV DNA copy numbers in the cells and in the supernatants of infected cultures. Entry assays pointed out that pre-treatment of human EC with DXS led to a complete block of entry. Moreover, DXS also abrogated HCMV entry and replication into porcine EC. Finally, *in vitro* analyses indicated that DXS may exert its antiviral activity through direct binding to the virions.
If a similar effect can be reproduced *in vivo*, DXS, in combination with conventional immunosuppressive protocols, may represent a promising agent to reduce the risk of HCMV reactivation following allo- and xenotransplantation.

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Cure of experimental Laron syndrome (LS) by microencapsulated Sertoli cell (SC) xenograft

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LS is a rare human inherited disorder caused by defects in the gene encoding the growth hormone (GH) receptor, with consequential suppression of IGF-1 production, leading to clinical dwarfism. The only actual available therapy consists of recombinant human IGF−1, injected daily, which unfortunately is burdened with side effects, other than being an “orphan drug”, hence poorly available for treating LS patients. Since IGF-1 is one of the most important

SC secretory products, we aimed to determine if alginate-encapsulated SC transplant (TX) would reverse GH deficiency in "Laron mice" (LM), a unique animal model of genetic GH deficiency, identical to human LS. 60 LM were grafted with encapsulated SC, while 20 untreated LM served as controls. SC, upon retrieval from pre-pubertal pigs, were enveloped in Barium-AG microcapsules (BaMCs), according to our methods. BaMCs were examined as far as: (a) SC morphology by light microscopy; (b) SC viability, by fluorescence microscopy after staining with ethidium bromide and fluorescein diacetate (EB+FDA); (c) pre-TX SC in vitro function (alpha-aromatase activity and IGF-I secretion); (d) post-TX inducedgrowth parameters in LM, were concerned. BaMCs exhibited excellent physical-chemical features, while the enveloped SC showed excellent viability (over 90%) and function in terms of a-aromatase activity and IGF-I secretion, prior to TX. Intraperitoneal TX of BaMCs into LM induced significant increase in growth parameters, in terms of body weight and nose-to-anus length, exceeding by 20% those of untreated controls. All retrieved organs showed significant growth as compared to those of untreated LM, indicating full correction of the GH deficiency. In conclusion, xenograft of morphologically intact and functionally competent SC with Ba-AG microcapsules into homozygous LM with overt dwarfism, induced an unprecedented and significant increase in all growth parameters in comparison with untreated controls.This result might definitely open new perspectives for the cure of human LS.

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Decarboxylated osteocalcin improves human islet function and induces beta cells proliferation in-vitro and in-vivo

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Objective: The osteoblast-specific secreted molecule osteocalcin infusion in wild type mice was shown to increase the insulin genes Ins1 and Ins2 expression and to induce the expression of the genes necessary for in vivo β-cell proliferation such as cyclin D2 and Cyclin-dependent kinase 4 (Cdk4). In our laboratory, we found that non-function human islet show an up regulation of oxytocin gene expression which inhibits cell proliferation, and down regulation of cyclin D which is involved in beta cell proliferation. In this study, we investigate the impact of osteocalcin on human islets function and beta cells proliferation, in-vitro and in-vivo.
Methods: Aliquots of human islets from 7 donors were cultured in serum free media with 0.3, 1.0, 4.5, 15 ng/ml of decarboxylated osteocalcin. After 7, 14, and 28 days in culture, islets were analyzed for insulin content, insulin secretion and beta cells proliferation using western blot as well as static incubation assay. In-vivo function of human islets from 5 isolations with and without decarboxylated osteocalcin (4.5 ng/ml/hr) was tested using NOD-SCID mouse model (500IEQ/mice).
Results: Human islet co-cultured with decarboxylated osteocalcin showed a significant increase in insulin content as early as 7-post culture, insulin content was two fold higher than that of the control islet. Western blots of human islet showed an increase in protein expression for Cyclin D1&Cdk4, which are necessary for beta cells proliferation and SUR-1 which functions as a modulator of ATP-sensitive potassium channels and insulin release in islets cultured in 4.5ng/ml decarboxylated osteocalcin supplemented media. Moreover, supplementing the transplanted human islets in-vivo with decarboxylated osteocalcin resulted in significant increase in human insulin and c-peptide production $p<0.05$.
Conclusions: Culturing human islet with decarboxylated osteocalcin, results in an increase of Beta cells number, insulin content, insulin processing and significantly increase in-vivo production of c-peptide.

Parallel Session 24
Stem Cells/Transdifferentiation

569
Insulin-secreting thyroid C-cells as surrogate beta cells for Type 1 diabetes

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The treatment of Type 1 diabetes mellitus (DM1) typically requires exogenous insulin. As an alternative, transplantation of autologous, genetically engineered insulin-secreting non-beta cells would alleviate the need for immunosuppression and avoid the recipient's autoimmune mechanisms. Human thyroid parafollicular C-cells are ideal candidates because, like islet beta cells, they contain dense core secretory vesicles and have a high capacity for protein synthesis and secretion. C-cells also express endopeptidases capable of processing pro-insulin to mature insulin, and they proliferate *in vitro*, permitting *ex vivo* expansion. Key challenges in using C-cells are engineering sufficient levels of recombinant insulin expression and secretory glucose responsiveness.
We have purified human C-cells from 8 fresh, operative thyroid specimens, obtaining 1.6 to 2.8 million C-cells/gm of thyroid tissue. (A normal 30 gram human thyroid yields approximately 60 million C-cells which can be expanded to obtain sufficient numbers of engineered cells to control glycemia.) The C-cells contained secretory granules and stained positively for calcitonin. When C-cells were transduced with viral vectors carrying the insulin gene, they co-expressed human insulin and calcitonin. Transduction efficiencies were generally >90%, and insulin expression appeared stable three weeks after transduction. Immunogold staining and electron microscopy showed that secretory granules contained human insulin, and insulin was detected in both media and lysates from the transduced cells. In addition, we have shown that *Ngn3,* a key islet beta cell transdifferentiation factor, is expressed in non-transduced, control C-cells.
We believe that transplantation of insulin-secreting autologous thyroid cells has the potential to improve glycemic control in over 1 million people affected by DM1 in the U.S., and to reduce the long term complications of diabetes.

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Inflammatory bowel disease: treatment with human MIAMI cells

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Crohn's disease (CD) and Ulcerative Colitis (UC) are the major types of inflammatory bowel diseases (IBD), a group of inflammatory conditions of the colon and small intestine. In the present study we used a multipotential sub-population of human bone marrow stromal cells, the marrow-isolated, adult, multilineage, inducible (MIAMI) cells to assess whether non-myeloablative therapeutic human MIAMI cells treatment could ameliorate the conditions of a chronic UC mouse model. Chronic ulcerative colitis was induced in C57BL/6 mice by the addition of 3% dextran sulfate sodium (DSS) in the drinking water from day 1 to day 7 and from day 15 to day 21. Two million human MIAMI cells were prepared and labeled with Quantum-Dots and injected on four different days: 8, 15, 29 and 36. Animals were sacrificed on day 43. A significant reduction of inflammation was observed with a colonoscopy only on mice treated with MIAMI cells. Control mice, treated only with PBS, showed a reduction on the length of the entire colon however, upon treatment with MIAMI cells the colon appeared to be normal in size. Ex-vivo organotypic cultures of intestinal tissue from mice treated with MIAMI cells showed a higher amount of IL-10 production and a significant reduction on the secretion of IFN-gamma compared to control mice treated with PBS. Mice treated with MIAMI cells showed a significant reduction of blood in the stool and improved histological colitis score compared to controls. Finally, human MIAMI cells engrafted in the sub-epithelial mesenchymal cells and intestinal tissue as epithelial. We conclude that these findings demonstrate for the first time that ex-vivo-expanded human MIAMI cells are effective in the treatment of chronic UC in a mouse model of DSS-induced. Our results suggest that IP infusion of exogenous human MIAMI cells might be a novel and effective anti-inflammatory treatment for IBD.

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Potential of amniotic membrane and its derivatives in tissue regeneration and repair

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Due to its anti-inflammatory, anti-scarring and wound healing properties, the amniotic membrane (AM) is now well-established as a valuable surgical material, and is now applied for several purposes including healing of skin wounds and management of ocular surface disorders. In recent years, AM has also emerged as a promising source of

stem/progenitor cells which display multilineage differentiation potential as well as interesting immunomodulatory and trophic features.
Our group is currently investigating the possibility of expanding the use of AM fragments to include treatment of pathological conditions other than skin/cornea wounds, as well as investigating the effects of AM-derived cells in pre-clinical models of diseases involving inflammatory and fibrotic mechanisms.
We have observed that transplantation of either allogeneic or xenogeneic fetal membrane-derived cells reduces lung fibrosis in mice with bleomycin-induced injury. In addition, we have shown that human AM patches reduce post-ischemic cardiac scars and liver fibrosis in rat models of coronary artery and bile duct ligation, respectively. In all of these studies, we detected rare or absent levels of donor cells in host tissues, suggesting that AM-derived cells might exert reparative effects mainly by releasing yet unknown paracrine factors. When we assessed fibroblast proliferation, collagen deposition and alveolar obliteration parameters in lung tissue sections of bleomycin-challenged mice treated with conditioned medium generated by AM-cells(AM-CM) or control medium, as well as inanimals that received no treatment, we found that AM-CM reduced the extent, severity and progression of lung fibrosis. This supports an important role for soluble factors in AM-mediated therapeutic effects, although the identity and mode of action of these factors remain to be elucidated.
On these bases, human AM can be considered a source of derivatives (AM-fragments, cells and soluble effectors) with multi-faceted properties that may be exploited for novel regenerative and reparative medicine approaches.

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The efficacy of human autologous adipose mesenchymal stem cells isolated with cGMP collagenase to obtain osteogenic-bone like tissue

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Background: Multipotent adipose-derived mesenchymal stem cells (AMSCs) can be isolated from animal and human subcutaneous tissue for osteogenic differentiation and bone tissue engineering. However, the collagenase digestion of adipose tissue remains a crucial step in view to obtain a maximal yield of stem cells in Good Manufacturing Practice (cGMP) for cell banking. This study investigated the impact of collagenase (crude or GMP collagenase) on human AMSCs in term of isolation, expansion and osteogenic-differentiation.
Methods: Human adipose tissues were digested with a standard static digestion method using GMP- (provided by Serva, n=12) or crude-collagenase (Sigma, n=7) and were compared to pre-clinical pig AMSCs isolation with crude collagenase (n=8) at the concentration of 1.5 mg/ml. Time course of Proliferation Phase (PP, Passage 0^{th} to Passage 4^{th}) and Osteogenic-Differentiation Phase (DP), AMSCs cellular yield at passage 4^{th} and the osteogenic phenotype expression (Red Alizarin, Osteocalcin,Von Kossa staining) were assessed for each experimental groups.
Results: No significant difference of adipose tissue weight (prior digestion) was observed between groups (p=0.784). Human AMSCs (isolated with GMP collagenase) demonstrated a significant shorter PP than pig AMSCs (28±10 vs. 60±25 days, p=0.003, respectively). No significant difference of proliferation phase was found for human AMSCs isolated with both GMP and crude collagenase.
A significant lower yield of AMSCs was found at Passage 4^{th} (after PP) from human adipose tissues isolated with crude collagenase in comparison to GMP collagenase ($2.3\pm0.6 \times 10^6$ vs. $9.8\pm3.5 \times 10^6$ cells, respectively, p=0.025). No significant difference of time (required for DP) was observed between experimental groups (mean of 30 days, p=0.131). All differentiated AMSCs expressed osteogenic phenotype with mineralization process at the end of the differentiation phase.
Conclusions: cGMP clinical grade collagenase can provide a safety procedure to obtain rapidly a high yield of human AMSCs differentiated in osteogenic-like tissue for bone reconstruction.

Posters

P002
Human hepatoma cell line conditioned medium promotes migration and increases alpha smooth muscle expression in multipotent mesenchymal stromal cells

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Introduction: Multipotent mesenchymal stromal cells (MSC) are currently used clinically as therapy for graft versus host disease. There is however evidence that MSC can be recruited to tumor sites and sustain tumor growth. To investigate the molecular mechanism and factors implicated in MSC migration we used a human hepatoma cell line (Huh-7) conditioned medium (CM).
Methods: We performed transwell chamber migration assays measuring cell movement by live-cell imaging of human bone marrow derived MSC. Inflammatory cytokines in Huh-7 CM were detected by an antibody cytokine array.Phosphorylation of Erk and Focal adhesion kinase (FAK) pathways as well as aSMA expression by MSC were measured.
Results: Huh-7 CM triggered 3.5-fold increase of MSC migration compared to control medium, whereas platelet derived growth factor BB (PDGF-BB), used as positive control, induced a 6-fold increase. Phosphorylation of Erk was induced by PDGF-BB and Huh-7 CM, whereas phosphorylation of FAK was only induced by Huh-7 CM. Live-cell imaging revealed that both Huh-7 CM and PDGF-BB increased directional migration. Inhibition of phosphorylation of Erk correlated with decreased migration. We identified high levels of Interleukin 8 (IL-8), Macrophage inhibitory protein 1b (MIP1b),1g (MIP1g) and RANTES in Huh-7 CM compared to control medium. However, recombinant IL-8, MIP1b, MIP1gand RANTES (CCL5) did not activate MSC migration in the transwell system. Finally, expression of aSMA by MSC maintained in Huh-7 CM or recombinant MIP1gincreased significantly compared to control and PDGF-BB conditions.
Conclusions: Huh-7 CM activates migration and aSMA expression in MSC. Inhibition of phosphorylation of Erk correlates with decreased migration suggesting that activation of this pathway is implicated in MSC migration induced by hepatoma cells.

P003
Intracoronary delivery of autologous bone marrow cells in patient with chronic ischemic cardiomyopathy

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Background: It has been shown that autologous bone marrow cells may contribute to myocardial repair after acute myocardial infarction. The aim of this study was to assess the beneficial effects of intracoronary transplantation of bone marrow cells in patients (n=9) with chronic ischemic cardiomyopathy.
Methods: Bone marrow was obtained and the cells were injected intracoronary after a brief balloon occlusion at a normal coronary segment. Dobutamine stress echo showed that all patients had left ventricular ejection fraction < 35%. Patients were followed up to 12 month. Clinical follow up was performed periodically and included electrocardiography, laboratory tests and echocardiography.
Results: Intracoronary bone marrow cell therapy improved ventricular performance, quality of life and survival in patients with chronic ischemic cardiomyopathy. No side effects were observed
Conclusions: Intracoronary transplantation of autologous bone marrow cells is safe and feasible in chronic ischemic cardiomyopathy.

P004
Autologous stem cell and hyperbaric oxygen therapy in type 2 diabetes Mellitus

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Study Design: A prospective phase I-II, SMT controlled clinical trial.

Objective: To determine whether intra-pancreatic infusion of bone marrow- derived autologous stem cells (ASC) in combination with hyperbaric oxygen therapy (HBO) of the recipient could improve metabolism in patients with Type 2 Diabetes (T2DM). The study hypothesis was that combination of autologous stem cell therapy and hyperbaric oxygen treatment could favor tissue remodeling and regeneration/expansion of insulin producing cells in the treated patients.
Methods: 23 patients at baseline, suffering from T2DM (duration 5-15 years) were randomized to treatment (13 HBO-ASC, 10 SMT). Metabolic outcomes were measured at baseline and at 180 days. Comparisons were made between groups over this 6 month- period post baseline. Metabolic outcome measures of interest included A1c, glucose, cpep/gluc.
Results: At baseline, there was no significant difference between groups in A1c, glucose, cpep, and cpep/gluc. However, at 180 days, in the intervention group,there were significantly lower levels of A1c(difference=-1.47, $p<0.001$) and glucose ((diff=-64.86, $p<0.0001$) and significantly higher levels of cpeptide (diff=1.07, $p<0.001$) and cpep/gluc (diff=0.01, $p<0.0001$). Changes in A1c over the 6 month follow-up were not significant in the SMT group, but were significant in the intervention group (change in intervention group =-1.46, $p<0.001$). Decreases in A1c in the intervention group was estimated to be 1.14 units greater than the estimated decrease in the SMT group ($p=0.01$). 30% of patients in the SMT group and 77% of patients in the intervention group demonstrated a decrease in A1c at 180 days compared to baseline of at least 1 unit, representing a significant difference between groups in the proportion achieving a 1 unit reduction of A1c in this study over 6 months ($p=0.02$).
Conclusion: This study validates the initial pilot study although more research is necessary for final findings confirmation.

P005
Bone marrow-derived stem cells in human liver diseases

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Bone marrow (BM) seems to play an important role in regeneration after liver injury. Several growth factors and cytokines such as HGF, SDF-1 and VEGF, released during injury act as powerful chemoattractantsto BM progenitor cells. One of the primary signals that the stemcells receive after tissue injury may be the hypoxia-inducedlocal activation of HGF that could act, in a gradient-dependent manner, to induce stem cell migration into thedamaged tissue. Although there are data supporting this hypothesis in animal studies, there is little information about these phenomena in humans.
Objective: To study the role and mechanisms of BM progenitor cells in liver injury in humans.
Patients and methods: Peripheral blood was recollected from 16 patients with acute hepatitis (AH), 7 with liver cirrhosis (LC), 7 with acute on chronic liver disease (AOC) and 7 healthy controls (HC). BM stem cells were analyzed by flow cytometry measuring CD34+, CD131+, CXCR4+, VEGF-2R+, c-KIT+, populations. Growth factors and cytokines (VEGF, HGF, SDF-1, G-CSF, SCF, MPP9, IL-3, IL-6 and IL-8) were quantified by luminex technology in peripheral and suprahepatic blood.
Results: Significant BM mobilization was detected in patients with HA. In this group CD34+ cells were 0.052%±0.03 versus 0.020%±0.01 in LC patients, and VEGFR-2+ cells were 0.078%±0.08 versus 0.017± 0.09. AH group had also higher levels of VEGF (933.4±824.7 pg/ml versus 464±730 pg/ml) and HGF (6700.9±4561 pg/ml versus 1930.6±886 pg/ml) than LC patients. All these differences were significant ($p<0,05$). HGF levels were three times higher in suprahepatic (24879±31119 pg/ml) than in peripheral blood (8038±8613 pg/ml).
Conclusion: Growth factors released in acute liver injury could induce bone marrow mobilization indicatingthat bone marrow stem cell could play an important role in liver regeneration in humans.

P006
Phenotype plasticity of mesenchymal stem cells from Lipostem™-derived human adipose tissue

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Human adipose tissue-derived mesenchymal stem cells (hADSCs) have recently generated a great interest based on their biological and clinical potential. It has been shown their ability to differentiate into various cellular lineages such as adipogenic, osteogenic, chondrogenic, myogenic and other mesenchymal lineages. Moreover, application of hADSCs promotes angiogenesis, and suppression of effector T cells and inflammatory responses in general. All together their transplantation is emerging as a novel therapeutic option for the promotion of functional recovery of diverse types of damaged tissues. Here, we report the phenotypic features of hADSCs isolated from adipose tissue with or without application of Lipostem™. Lipostem™ is a registered technological apparatus for the preparation of ready to use adipose tissue. hADSCs migrate out of fragmented adipose tissue, and can be grown in culture in presence of different media. To mimic different extracellular conditions hADSCs were also plated in presence or absence of various adhesion substrates such as laminin, polylysine, polyornithine and Matrigel®. Cells grown in mesenchymal classic medium (αMEM) were flat, large with few short processes, while those grown in human neural medium (HN) were slim and elongated with one or few prolonged processes. The growth rate was comparable in both media. When cell culture dishes were coated with Matrigel® and laminin, the growth rate in αMEM was far greater, and the morphology changed since cells were smaller and slender with few, short medium size processes. In presence of positively – charged dishe coating (polylysine and polyornithine) the rate of cells growth was lower than normal. Growth wasn't affected by substrates when HN was used as medium, although the presence of laminin substrate allowed the formation of neuronal-like longer and slender processes. Lipostem™ -derived adipose tissue was a good source of stem cells even in absence of enzymatic treatment, and when kept frozen for several weeks. This initial results strongly support the idea that Lipostem™ -treated adipose tissue may represent a powerful source of adult mesenchymal stem cells.

P007
Comparative characterization of human umbilical cord blood and adipose tissue–derived multipotent mesenchymal stem cells

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Cell-based therapies will benefit from the definition of appropriate sources of pluripotent adult stem cells. The mesenchymal stroma harbors a population of cells that can self-renew and differentiate into multiple lineages.The bone marrow contains mesenchymal stromal cells (AKA mesenchymal stem cells, MSCs),which have potential to differentiate intofat, bone and cartilage, and could therefore represent a good source of MSC. However, autologous bone marrow procurement has potential limitations and therefore alternative, less invasive sources of MSCs are highly desirable. The umbilical cord-blood and adipose tissue have been suggested as viable options.The aim of this study was to compare selected biological characteristics of MSCs derived from cord blood (CB-MSCs) and adipose tissue (AT-MSCs).Cells were analyzed for proliferation, surface marker expression, stem cell gene expression and multi-lineage plasticity.MSCs derived both from CB and AT were similar in morphology and antigenic phenotype; however, the proliferation potential and yield were superior in CB-MSCs than in AT-MSCs. Also, there were significant differences in proliferation, population doubling time and senescence associated with the age of the donor of AT-MSCs; younger donors' cells showed better proliferation and less senescence. The adipogenic, osteogenic and chondrogenic differentiation potential was not grossly different between studied populations (CB-MSCs vs. AT-MSCs). MSCs from both sources expressedthe pluripotent stage–specific nuclear transcription factorOCT4 and surface stemness markers SSEA-3 and SSEA-4. However, the frequency of MSCs expressing these stem cell genes was higher in CB-MSCs than in AT-MSCs (OCT4: 80-90% for CB-MSCs and 15-30% for AT-MSCs; SSEA3 and SSEA4: 15-30% for CB-MSCs and 3-15% for AT-MSCs). The features seen in the MSCs derived from the umbilical cord may reflecttheir early developmental stage in ontogenesis. Further refinement of the characterization and functional studies are necessary to consider these MSCs for use in regenerative medicine.

P008
Lineage analysis and in vitro differentiation of human mesenchymal stem cells (MSCs) into insulin-producing cells: A comparison of cord blood- and adipose tissue-derived MSCs

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Generation of insulin-producing cells in large numbers is a major goal for their widespread use in the treatment of diabetes. Lineage analyses allows for the identification of progenitor cells from which mature cell types differentiate.Defined “progenitor cells” are cells produced during differentiation of a stem cell that have some, but not all, of the characteristics of their terminally-differentiated progeny. Transcription factors (TFs) may provide a key tool for uncovering how stem cells attain their phenotype, and how lineage-specific differentiation is initiated. In this study, we explored the presence of such defined “endocrine progenitor cells” in human mesenchymal stem cells (MSCs) derived from cord blood (CB) and adipose tissue (AT). Our results demonstrate thatundifferentiated CB-MSCs and AT-MSCs express key pancreatic TFs such as PDX1, NGN3, NEUROD1, ISL1 and NKX6.1, and maintain them during in vitro expansion at early passages. Co-expression of the MSC marker vimentin together with selected pancreatic TFs in ~20-50% of the cells suggests the presence of a subpopulation of cells within the undifferentiated MSCs that may be pre-committed towards the pancreatic lineage. We also explored the potential of CB-MSCs and AT-MSCs todifferentiate into insulin-producingβ cells. MSCs were induced in vitro to differentiateinto the pancreatic endocrine lineage followinga three-step differentiation protocol as described for murine adipose tissue-derived stem cells to differentiate into functional islet-like cell aggregates. Both CB-MSCs and AT-MSCs responded to this protocol witha) down-regulation of MSC markers and stemness markers and, b) up-regulation of selected key markers of pancreatic differentiation (definitive endoderm, primitive gut tube, posterior foregut and pancreatic endoderm) but not endocrine hormone expression. By further analysis of the effectiveness of the differentiation protocols applied, and with improved experimental settings, these “pancreas-committed” sub-populations from CB-MSCs and AT-MSCs may be efficiently directed towards the endocrine pancreatic lineage.

P009
Bone marrow-derived Mesenchymal stem cells accelerate tissue repair for hepatic ischemia reperfusion injury

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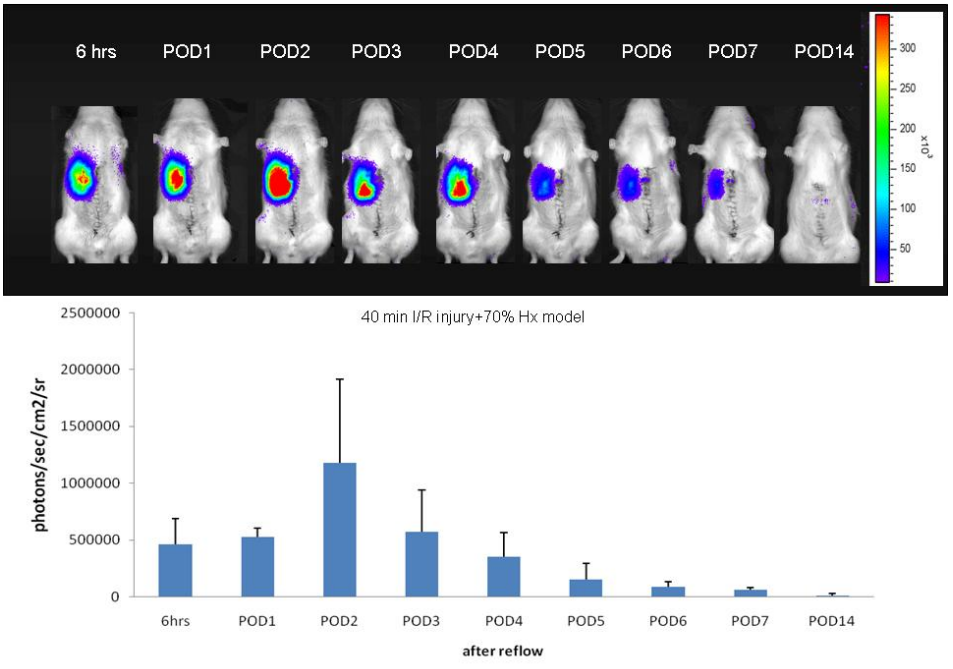
Background: Ischemia-reperfusion (I/R) injury associated with living donor liver transplantation impairs liver graft regeneration. Mesenchymalstem cells (MSCs) are presently considered to the most promising cell source for the cell therapy of various diseases.In this study, we demonstrate the impact of MSCs against hepatic I/R injury and hepatectomy.
Methodology/Principal Findings: We used a new rat model in which major hepatectomy with I/R injury was performed. Adult-Lewis rats were separated into two groups: MSC group given MSCs after reperfusion as treatment, and Control group given phosphate-buffered saline after reperfusion as placebo. The results of liver function tests, pathologic changes in the liver, and the remnant liver regeneration rate were assessed. The fate of transplantedMSCs in the luciferase-expressing rats was examined by in vivo luminescent imaging.
The MSC group showed peak luciferase activity of transplanted MSCs in the remnant liver 48 h after reperfusion, after which luciferase activity gradually declined. The elevation of serum alanine transaminase levels was significantly reduced by MSC injection. Histopathological findings showed that vacuolar change was lower in the MSC group compared to the Control group. In addition, a significantly lower percentage of TUNEL-positive cells was observed in the MSC group compared with the controls. Remnant liver regeneration rate was accelerated in the MSC group.
Conclusions/Significance: These data suggest that MSC transplantation provides trophic support to the I/R-injured liver by inhibiting hepatocellular apoptosis and by stimulating regeneration.



P010
Endothelial cells promote pancreatic stem cell activation during islet regeneration

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Objectives: Diabetes is the clinical consequence of the loss of the majority of the β cell population and failure to regenerate new pancreatic β-cells. The current therapies based on β cell replacement have failed to achieve β cell renewal and thus, long-term insulin freedom. We have hypothesized that early rejection of endothelial within the islet grafts may seriously hampered islet regeneration of both native and islet grafts.
Methods: In the present study, we analyzed the role of endothelial cells in the activation of pancreatic stem cells during islet regeneration. Mice were pretreated with or without endothelial pharmacological ablation of endothelial cells, followed by an acute β cell injury using a single intraperitoneal injection of streptozotocin.
We performed a comparative morphometric analyses within the recovered pancreata on days 3rd, 7th, 10th and 30th after streptozotocin injury, and thus stained for representative cell types, β cell, endothelial and stem cells co-stained with BrdU. Blood glucose levels were measured continuously after injury to monitor the capacity of metabolic control.
Results: Morphometric analyses revealed an increased number over time of cells stained with a stem cell marker and BrdU marker in animals only injured with streptozotocin but no endothelial ablation. Notably, on day 10th stem cell marker dramatically decrease nearly basal levels, and instead numerous insulin-positive cells appeared. Intact vessels with cobblestone-shaped endothelial were observed in direct proportion with better outcomes, in both morphometric and metabolic parameters. In contrast, fewer insulin positive cells were found on pancreata, which were ablated of endothelial cells, and showed an extensive collapse of the entire endocrine functions.
Conclusions: We have found that endothelial promote stem cell proliferation and islet regeneration after β cell insult. We believe that preservation of endothelial cells may positively affect the process of pancreatic regeneration.
Keywords: Islet regeneration, pancreatic stem cells, endothelial cells.

P011
Role of progerin accumulation in stem cell self-renewal and differentiation

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Inefficient tissue repair observed in aging can disrupt tissue homeostasis and lead to many age-associated disorders. Little is known about the molecular mechanisms that contribute to the aging process in stem/progenitor cells. Recently, progerin, the altered protein known to be the cause of the advanced aging disease Hutchinson-Gilford Progeria Syndrome (HGPS), has been implicated in physiological aging. Progerin is a mutated, permanently farnesylated form of lamin A. Normally, lamin A inserts into the nuclear lamina, providing nuclear structural stability while regulating other important cellular and nuclear activities. In HGPS patients, progerin is produced by a mutation that activates an alternative splice site, altering normal processing. Progerin also accumulates in aged, non-HGPS patients, at much lower rates, by random utilization of the same cryptic splice site. We hypothesize that age-induced progerin expression significantly contributes to inefficient stem cell-mediated tissue repair processes.

To investigate the effects of progerin expression on critical stem cell processes, we retrovirally transduced marrow-isolated adult multilineage inducible (MIAMI) stem cells with GFP-progerin, GFP-lamin A, and an empty vector control.

By using immunofluorescence, real-time qPCR, and immunohistochemistry, we demonstrate that progerin incorporation causes abnormal nuclear structure (blebbing) while altering self-renewal transcription factor expression, DNA damage and repair, proliferation, and differentiation, but does not seem to affect cell growth.

We conclude that progerin incorporation significantly alters vital stem cell processes. Future goals include developing induced pluripotent stem cells (iPSCs) from HGPS and non-HGPS patients and, together with the lines we have already generated, determine effects of progerin accumulation on migration, recruitment, and differentiation *in vivo* in the context of tissue repair.

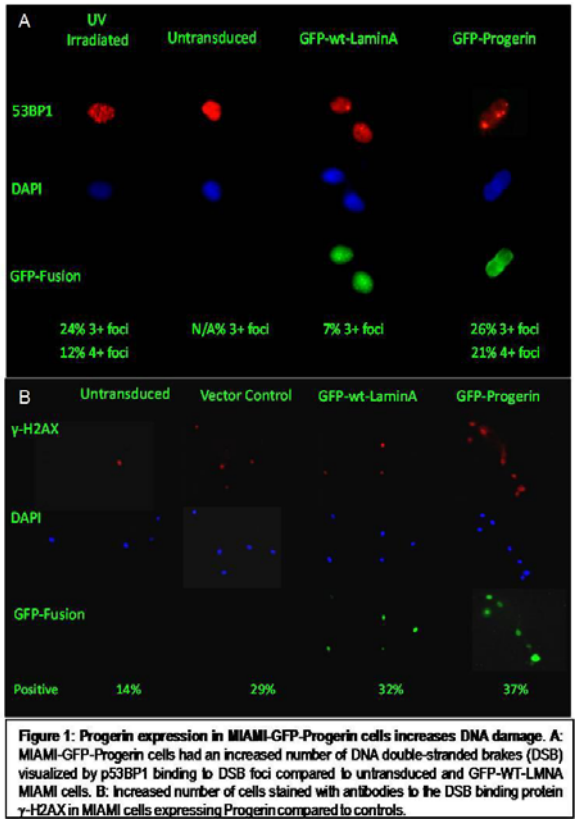

Figure 1: Progerin expression in MIAMI-GFP-Progerin cells increases DNA damage. A: MIAMI-GFP-Progerin cells had an increased number of DNA double-stranded brakes (DSB) visualized by p53BP1 binding to DSB foci compared to untransduced and GFP-WT-LMNA MIAMI cells. **B:** Increased number of cells stained with antibodies to the DSB binding protein γ-H2AX in MIAMI cells expressing Progerin compared to controls.

P012
Improving the ischemic reperfusion injury of small intestine with intra-arterially infusion of adipose-derived mesenchymal stem cells

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Introduction: Mesechymal stem cells (MSCs) as somatic stem cells, are known to have the power to repair damaged tissue and the immunomodulatory effect. Ischemic reperfusion injury in small intestinal transplantation remains to be a major problem because of its destructive effect of intestinal mucosa. Here we demonstrated whether intra-arterially infusion of MSCs improve ischemic reperfusion injury of small intestine and can be done in safely.

Materials and Method: We establish adipose-derived MSCs of Luciferase-Transgenic LEW rat. Wild-type LEW rat was anesthetized and abdominal laparotomy was performed. After ligating the marginal artery of upper ileum and terminal ileum, superior mesenteric artery(SMA) was occluded by vascular clip for 60minutes.During the ischemic period, micro-catheter was inserted through the femoral artery so as to the tip was located near the SMA bifurcation area.Then the clip was removed, MSCs (3x10(6) cells/2mL) was administered from the tip of the catheter. At the end of 3hrs reperfusion period the rats were sacrificed and the middle of the small intestine was removed for the assessment of histological damage, several kinds of gene expression profile and content rate of polyamines. Additionally, macroscopic grading(by using macroscopic grading 0-3, Petrat 2010)was evaluated for 10cm-long segment of the terminal ileum at the same time.
Results: In MSCs administered group, transplanted MSCs were confirmed to exist in the small intestine by IVIS imaging. Ischemic reperfusion injury was improved in histological scoring and macroscopic grading. In the experimental group, the content of polyamines were shown to increase. Similarly, tissue regeneration and restraining apoptosis was suggested by semi-quantitative PCR analyses.
Conclusion: Our results suggest the efficacy of intra-arterial infusion of MSCs in rat small intestine ischemic reperfusion model.

P013
MIAMI cells promote neuronal precursor proliferation and increased neurite complexity of human fetal brain-derived neuroepithelial cells

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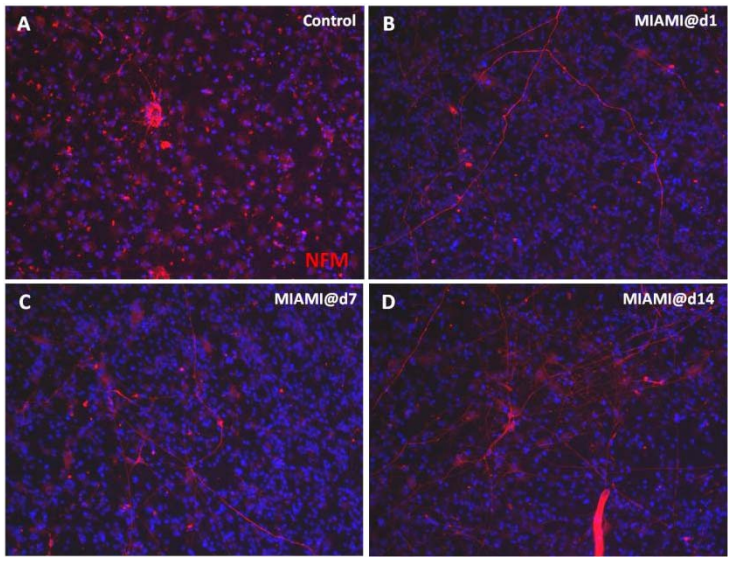
Marrow-Isolated Adult Multilineage-Inducible (MIAMI) cells are a homogeneous subpopulation of immature hMSC, with the ability to differentiate into tissues derived from all 3 germ layers, including neuronal-like cells, and secrete cytokines known to be involved in neuroprotection, cell survival, progenitor cell recruitment and angiogenesis. We tested the effect of MIAMI cells on differentiating neuroepithelial progenitor (NEP) cells in culture, with the future aim of their utilization to prevent neuronal damage and promote repair in injured central or peripheral nervous system.
Human fetal NEP cells were grown for 21 days in the absence (controls) or presence of MIAMI cells. MIAMI-eGFP cells were added to the NEP cell cultures on day 1, day 7, or day 14 and grown until day 21 to determine the effect of MIAMI cells on NPC growth and differentiation. On day 21, cultures were fixed and stained with either βIII-tubulin or neurofilament-M (NFM) and anti-GFP. Cell counts and neurite lengths were measured on βIII-tubulin or NMF positive NEP cells using SigmaScan Pro-software.
βIII-tubulin positive NEP cells co-cultured with MIAMI cells had significant increase in mean neurite length/cell when compared to control for all three incubation times (n/c 60mm vs. 66-69mm). Mean number of neurites per cell was also significantly increased compared to control (n/c 1.4 vs. 1.73-1.9). The total NEP cell counts were 2-fold higher when co-cultured with MIAMI cells. NFM-positive neurites increased 3.5-fold, and between 2-2.4-fold, in length when NPCs were cocultured with MIAMI cells since day 1, or from day 7 or 14 on, respectively.
Data suggest that MIAMI cells impact the neurogenic microenvironment to promote neuronal precursor proliferation and increase neurite complexity in differentiating human NEP populations. These specific neuroprotective and repair properties make MIAMI cells a strong candidate for cell-based therapy in neurodegenerative diseases, cerebral ischemia, and traumatic brain or spinal cord injuries.



P014
Vocal fold lipostructure: preliminary results changing from Coleman to Lipostem technique

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Glottic insufficiency is a common problem in ENT patients, usually as a result of recurrent nerve lesion or of laringeal cancer treatment. The usual surgical treatment relies mainly on Colemans lipostructure technique, which allows the medialization of the lateral laringeal wall thus facilitating glottic continence. The results though, are highly unpredictable because a large part of the implanted fat is often reabsorbed in the first months after surgery and the revitalising effect due to adipose tissue stem cells is often minimal and of late onset.
Trying to improve the predictability and durability of results, our group started to apply the lipostem technique, a method we already used succesfully to perform lipostructure in different areas of the face and neck. The collected fat is inserted, in the operating room, directly in a single use, sterile, closed device where, with minimal manipulation, it is thouroughly washed to eliminate oils and is then filtered to obtain small omogenous fat fragments, easy to inject through fine needles. The fat obtained is rich in adipose stem cells that will be implanted in the receiving tissue. The use of fine needles guarantees minimal surgical traumatism and allows the injection of small amounts of oil free, stem cell rich, fat, thus reducing reabsorption to the minimum and facilitating maximal survival of the stem cells. The procedure is safe as it implants only autologous fat and the water contained in the solution is absorbed within the first hour postoperatively, thus the results are quickly observable.
Already after the first month the revitalizing effect of the implant is clear, and though long term results are still to be avaluated, the preliminary impressions are surely positive and the simplicity of the technique makes it apt for Day Surgery treatments, in the future even in local anesthesia.

P015
Expression of surface and intracellular specific markers by human stem cells derived from Lipostem™-treated adipose tissue

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Adipose tissue is derived from the embryonic mesoderm, and the human adipose tissue derived stem cells (hADSCS) are multipotent stem cells able to differentiate into different cell lineages such as bone, muscle and neural cells. This study is aimed at isolation and in vitro and in vivo characterization of hADSCs obtained from

adipose tissue without enzimatic digestion. Adipose tissue was fragmented by means of Lipostem™. This is a system the allows the fragmentation of adipose tissue into clusters as small as 500μm. The reduced particle size of adipose tissue enriches he content in available stem cells that creep out of the tissue tissue and within 7 days are ready for the first passage. hADSCs are 100% positive to surface markers tipical of mesenchymal stem cells such as CD44, CD73, CD90, CD105, CD146 and CD166. About 50% of these express also CD34 and CD45. The phenotypical expression of hADSCs is sensitive to growth medium and to adhesion specific substrates. For instance these cells express Vimentin, Nestin, GFAP and TUJ1, but the switch to different adhesion substrate can modify such expression. For instance the use of Matrigel™ eliminates the expression of GFAP. Thus in addiction to changes in morphology these stem cells change intracellular phenotype according to medium and surface substrates. All hADSCs espresse the potein O4 typical oligodendrocyte precursors when these cells were grown in presence of Human Neural medium. In conclusion our study shows that hADSCs obtained from adipose tissue by means of Lipostem™ grow nicely in vitro, show typical mesenchyme surface proteins, and modify their phenotype accrding to growth medium and surface substrates.

P016
An improved method for adipose tissue grafting (Lipostem system) in facial aesthetic and orthognatic surgical procedures

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Lipofilling has been used extensively by plastic and maxillofacial surgeons, either alone or to improve the results of other facial surgical procedures. However, previously reported results have been mixed, because of challenges related to uneven and/or early resorbtion of the grafted adipose tissue. We report the results of 168 patients treated since May 2010 using a novel system for enzyme-free adipose tissue preparation. Autologous adipose tissue was obtained from selected subcutaneous areas by manual liposuction. The tissue was processed using the Lipostem device to obtain adipose tissue cell products with a reduced cell cluster diameter size. Importantly, the method allowed for reduction of the oil residues from the supernatant of the final cell products. The final cell products were implanted in facial tissues using significantly smaller diameter cannulas which were substantially less traumatic compared to previously described methods. This novel method for fat transfer in plastic and reconstructive surgery applications appears to be not only faster and more practical, but also produced improved clinical results (3-12 month follow-up) compared to conventional technique of lipofilling. The volumetric correction results appeared to be more stable over time and significant improvement of skin texture was virtually always observed within a few weeks from the surgical procedure. It is conceivable that the observed clinical effect could be mediated by progenitor cell components that are present in the final cell products implanted. It is possible that the improved results could be associated to the smaller size of the cell clusters that not only allowed for a better revascularization and engraftment of the transplanted tissue, but also could have provided a better bioavailability of the adipose tissue stem cells present within the final preparations.

P017
Cell-transplantation therapy of the aggregation-free infusion solution with mesenchymal stem cells

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Stem cells are increasingly seen as potential therapies for organ and tissue failure. Recently, stem cells transplanted two Korean patients, the auto-mesenchymal stem cells (MSCs), died of pulmonary embolism. It is very important to consider the cause of their deaths. One of the major risks of MSCs therapy include pulmonary embolism at the very early phase following treatment. It is important to note that MSCs suspended in saline or culture media easily, leading to cell aggregation in less than 1 hour because of sedimentation. This is related to the risk of embolism-related complications after their intravenous injection. Herein, we developed a transplantable novel cell solution to patients, and we examine the safety of the cell transplantation therapy using the animal models of mouse, rat and swine. We foundthat our novel solution is effective for inhibition of cell-aggregation for rat, swine and human adipose-tissue (AT)-MSCs, maintaining the favorable condition for long time. Furthermore, we found that the

potency of cell suspending conservation into the infusion bag. These results indicate that our cell-suspension solution is safe and effective for cell-transplantation therapy containing of MSCs-, ES cells- and iPS cells-derived functional cells for the patients suffering from several diseases.

P018
Assessment of human adipose tissue derived cell products obtained using a novel non-enzymatic method

Erika Leonardi[1,2], Armando Mendez[1], Giacomo Lanzoni[1,3], Kamalaveni R. Prabakar[1], Alice A. Tomei[1], Luca Inverardi[1], Carlo Tremolada[8], Camillo Ricordi[1,4,5,6,7]

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Adipose tissue (AT) represents a desirable source of stem cells for regenerative medicine approaches: it is accessible in the prospective recipient through minimally invasive procedures, can be harvested in large amounts and multiple times, and it contains several types of stem cells, including cells of pericytic nature that have been shown to give rise to mesenchymal stem cell cultures.
Current methods for the manipulation of AT are based on enzymatic digestion of the lipoaspirate followed by administration to patients after minimal manipulation. Potential drawbacks of traditional enzyme-based methods include the cost associated with the procedure, which requires cGMP grade enzyme solutions, specialized equipment and a significant time for processing. We have assessed a recently developed method where lipoaspirates, obtained by minimally invasive mini-liposuction procedures, were processed by a mechanical non enzymatic apparatus that allows for reduction of adipose cell cluster size, while eliminating oil residues from the final cell product suspension.
Preliminary data suggests that AT can be efficiently reduced in size in a significantly shorter processing time, compared to traditional enzyme based methods, utilizing a fully enclosed, disposable device that doesn't require specialized laboratory equipment or a cGMP facility. Characterization of the processed tissue was performed after digestion and in-vitro culture expansion of plastic-adherent cells. The expanded cell population was composed by cells with a mesenchymal stem cell phenotype (CD44+ CD73+, CD90+, CD105+, CD146+, CD14-, CD19-, CD31-, CD34-, CD45-, HLA-DR-) and was highly enriched in putative progenitors of pericytic nature, displaying an MSC phenotype and CD34 positivity. Cultures appear devoid of contaminating endothelial cells.
This novel non-enzymatic method for human AT processing, which is already in clinical trials of plastic and reconstructive surgery, may also represent a viable alternative to current enzyme-based approaches for additional regenerative medicine applications. These observations warrant further investigations.

P019
Preliminary assessment of a spiral needle to deliver cell products into tissues

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Regenerative medicine using cellular therapies is a rapidly evolving field of investigation. Direct infiltration of tissues with stem cells or specialized cellular products has the potential to enhance tissue repair/regeneration. Adequate 3-D distribution of the cell inoculum in the tissue may also allow for better engraftment and functionality.
We have engineering a novel injection needle with a spiral shape designed for the delivery of cellular products into tissues. The working hypothesis of our approach is that the spiral shape of the needle would distribute the same amount of inoculum in a larger surface area when compared to conventional injection means.
Preliminary assessment of tissue distribution was done using an ex vivo intramuscular site. To this aim, primary human mensenchymal stromal cells were expanded in vitro and pre-loaded with fluorescently labeled MRI nanoparticles (MoldayION Rhodamine B™) containing either 30-50µg of iron per ml. After overnight incubation, labeling of 100% of the cell preparation was confirmed by flow cytometry analysis. To assess distribution into an

intramuscular site, labeled cells were resuspended at a density of either 10x105 or 5x106 and injected manually using spiral or straight needle with comparable inner and outer diameters. Inoculum was uneventful although backflow appeared more pronounced using straight needles. After cell injection, distribution of labeled cells injected in the muscle was evaluated on a 1.5T MRI. Optimal scanning sequences and imaging parameters were determined based on contrast-to-noise ratio and contrast modulation. Preliminary analysis of T2 and T2* weighted sequences indicates distribution in the muscle of MRI contrast (indicating labeled cells) through the tract generated by the needle.
Our preliminary ex vivo data indicates that delivery of cells in target tissues can be achieved using the newly engineered spiral needle. Further studies will address the efficiency of engraftment and function of implanted cells using this device.

P020
In vivo grafting of human Hepatic Stem Cells (hHpSCs) in Hyaluronan hydrogels for use in liver failure therapies

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After acute injury, the liver is able to regenerate rapidly, but severe cellular damage may result in liver failure. Cell transplant therapies can provide an alternative to whole organ transplants, but are limited by inefficient engraftment, poor survival of the cells, and propensity for formation of life-threatening emboli. Transplant success necessitates grafting methods, accomplished by co-embedding HpSCs into/onto biomaterials mimicking the liver's stem cell niche forming grafts, increasing the survival, expansion and regeneration of tissue. This study investigated grafting technologies as strategies for transplantation of hHpSCs and their mesenchymal cell partners into the livers of immunocompromised athymic nude mice. Cells were embedded in a hyalunonan complex, and then grafted into the liver. The extent of humanization of the livers was compared between novel grafting methods versus direct injection of cell suspensions into the liver. Injury models to enhance regeneration mechanisms consisted of treating mice with carbon tetrachloride <24 hrs after cell transplantation.
In vivo luminescent imaging of luciferin-expressing hHpSCs showed that traditional cell injection resulted in cell dispersal throughout the abdomen and evidence of human cells in multiple ectopic sites. By contrast, grafting methods resulted in hHpSCs confined to the livers of the hosts. Human albumin in the host serum showed increased production in injury models due to increased numbers of human cells within the host livers. Histology staining of liver sections for human albumin showed large masses of transplanted cells when grafted in the host liver as opposed to smaller single cell groups evident using traditional methods with transplantation of cell suspensions. Grafting of hepatic progenitors in hyaluronan hydrogels localized cells specifically to the target liver tissue, providing an efficient method for cell transplantation for use in liver regeneration, utilizing GMP materials making grafting technologies rapidly translatable to therapeutic uses in patients.

P021
Autologous skeletal-myoblast-sheet transplantation improved porcine cardiac function without Increasing risk of arrhythmia

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Background: Autologous skeletal myoblast (SkM) injection has been used for repairing infracted myocardium. However, some clinical trials have been plagued by ventricular tachyarrhythmia. This study is investigated the cardiac electrical stability of porcine model after SkM sheet transplantation in infarcted myocardium.
Methods: Male mini-pigs underwent the left anterior descending coronary artery occlusion by a balloon catheter for 2 h, followed by reperfusion. After 4 weeks, 12 SkM sheets were implanted onto infarcted myocardium (Sheet-group n = 6), the same amount of cells were injected into the myocardium (Injection-group n = 5) and sham operations were performed (Sham-group n = 5). At 4 weeks after the transplantation, we assessed systolic function with MDCT, interrogated implantable ECG loop recorders (ILR), and performed programmed ventricular stimulation (PVS). For assessing the inflammatory and injury response in coronary sinus blood samples,

inflammation factors, and high-sensitive CRP and Troponin-I were measured after the transplantation by cytokine array method and ELISA, respectively. For observing inflammation, the tissues were stained immunochemically with anti- macrophage.
Results: Sheet-group improved their cardiac function compared with Injection-group and Sham-group (left ventricular ejection fraction change: 5.0 ± 3.2, -1.5 ± 3.8, -3.5 ± 1.7 % in Sheet, Injection, Sham-groups, respectively). No VF was detected by ILR in all groups, while VT was detected in one pig of Injection-group. No sustained VT was induced in all groups, while VF was induced in one pig of the Sheet-group and 3 pigs of the Injection-group by PVS. Toroponin-I and IL-6 levels tended to increase in Injection-group compared with Sheet and Sham-groups. Although there are no migrating cells into myocardium in Sheet-group, macrophages were observed around injected cells in myocardium in Injection-group.
Conclusions: SkM sheets transplantation in infarcted myocardium improved cardiac function over SkM Injection without increasing arrhythmogenicity. Myocardium inflammation may increase the arrhythmia risk.

P022
Cell surface glycsphingolipid antigens of human embryonic stem cells

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Background: Cell surface carbohydrate antigens, such as AB(O)H and related blood group antigens, are potential strong immune barriers in cell transplantation. To bring treatment using stem cells to the clinic, cell surface antigen expression and chemical structures of these molecules have to be explored. In this project, glycosphingolipid antigens isolated from human embryonic stem cells (hESC) are structurally characterized.
Experimental: Human ESC lines (SA121, SA181) were generated from leftover *in vitro* fertilized embryos. Cells were cultured in mono-layer cultures under serum and feeder-free conditions. Total neutral glycolipid fractions were isolated and structural characterized by thin-layer-chromatography (TLC) detected with chemical reagent and immunostaining using antibodies/lectins. Saccharides liberated by endoceramidase, were structurally characterized by liquid chromatography-mass spectrometry.
Results: Total amounts of neutral glycolipid isolated from each cell line were about 1-2 mg from $1x10^9$cells. Chemical staining of TLC plates revealed several glycolipid components containing one to approximately eight sugar residues. TLC immunostaining identified one single blood group A glycolipid band migrating as a six sugar component in both cell lines most strongly seen in SA181. The anti-Le^yantibody revealed several components with six to approximately 16 sugar residues in both cell lines. Small amounts of Le^x components were detected in SA181 and only trace amounts in SA121. *N*-Acetyllactosamineterminated compounds with 4 and 6 sugar residues were detected with the *erytrina cristagalli* lectin. The initial mass spectrometry study of released oligosacchrides showed, in both cell lines, the presence of several saccharides of which one was tentatively identified as a five sugar blood group H type 1 core chain compound.
Conclusion: Human ESC expresses, a cell line specific, complex pattern of glycolipid antigens including blood group antigens known to be strong immune barrier in allotransplantation.

P023
Galectin expression of human embryonic stem cells and hepatocyte-like cells

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Background: Galectins are a family of carbohydrate binding proteins involved in many physiological and pathophysiological processes including differentiation, and inflammation. In the cell, each galectin directs trafficking of a subset of glycoproteins, which in turn affects cell differentiation and sensitivity to surrounding factors. It is expected that such effects will be important in function of stem cells, but this has not been studied much before. Therefore, this study explores galectin expression in human embryonic stem cells (hESC) and hepatocyte-like cells differentiated from one of the cell lines.
Experimental: Expression of galectin-1, -2, -3 and -4 in hESC line SA002, grown on feeder cells, and hepatocyte-like cells derived from this cell line was analysed by immunohistochemistry with antibodies specific for each

galectin. In addition, hESC lines SA121 and SA181, grown under serum and feeder-free conditions in mono-layer cultures, were studied by FACS. All hESC lines were generated from leftover human *in vitro* fertilized embryos.
Results: In the SA002 cells, no galectin-1 to -4 staining could be identified by immunohistochemistry. In the hepatocyte-like cells derived from the SA002 cells, galectin-2 and -3 were present but galectin-1 and -4 were negative. In adult liver, galectin-2 and -4 showed clearl positive staining, trace amounts of galectin-1 and no galectin-3 staining. In the two hESC lines SA121, SA181, FACS analysis revealed galectin-2 expression in SA181, galectin–4 expression in both cell lines while no staining for galectin-1 and -3 was found.
Conclusion: This study shows that hESC have a specific expression of different galectins that varies between the cell lines as well as at different stages of cell maturity. The specific induction of galectins-2 and -3 in hepatocyte-like cells and absence of galectin-3 in healthy adult liver is interesting.

P024
A high-throughput procedure for improved microencapsulation of Islets

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Purpose: Major factors limiting the success of encapsulated islet transplantation include impaired O_2 and nutrient supply to islets, uncertain stability of microcapsules and the lack of high-throughput encapsulation devices. Large capsules impair oxygen and nutrient diffusion, hence detrimental to islet viability and function; while long duration of encapsulation also affects islet cell viability. This study describes a novel multi-nozzle high-throughput microfluidic device that produces uniform, smaller and mechanically-strong alginate microcapsules with a liquefied-core to enhance diffusion of molecules for encapsulated islets.
Methods: Using an 8-outlet microfluidic device, we optimized the conditions to achieve uniform alginate microcapsules (diameter ~300 um) at high flow rates (1.2 - 2 ml/min) with 1.5 wt% ultrapure-alginate solution containing isolated rat islets. The microcapsules were then perm-selectively coated with poly-L-ornithine (0.1 wt% PLO), and the inner-alginate core of microcapsules was partially-liquefied (hollow) using 55mM sodium citrate. Finally, an outer layer of alginate was applied to obtain alginate-PLO-alginate (APA) microcapsules. These microcapsules were subjected to mechanical stress test while the islets contained in them were tested for viability and function.
Results: Bose-Compression test showed that there was no significant difference in the mechanical strength of hollow vs. non-hollow microcapsules. Islets in microcapsules were imaged (Live-Dead assay, Confocal Microscopy) and viability was quantified with z-stack images (ImageJ, NIH). Islets in hollow-microcapsules had the same %viability (60.4±3.6) as naked (unencapsulated) islets (62.5±1.7), which was significantly higher than islets in non-hollow microcapsules (41.6±1.7, $p<0.05$, n=3). The microcapsules also maintained the full islet mass, while unencapsulated islets began to fragment and decrease in size with culture. Following culture, *in-vitro* glucose-challenge test in both static and perifusion conditions showed better insulin response to high-glucose by islets in hollow-microcapsules compared to unencapsulated islets.
Conclusions: Our data suggest that this novel high-throughput microfluidic device can produce stable APA-microcapsules with viable and functional islets for transplantation.

P025
Human islets encapsulated in alginate sheets survive and function after 8 weeks in the subcutaneous space of rats

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Islet encapsulation in macroscopic polymer devices offers a means to protect transplanted islets from the host response while having the ability to retrieve the graft. This study assessed the function and viability of human islets encapsulated in an alginate sheets, after transplant, in the subcutaneous space of rats.
Human islets were isolated from cadaveric organ donors at UC Irvine using purified collagenase HA and BP Proteasedissociation and continuous Ficoll-UWD purification. After overnight tissue culture, islets were encapsulated in alginate sheets and either transplanted subcutaneously into Lewis rats or maintained in tissue

culture, as a control (37°C/5% CO_2). At 1, 2, 4 and 8 weeks islet sheets were retrieved and assessed for viability using FDA/PI and glucose stimulated insulin release using incubation for 1 hr in low (2.8mM), then high glucose (28mM), then high (28mM) plus 50mm IMBX (3-isobutyl-1-methylxanthine) and then low glucose (2.8mM).
Encapsulated human islets were 95±0.2% viable (mean±SEM) after 1 week in culture. The initial stimulation index (SI, ratio of insulin produced in high over low glucose) was 3.8±0.2 and the maximum secretion (MX, ratio of high glucose + IBMX over high glucose), was 2.0±0.1. At 1-week post transplant the sheet remained intact and viability was 90± 4%. Islet function was maintained with a SI of 3.0±0.5 and MX of 2.1(p=ns, t test). At 2-weeks post transplant, explanted sheets were 85±0.8% viable and maintained glucose responsiveness with a SI of 3.0±0.4, MX of 1.8. At 4 and 8 weeks post transplant, explanted sheets remained both viable (86.0±2.2%, 73.0±1.5%; respectively) and continued glucose responsiveness (SI=1.5±0.5 and MX=1.5±0.1, SI=1.5±0.3 and MX=1.6±0.6; respectively).
These studies show that xenotransplanted human islets, encapsulated in alginate sheets, can be easily retrieved after implantation, quantitatively assessed for viability and metabolicfunction, thus showing that grafts survive and function over an extended period of time.

P026
Dynamic response of encapsulated young porcine islets using a perifusion system

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Xenotransplantation, using porcine islets, offers the potential of treating patients suffering from Type 1 Diabetes. We are currently developing a program of clinical islet transplantation by incorporating a novel method of isolating and maturing young porcine islets followed by encapsulation in small alginate capsules. It is key to demonstrate that encapsulated porcine islets respond to various secretagogues in a dynamic and rapid fashion. It was the aim of this study to examine the dynamic insulin secretion responses of encapsulated porcine islets when placed in a perifusion system.
Islets were isolated from Yorkshire pigs (6-10 kg) and allowed to mature in tissue culture (37°C/5%CO_2) for 7-10 days. Young porcine islets (YP islets) were then encapsulated in ultra pure, high mannuronate sodium alginate using an electrostatic pump generator and allowed to crosslink in 100mM $CaCl_2$. Groups of 40 un-encapsulated (control) and encapsulated YP islets were perifused for 90 minutes at 37°C with buffers supplemented with glucose (2mM, 7mM, 15mM) and with 15mM glucose plus 500mM carbachol. Samples were collected every 2 minutes and insulin levels determined using RIA.
Both un-encapsulated and encapsulated YP islets showed concentration-dependent increases in insulin secretion in response to glucose that were rapid in onset and readily reversible, but encapsulated YP islets demonstrated a blunted insulin response to all stimuli. Encapsulated islets had an approximate 3.5-fold increase in insulin secretion in response to carbachol at 15mM glucose whereas the control islets demonstrated a 4.6-fold increase (Un-encapsulated 15mM glucose platueau: 0.57±0.07pg/islet/min; +500mM CCh: 2.60±0.35, n=4, $P<0.01$; encapsulated 15mM glucose platueau: 0.23±0.03pg/islet/min; +500mM CCh: 0.81±0.12, n=4, $P<0.01$).
This study demonstrates that isolated encapsulated young porcine islets respond to glucose in a dynamic fashion during perifusion, although the amplitude of insulin secretion is reduced. Future directions will focus on in vivo transplantation studies including dose escalation in diabetic animal models.

P027
In vitro maturation of viable islets from partially digested pig pancreas

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Porcine pancreatic islets have been explored as a source of tissue for xenotransplantation. The isolation of islets from mature market weight pigs is costly since the islets often fragment during and after the tissue dissociation and islets recovered from neonatal pigs take time to mature before becoming insulin responsive. The aim of this study was to develop a reproducible and scalable procedure for isolating porcine islets that have high viability and are insulin responsive.
Pancreases from Yorkshire pigs (range 14 to 28 days) were recovered using a rapid surgical procurement (< 5 min). Partial enzymatic digestion of the pancreas was performed using low dose purified collagenase MA/BP protease. Then the tissue was cultured at 37°C and 5% CO_2 for up to 14 days with media changes every 48 hrs. Viability was

assessed using FDA/PI or Newport Green and function was assessed using glucose stimulated insulin release (GSIR) assay.
Islet yield immediately following dissociation was $12.6x10^3$±183 IE (mean±sem) per organ which then increased to $30.6x10^3$±170 and $33.3x10^3$±136 after 5 and 7 days of culture, respectively. At day 7 yields were 6.4 $x10^3$±77 IE/gm tissue (average tissue 5.18±0.1g). Viability was >98±0.03% (FDA/PI) and >90±0.4% (Newport Green) at day 7 of culture. Islet function (GSIR) initially after isolation was 1.3±0.1 (SI, ratio of insulin during high glucose (28mM) stimulation/low glucose (2.8mM)). At day 2, insulin response increased to an SI of 1.9±0.5 which further improved to 2.6±0.2 after 7 days of culture.
This model provides a reproducible and scalable method of isolating and maturing porcine islets in culture. Partial pancreas dissociation, followed by extended culture allows for islets to gently separate from the surrounding exocrine matrix and may be a key factor in this protocol success.

P029

The survival and engraftment of human Schwann cells within the thoracically contused spinal cord of athymic nude rats

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Schwann cell (SC) implantation has been demonstrated to provide anatomical and functional restitution in numerous experimental spinal cord injury (SCI) models, facilitating neuroprotection, axon growth support, remyelination repair and improvements in limb function. In studies employing clinically-relevant contusion SCI models, to date, only rodent SCs have been evaluated. The current work sought to examine the survival, proliferation, tumorgenicity, biodistribution and host responses to exogenous human SCs (hSCs) using a contusive SCI xenotransplant paradigm. For these studies, adult female nude rats received a mild contusive SCI at the thoracic level using the MASCIS impactor. At 4 wk post-SCI a single stereotactic injection of 300,000 hSCs suspended in media was made into the injury site. The hSCs were obtained from the sural nerves of human donors (cadaveric or organ donor), cultured to P2 and used either fresh or from cryopreserved stocks. To enhance the long-term survival of hSCs, animals were further immunosuppressed with anti-asialo GM1 antibody to inhibit natural killer cell activity. Animals were perfused at 3 d, 6 wk or 6 mo for histological and immunohistochemical analysis. Using the human nuclear antigen NuMA to identify implanted hSCs, the number of original hSCs persisting post-implantation was determined to be as high as 38% (3 d), 23% (6 wk) and 5% (6 mo), though significant variation in hSC numbers within the injured spinal cord was observed both among and within donor-specific cohorts. Neuropathology review of spinal cord samples for tumorgenicity revealed no presence of aberrant hSC mitosis. Host responses were evaluated by immunochemistry; including astrogliosis, immune cell activation, scarring and axon growth and myelination within the implant and related to numbers of persisting hSCs. These investigations comprise important pre-clinical safety and toxicity data for the support of an IND to the FDA for Phase 1 safety trials of autologous SCs in human SCI.
Source of Funding: Dept. of Defense Congressionally Directed Medical Research Programs, The Miami Project to Cure Paralysis.

P030

In Vivo study of humoral/cellular immune responses to osteoblastic differentiated Adipose Mesenchymal Stem Cell xenografts: Galactosyl Knock-Out vs. Gal positive pigs

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Objectives: In bone tissue reconstruction, pig Adipose Mesenchymal Stem Cells (AMSCs) could be proposed to constitute a "Ready to Use" cell bank. However, pig AMSCs remain associated with the expression of the Galactosyl epitope inducing an hyper-acute xenograft rejection. Therefore, we investigated *in vivo* the potential of α-1,3-Galactosyltransferase Knock-Out (Gal-T-KO) pig osteogenic differentiated AMSCs in an immuno-competent rodent model, without any immunosuppression.
Methods: Osteoblastic differentiated AMSCs from Gal+ (Landrace)/Gal-T-KO pigs were implanted in 30 adult Wistar rats using a spinal fusion model. The Galacosyl expression was assessed by anti-Gal Isolectin

immunohistochemistry, for non-/osteoblastic differentiated AMSCs. Three experimental groups were investigated: Group I=sham animal; Group II=Gal+ osteoblastic differentiated AMSCs and Group III=Gal-T-KO osteoblastic differentiated AMSCs. The humoral response (anti-pig IgM/IgG antibodies and Gal specificity) was assessed on sera samples at day 0/7/28 post-transplantation by Flow Cytometry and ELISA, respectively. Cellular and humoral responses were assessed by immunohistochemistry for CD3/CD68/C3d at day 7 and 28 post-implantation.
Results: Although a significant reduction of Gal expression was found between non-/osteoblastic differentiated AMSCs (from Landrace pig) at passage 4 (by -33.5%,$p<0.005$), this epitope remains expressed by 66.5%of the cellular graft. In contrast, no Gal expression was found for non-/osteogenic AMSCs from Gal-T-KO donor.
Although no significant difference was observed for anti-pig IgM sera levels between groups, a significantly lower anti-pig IgG level was found for Group III at day 28, without specificity for the Gal epitope (MFI: 513±149 vs. 323±112 for Gal+ and Gal-T-KO, repectively, $p<0.05$). A significant reduction of CD3/CD68 infiltration was found, in Group III (Gal-T-KO), at day 7 ($p<0.05$) whereas no difference was found at day 28 post-transplantation.
Conclusions: Our preliminary results show that a Gal-T-KO differentiated AMSCs xenograft induced a lower humoral response and delayed cellular infiltration in comparison to Gal+ AMSCs in a "pig to rodent" model.

P032
How to write a functional SOP

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A step by step outline for staff to follow that guarantees the same outcome independent of the operator or the key personnel responsible for maintaining and completing said task. All procedures can begin with an introduction, so the responsible staff has a basic idea of what they are going to be doing. It can include some historical background or just the basic idea of said procedure. Many times the procedures are novel and there is no history involved in these cases, a basic explanation can suffice. It is a good idea at this time to include definitions of all the terms related to a procedure (i.e. SOP). List equipment, reagents and materials to be used and referred to in the procedure. Consider describing limitations or special considerations if any. Proceed to describe the procedure: describe what needs to be reflected in the Header and footer information on each page. Add an effective date- original version date-number of revisions. List any worksheets or attachments. The title should be descriptive and short of the procedure. Detail the purpose, responsibility and scope of this procedure. Step by step in a clear, precise manner, describe the content of the procedure, user friendly and understandable. Follow this section with notes and interpretation which include acceptance criteria, results, end points and any further actions needed. All sop's need some quality control (annual revisions, maintenance of equipment, etc) and record review (how frequent and by whom), record retention (how long must we keep our records), references, approval page (usually signed by the author and other staff members including the responsible heads. And last to make life a little easier you can include a review and revision page where on yearly bases the reviewer can sign.

P033
Regulatory, economic, academic, legal and other impediments to innovation and to the development of novel cell based therapies

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Annual health care costs of in the USA are now $2.5 Trillion (>17.5% of the GDP), vs. $250 Billion in 1980. Dramatically, while this economically unsustainable situation unfolds, an increasing tide of impediments and challenges to innovation are blocking the development of cures. R&D investments are targeting treatment rather than cure and eradication of chronic disease conditions, which represent the market base for newly developed proprietary molecules or technological improvements. However, very little is invested in prevention and cure focused research. Regulatory impediments to innovation and new treatments are now raising the cost and time required to develop any new strategy to a level which is unsustainable by any physician/scientist, academic laboratory or even small biotech industry, with $52 Billion of Pharma R&D spent in 2008 in the US alone to produce less than 20 new drugs approved. Regulatory risk avoidance philosophy and performance indicators are not balanced by a risk analysis of the cost of paralysis to innovation. Academic impediments include performance indicators for promotion and tenure that prioritize indirect cost recovery to the institutions rather than faculty contributions towards the definition of a cure or eradication/prevention of a disease condition. Without entering the details of religious and political impediments, the increasingly litigious legal system imposes an additional set of

challenges, as institutions and hospitals are becoming so risk adverse for fear of malpractice lawsuits, that multiple deterrents are set against any academic physician-scientist who would actually dare to attempt treating a patient based on the best of his/her knowledge, outside the limits of evidence-based medicine. The net result is an overall neuronal network of impediments that would have blocked most major medical breakthroughs of the last century. Practical alternatives and solutions to the current system paralysis will be discussed.

P034
Cure focus research alliance: inception and progress report

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The Cure Focus Research Alliance (Alliance) is a recently established international not-for-profit, collegial association of scientists, physicians, surgeons, other professionals or individuals, who share the vision and primary objective to develop effective strategies for the cure and eventual eradication of disease conditions now afflicting humankind, and to do so in the fastest, most efficient and safest ways possible. To achieve its primary objective, the Alliance will further and catalyze collaborative research and translational clinical work, including but not limited to, the development and effective application of initiatives and technology aimed at the elimination of geographical barriers to collaborative scientific and research efforts. The Alliance will also promote strategies to alleviate and resolve the challenges imposed by institutional, regulatory, political and philosophical barriers to collaborative translational research efforts towards achievement of the Alliance's primary objective. The Alliance will participate and assist requesting academic centers, institutions, companies, funding agencies and individual scientists in the selection and coordination of the most appropriate, timely and cost-effective strategies for cure-focused research. In this direction, the Alliance will provide support, from the definition of the most effective pre-clinical strategies, to translational efforts towards clinical implementation of strategies for the cure and eradication of disease conditions, including pilot clinical trial design and implementation, identification of the most appropriate geographic locations for execution of the proposed pre-clinical or clinical trials, selection of qualified expert teams, validation and registration trials to eventually bring the novel therapeutic or preventive strategies to all patients who could benefit from them. Membership in the Alliance is by invitation from its Board, or by application and review process. The Alliance currently comprises over 100 distinguished scientists, physicians, surgeons, professionals, business and founding agency leaders, as well as committed individuals and supporters from the film and media industry and from the performing and visual arts.

P035
Sustained expression of insulin by immune-privileged Sertoli cells

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Sertoli cells are immune-privileged cells that exhibit long-term survival after allo- or xeno-transplantation, suggesting that they can be used as a potential vehicle for gene therapy. In a previous study, neonatal porcine Sertoli cells transduced with a recombinant adenoviral vector containing furin-modified human proinsulin cDNA were transplanted into diabetic SCID mice, resulting in a short-term decrease in blood-glucose levels. Immunohistochemical analysis of the grafts indicated that insulin expression was also transient and that insulin loss corresponded with the rise in blood-glucose levels. The adenoviral vector used is ideal for efficient high expression of the gene of interest; however, it does not integrate into the host genome, which could explain the loss of insulin expression. Thus, the goal of our current study was to use a lentiviral vector to achieve long-term, stable expression of insulin in Sertoli cells (LVhInsSC). Using a mouse Sertoli cell line as a model, we transduced these cells with a recombinant lentiviral vector containing furin-modified human proinsulin cDNA to create LVhInsSC. *In vitro* expression of insulin by the LVhInsSC was observed for a period of at least 11 months, as verified by the use of RT-PCR and immunohistochemistry to detect insulin mRNA and protein, respectively. Six million LVhInsSC were transplanted as allografts into diabetic BALB/c mice to determine cell survival and insulin expression *in vivo*. Immunohistochemical staining for large-T antigen (a marker for the Sertoli cell line) showed 100% (2/2) graft survival at 20 days and 75% (3/4) graft survival at 50 days post-transplantation. Insulin mRNA expression was detectable via RT-PCR at 20 (2/2) and 50 (3/4) days post-transplantation; however, insulin protein was not detected by immunohistochemistry at any time point. Consistently, there was no significant change in blood glucose levels. This suggests that Sertoli cells can be altered to produce low levels of insulin for a sustained period of time and

continue to survive as allografts after genetic manipulation. Now, that we have achieved long-term production of insulin by Sertoli cells, our future studies will be aimed toward increasing the insulin secretion to levels high enough to normalize blood glucose levels long-term in diabetic mice.

P036
Human stem cell recombineering

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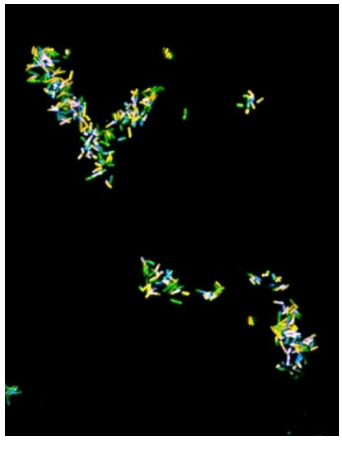
Human stem cell recombineering using viral proteins to catalyze homologous recombination could transform medicine. Bacterial recombineering catalyzes in vivo homologous recombination by using a complex of two bacteriophage proteins: an exonuclease that processes linear DNA to create single-stranded DNA (ssDNA) ends and a synaptase that binds to ssDNA and catalyzes homologous DNA recombination with efficiencies approaching 50%. Mammalian recombineering using bacteriophage recombinases is much less efficient, limiting its utility for producing disease models and gene therapy. We hypothesize that recombineering is host-specific because viral recombinases co-evolve with host proteins. Previously, we discovered related recombinases in human viruses and showed that one such complex catalyzes recombination in vitro. We predict that the human virus recombinase will be specific to human cells while the bacteriophage λ recombinase will be specific to *E. coli*.
To create a quantitative assay for ssDNA recombineering, we created a series of green fluorescent protein (GFP) variants that shift the fluorescence spectrum by changing amino acids in or nearby the chromophore region. We changed GFP to CFP, BFP, YFP and vice versa via ssDNA recombineering in bacteria and created fluorescent lentiviral reporter vectors for human cells. We used the fluorescent reports to evaluate aspects of the design/protocol that drastically affect recombineering efficiency in bacteria. We found them to be: outgrowth time, targeted strand bias, DNA mismatch repair and transformation efficiency.
In order to evaluate recombineering in mammalian cells, we transduced human marrow-isolated adult multilineage inducible (MIAMI) stem cells with lentiviral vectors to introduce recombineering target genes (CFP, BFP, YFP and GFP). We determined conditions that yield 100% nuclear transfection efficiency with a model recombineering substrate oligonucleotide with no apparent harm to MIAMI cells. We also determined that the human viral recombinase is properly localized in MIAMI cells and are currently testing whether recombineering is host specific.



P037
Cell surface antigen expression of human embryonic stem cells

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Background: A prerequisite for use of human embryonic stem cells (hESC) in regenerative medicine is that the grafted cells are not rejected by the recipient immune system. Therefore, a detailed knowledge regarding hESC surface antigens as well as their interaction with the human humoral and cellular immune systems is needed. This project explores the antigen expression of two different hESC lines using FACS, immunohistochemistry (IH) and carbohydrate biochemical techniques.

Experimental: Human ESC lines (SA121, SA181) generated from leftover *in vitro* fertilized embryos were cultured in mono-layer cultures under serum and feeder-free conditions. Cells were analysed by FACS, IH after paraformaldehyde fixation and glycolipid fractions were isolated and characterized by thin-layer chromatography (TLC) immunostaining.
Results: Both cell lines were positive for blood group A antigens by FACS and IH (both intracellular and at the cell surface). The blood group A glycosyltransferase gene was identified in both cell lines by PCR. Regarding other carbohydrate antigens, FACS analysis revealed that both cell lines expressed Le^yantigens, SA181 expressed Le^x(CD15) while both cell lines were negative for blood group B, Le^a/ $Le^b$and sialyl-Le^xantigen. TLC immunostaining of glycolipid fractions revealed large amounts of Le^ycomponents in both cell lines. A single blood group A component was present in SA181 but only weakly seen in SA121. Le^xglycolipids were present in SA181 but almost absent in SA121. Furthermore, FACS analysis revealed HLA-ABC on both cell types. HLA-DR was strongly expressed on SA121 but weakly on SA181. Both cell types were positive for CD34, CD133, CD54/ICAM and negative for CD80, CD86, CD152 and ICOS.
Conclusion: Human ESC express HLA protein antigens as well as blood group ABO, $Le^y$and Le^xcarbohydrate antigens. These antigens may be of importance when these cells, or cells derived there from, are grafted to immune competent recipients.

P038
Studying the infiltration kinetics and movement dynamics of macrophages in pancreatic islet grafts in vivo

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Macrophages are among the first and earliest immune cells to infiltrate pancreatic islets after transplantation. Macrophages participate in mediating an inflammatory milieu by locally producing cytokines and chemokines that result in T-lymphocyte recruitment to the target tissue; while syngeneic tissue is typically preserved, allogeneic tissue is ultimately rejected. In pancreatic islet transplantation, cytotoxic T-lymphocytes play a primary role in graft damage, however, oxidative stress mediated by the activation of infiltrating macrophages has been shown to play a part in allorejection. Here, we investigated the role of infiltrating macrophages and T-lymphocytes in islet allograft rejection by studying their infiltration kinetics and movement dynamics in the living organism. We performed noninvasive *in vivo* imaging of recipients' GFP-expressing macrophages or T-lymphocytes as they infiltrated either syngeneic or allogeneic islets transplanted into the anterior chamber of the mouse eye. Intraocular transplantation enabled longitudinal studies in the same islet grafts revealing the kinetics of islet infiltration by immune cells and their movement dynamics within the islet grafts. While macrophage infiltration was observed in both syngeneic and allogeneic islet grafts, only allogeneic islets were rejected with progressive accumulation of T-lymphocytes. Longitudinal imaging revealed early and sustained infiltration of islet grafts by macrophages. While the number of infiltrating macrophages within syngeneic grafts remained constant throughout the follow up period (50 days), allogeneic islets showed noticeably higher macrophage infiltration, which significantly increased during the rejection phase. Also, the macrophage infiltration kinetics was slower than that of effector T-lymphocytes. These results suggest that macrophages may play different roles during the early post-transplant period and later during the rejection phase. Noninvasive imaging and the intraocular transplant model used in our studies are invaluable tools toward a better understanding of the role of macrophages in islet transplantation.

P039
Optimization of the in vitro expansion of the human T regulatory cells for the purpose of the clinical co-transplantation

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Objective: Tregulatory cells (Tregs) have been recently utilized as therapeutic modality in a systemic adoptive transfer in order to control excessive immuno-reaction in graft versus host disease and may also be applied for immunoprotection of transplanted cells or organs. Although, technique for *in vitro* Tregs expansion has been already

described, obtaining sufficient yield and quality of the cells remains a challenge. In current study, we tested novel culture media for the purpose of improved yield and purity of expanded Tregs.
Methods: Peripheral blood mononuclear cells were isolated from buffy coats obtained from volunteer blood donors by Ficoll/Uropoline gradient centrifugation. Then, negative immunomagnetic sorting was applied in order to achieve CD4+ T cells (96–99% purity). Subsequently, CD4+ T cells will stained with the cocktail of monoclonal antibodies and sorted with FACS sorter to the following phenotype of Tregs: CD3(+) CD4(+) CD25(high) CD127(−) doublet(−) lineage(−) dead(−). During the *in vitro* expansion Tregs were cultured for extended period of time- 3 weeks in RPMI 1640 or cGMP grade medium supplemented with 10% fetal calf serum in high concentration of interleukin 2 and anti-CD3/anti-CD28 beads in 1:2 ratio. The quality check for Tregs consisted of FoxP3 staining, phenotypic features and functional assay- mixed lymphocyte reactions read out as IFN-gamma ELISPOT.
Results: After 2 weeks of *in vitro* expansion in cGMP media, 80% of cells were still expressing Tregs phenotype including FoxP3, whereas only 40% of cells cultured in RPMI. After additional week, the same high percentage of Tregs remained in the cGMP culture, whereas it declined to only 10% in RPMI. The yield of the Tregs was 20-fold higher in cGMP media comparing to RPMI, with proper function confirmed by IFN-gamma secretion inhibition detected in ELISPOT.
Conclusion: cGMP media allows obtaining higher yield of functional Tregs during the extended 3-week culture comparing to RPMI media. Such improvement in Tregs *in vitro* expansion allows to obtain clinically significant numbers of Tregs from limited amount of blood from the patient.

P040
Long-term treatment of a healthy nonhuman primate with steroid-free immune suppression (SFIS): reversible metabolic efects

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SFIS allows for extended pancreatic islet allograft survival in some patients with type 1 diabetes but has also been shown to induce marked insulin resistance and beta-cell toxicity in normal rodents. We evaluated the effect of long-term SFIS in a healthy cynomolgus macaque (Macaca fascicularis). SFIS (started on day 0) consisted of 0.025-0.050 mg/kg IM rapamycin BID and 0.02 mg/kg IM FK506 QD to achieve trough levels of 12-10 and 4-6 ng/ml, respectively; 5 doses of 1 mg/kg IV dacluzimab were given every other week, starting on day 1. After 344 days, SFIS was discontinued for almost 7 months (205 days) and restarted on day 550 for approximately 14 months. Comparison of metabolic parameters before and after the first SFIS long-term treatment showed a 25% increase in average fasting blood glucose (FBG, 45.8 ± 5.9 vs 57.4 ± 11.2 mg/dl); 36% increase in insulin levels (21.0 vs 28.5 μU/ml); 28% decrease in fasting c-peptide levels (4.00 vs 2.88 ng/ml) and 12% increase in %A1C (4.0 vs 3.5%) with no change in body weight (3.82 vs 3.70 kg). Following discontinuation of SFIS, FBG, A1C and fasting c-peptide reverted to levels similar to those prior to SFIS (49.8 ± 5.9 mg/dl, 3.6%, and 4.11 ± 1.12 ng/ml, respectively); with a 24% increase in body weight (4.60 ± 0.33 kg). Changes following SFIS from days 550 – 954 were similar to those observed after the first series of treatments, i.e., glycemic destabilization, with an increase in FBG and A1C, decrease in fasting c-peptide levels and body weight. A similar response pattern to SFIS was observed for some markers of liver function: total bilirubin, GGT and ALP. These deleterious effects of SFIS on glucose control in a healthy animal suggest this treatment may impair islet allograft function in transplanted type 1 diabetic patients.

P041
Down-regulated Txn1 by MPA treatment induces ROS-mediated apoptosis in insulin-producing cell

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Introduction: Mycophenolic acid (MPA) is one of many effective immunosuppressive drugs. However, MPA may induce cellular toxicity and impair cellular function in β-cells. The mechanisms underlying cell death following MPA treatment have not been fullyexplored. To address this issue we have used diverse technologies including illumina-microarray to examine which genes are regulated in a time-dependent manner during pancreatic β-cell

death, following MPA treatment. Critical functions of genes closely related with apoptosis after MPA treatment were thoroughly investigated.
Methods: Pancreatic β-cell line, INS-1E cell, was treated with MPA for 12, 24 and 36hrs. Microarray was performed according to the Macrogen rat BeadChip technical manual using Illumina RatRaf-12 Expression BeadChip. The peroxide-sensitive fluorescent probe 2, 7-dichlorodihydrofluorescein diacetate (DCF-DA) was used to assess the generation of intracellular reactive oxygen species (ROS). Functional screening was determined by using small interference RNA (siRNA)-mediated knockdown and over-expression of *txn1* gene in INS-1E cell line.
Results: MPA significantly increased cell death through Caspase-3 and p-JNK activation. We found that thousands of genes, especially *txn1,* were significantly altered during MPA-induced apoptosis. It was also observed that ROS levels were increased by MPA treatment. Over-expression of *txn1* increased cell viability and decreased activation of p-JNK and Caspase-3 after MPA treatment. However, knockdown of *txn1* by siRNA increased MPA-induced cell death and p-JNK and Caspase-3 activation. Moreover, N-acetyl-cystein (NAC) co-treatment increased cell viability in MPA-induced apoptosis.
Conclusion: MPA significantly induces apoptosis in insulin-secreting cells via down-regulation of *txn1*. Furthermore, reduced *txn1* expression by MPA treatment is related with increased level of ROS. We suggest that controlling the down-regulated *txn1* and ROS-mediated islet apoptosis by MPA is critical for successful islet transplantation.

P042
Effects of systemic immunosuppression on islet engraftment and function in syngeneic and allogeneic murine models of intrahepatic islet transplantation

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Systemic immunosuppression (IS) in islet transplantation (Tx) is a double-edged sword. On the one-hand, it can prevent alloreaction; on the other hand it can prevent engraftment and impair islet survival. The aim of this study was to identify the IS platform with the best balance between these two opposite effects.Methods: To study impact on engraftment and islet survival diabetic C57BL/6 mice were transplanted with autologous 350 intrahepatic islet and treated once-daily with RAPA (0.1-0.5-1 mg/kg ip), FK506 (0.1-0.5-1 mg/kg ip), MMF (60-120-300 mg/kg oral) or vehicle for 14 days. Islet function was evaluated by measuring not-fasting glycaemia and by performing an IVGTT on days 15 and 30 post-Tx. The lower dose of IS that did not affect engraftment was tested for preventing rejection in full mismatch allogenic Tx.Results: In autologous model a significant impairment in the ability to revert diabetes was evident with RAPA >0.5 mg/Kg, FK506 >0.5 mg/Kg and MMF >120 mg/kg. Graft dysfunction was irreversible after IS withdrawal (post-transplant day 15–40). Concordantly IVGTT was significantly impaired both during IS (day 15) and after IS washout (day 30). In allogenic model diabetic mice were treated with RAPA (0.1mg/kg) and/or FK506 (0.1mg/kg) + anti-IL2R mAb (100 µg) or, alternatively, with MMF (60mg/kg) and/or FK506 (0.1mg/kg). Animal treated with RAPA and/or FK506 rejected their grafts at the same time of control animal even if anti-IL2R mAb was added. On the other hand, The combination of MMF+FK506 led to significantly prolonged timing of rejection: the median survival time was 9 (3-14) and 3 (1-4) days respectively for MMF+FK506 and control treated mice (both treatment n=11; $p<0.01$).Conclusions: IS showed profound dose dependent deleterious effects on islet cell engraftment and survival. MMF/FK506 combination shows the best balance with less toxicity and more efficacy in controlling graft rejection

P043
Cellular function of RhoGDI- mediates the distinct cycling of RhoA, Rac1 to regulate pancreatic beta cell death by MPA

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Introduction: Mycophenolic acid (MPA) is an immunosuppressive agent widely used in clinical therapy, including pancreas and islet transplantation. However, potential cytotoxic effect of this drug on islet cells have been observed in vitro, including impediment of insulin secretion and reduction of the DNA content of rat islets. Previously, we showed that MPA induces significant apoptosis in an insulin-secreting cell, through RhoGDI-α down regulation

linked with an increase in JNK expression. In this study, we investigated the protein directly associated with RhoGDI-α during MPA-induced apoptosis in the insulin-secreting cell line, INS-1E.
Methods: INS-1E cells, pancreatic β-cell line, were treated with MPA for 12hr, 24hr and 36hr. Microarray was performed according to the Macrogen rat BeadChip technical manual used by Illumina RatRaf-12 Expression BeadChip. Functional screening was determined by using small interference RNA (siRNA)-mediated knockdown and over-expression of *RhoGDI-α* gene in INS-1E cell line. Immune-precipitation was examined to test the physical interaction between RhoGDI-α and Rac1 protein. RhoGDI-α related protein was found by using the yeast two hybrid assay.
Results: We found that thousands of genes were altered during MPA-induced apoptosis. Among them RhoGDI-α gene expression pattern was significantly decreased.RhoGDI-α over-expression significantly suppressed Rac1 expression, and Rac1 silencing with siRNA reduced MPA-induced cell death and altered expression of MKK4/7, p-JNK and cleaved caspase-3. Furthermore, Using the yeast two hybrid assay and immune-precipitation, we have found that RhoA and Rac1 correlated closely with RhoGDI-α.
Conclusion: MPA induces down-expression of RhoGDI-α, which is involved with small GTPase, RhoA, Rac1. And then, down-stream pathway of Rac1 activates JNK signaling pathway. Therefore, this novel pathway induced cellular apoptosis in transplanted islet cells after MPA treatment. The exact understanding of RhoGDI-α/RhoA,Rac1/JNK pathway could be helpful in improving the results of pancreatic islet transplantation.

P044
Polyclonal serum immunoglobulin M (IgM) therapy: Its role in preventing the onset of type 1 diabetes and recurrence of the disease in islet transplantation

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Objective: The objective is to investigate if administration of purified polyclonal serum IgM therapy can prevent the onset of autoimmune type 1 diabetes (T1D) as well as the recurrence of the disease following islet transplantation.
Methods: C57Bl6 mouse polyclonal IgM was purified using Sephacryl S-300 HR column chromatography. Eight weeks old NOD litter-mates were distributed into saline-injected control mice (n=20) group and IgM-injected test mice (n=13) group. Test mice received purified serum IgM (100ug/100ul PBS; IP) initially, followed by 50ug/100ul biweekly maintenance dose. Control mice received saline.
Results: Preliminary results indicated that while 80% of the control mice became diabetic, none of the mice in the IgM-treated test group became diabetic over the same time period (t-test $p<0.001$). Discontinuing IgM therapy resulted in the appearance of T1D in only three mice at 3 months time-point (experiment in progress). Examination of aldedhyde fuchsin-stained pancreas sections indicated severe insulitis as well as severe periductal and perivascular inflammation in islets from the control diabetic mice. In contrast, normal appearance or mild early stage insulitis is observed in the IgM-treated mice islets accompanied by some periductular and perivascular inflammation. Also, incubation of human islets in the presence of 2-8ug human IgM in vitro did not affect insulin secretion in response to glucose challenge when compared to controls as determined by glucose stimulated insulin secretion assay.
Conclusions: Purified polyclonal serum IgM therapy has tremendous therapeutic potential in the prevention of the onset of autoimmune T1D and may prove a beneficial intervention in preventing recurrence of the disease following islet transplantation.

P045
A summary of 12 autologous-islet transplant isolations at the University of Virginia

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Objective: The objective is to present islet isolation data from a group of 12 patients qualified for autologous pancreatic islet transplants following chronic pancreatitis at the University of Virginia.
Methods: Patients with chronic pancreatitis were referred to the UVa surgical team for autologous islet transplant. The pancreas was surgically removed and sent to the GMP pancreatic islet processing facility. The islets were isolated using the Ricordi method, purified using Biocoll gradient and loaded into a sterile infusion bag with transplant media and heparin. Adjustments were made to enhance islet yield in chronic pancreatitis patients by

increasing the neutral protease concentrationProlonged enzymatic organ inflation Cut the fibrotic organ into smaller pieces.
Results: 12 of 13 preparations were transplanted. Patients ranged from ages 15 to 62. The average organ weight from pancreatitis patients was 43g of hard tissue, digestion time averaged 25 minutes, viability 90%. The number of islet equivalents transplanted ranged from 52,000-357,000 but was independent of the original pancreas weight. Purity of autologous transplants averaged 30%. Age matched normal organ donor data collected in our facility noted an average pancreas weight of 84 grams of soft tissue, an average of 350,000 islet equivalents, 95% viability and 50% purity. Clearly, the condition of chronic pancreatitis affects the success of islets isolation.
Summary: 6 of 12 of patients receiving autologous islet transplants required no insulin after transplant. Success rates varied between the different enzymes used. 2 patients with pancreatitis caused by bacteremia resulted in contaminated islet preparations and higher insulin requirements after transplant. 2 patients were diagnosed with type 2 diabetes prior to islet transplant. 1 remains insulin free although she requires 1000mg of metformin per day.

P046
Modulation of the islet transplant microenvironment via hepatic ischemic preconditioning

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The acute loss of functional islet mass following intrahepatic infusion affects both engraftment and long-term outcome after transplantation. Hampering the induction of early inflammation may be of assistance in improving the success rate of intrahepatic islet transplantation using lower islet numbers.
The beneficial impact of ischemic preconditioning (IPC) of organs, including the liver, prior to a subsequent exposure to noxious stimuli has been recognized both in experimental models and clinical settings.
Working hypothesis of the present study is that hepatic IPC may contribute in reducing inflammation generated upon islet embolization in the portal system, therefore leading to improved engraftment. To test this hypothesis, we have performed intrahepatic syngeneic islet transplantation in chemically-diabetic Lewis rats exposed or not to 10-min warm ischemia achieved by applying vascular clamp on hepatic artery and portal vein, followed by 10-min reperfusion before islet transplantation via portal vein injection.
Transplantation of a suboptimal mass of syngeneic islets resulted in primary nonfunction in control recipients (n=6), while reversal of diabetes was observed in 43% of IPC-treated recipients (n=7). To evaluate the impact of syngeneic and allogeneic islets on inflammation generated upon intra-hepatic islet transplantation, we assessed cytokines and chemokines panels (Luminex) on serum samples (collected at baseline, 3- and 6-hrs post-transplantation) as well as apoptosis and inflammation gene expression panels (GeneArray) on liver biopsies. Significant differences in marker expression were identified when comparing samples obtained from recipients of syngeneic and allogeneic indicating a more profound inflammation response in the latter. Modulation of expression of several markers in both syngeneic and allogeneic recipients appeared modified by IPC.
Our data suggests that intrahepatic islet transplantation results in the activation of pro-inflammatory and pro-apoptotic pathways that are more pronounced in MHC mismatched combinations. The use of IPC may contribute to modulating the activation of inflammation resulting in improved islet engraftment.

P047
Assessment of the effect of immunosuppressive drugs on engraftment and function of human islet into immunodeficient mice

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Dissecting the impact of immunosuppression on islet potency in the clinical setting is cumbersome, since confounding variables affecting function (including insulin resistance, autoimmunity, amongst others) may interfere with the analysis. Rodent models of islet transplantation have been invaluable to assess the potency of human islets and to assess the effects of interventions on the islets or the recipients on graft performance.
To evaluate the impact of immunosuppressive drugs on human islet engraftment and function, athymic nu/nu (nude) mice induced diabetic by streptozotocin received a standardized mass of human islets obtained from the same cellular preparation under the kidney capsule either 'full' (2,000 IEQ) or 'suboptimal' (1,000 IEQ).
Recipients of full islet mass showed comparable outcomes both in terms of glycemic control and diabetes reversal when comparing untreated controls to animals treated with sirolimus and tacrolimus alone or in combination. When evaluating recipients of suboptimal islet mass, impaired glycemic control was observed in most of the animals, with stabilization of glycemic control being achieved in the group receiving sirolimus treatment alone. Animals in all groups showed normal fasting glycemic values despite lack of normalization of nonfasting glycemia values during the >30-days follow up. Intraperitoneal glucose tolerance test performed in recipients of suboptimal grafts with normal fasting glycemic values showed that animals treated with sirolimus alone had glucose clearance profile similar to that of controls, while treatment with tacrolimus alone or in combination with sirolimus resulted in slower glucose disposal rates than controls. These preliminary observations suggest that tacrolimus alone or in combination with sirolimus may result in impaired islet graft function in this model.
Using standardized islet mass and comparing the very same human islet preparation allows performing meaningful comparisons on the impact of treatments on human islet performance in vivo.
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P048
Vildagliptin administration protects islet cells against hydrogen peroxide induced injury

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Introduction: Isletcell transplantation promises cure of diabetes mellitus type 1.However, islet cells should be of good quality for better results of transplantation. Oxidative stress is one of the factors which have roles in mechanism of islet cell apoptosis. Vildagliptin increases insulin secretion and inhibits glucagon secretion via increasing the level of active GLP-1. In this study, we investigated effects of vildagliptin on islet cell viability and oxidative stress parameters.
Method: Isletcellswereisolated after rats were given vildagliptin at a dose of 100 mg/kg/day for four days respectively. Islets were kept in medium containing 0 µl H_2O_2 or 300 µl H_2O_2at +4 ° C for 15 minutes. The islets cells viability was examined with fluorescein diacetate (FDA) and propidium iodide (PI) mixture by using a fluorescence microscope. The rest of cells were stored for lipid peroxidation, protein oxidation and superoxide dismutase (SOD) activity assessment.
Results: The cell viability was decreased significantly in control and vildagliptin groups with 300 µl H_2O_2 (P<0,05). The viability of islet cells was decreased in control and vildagliptin groups from 90,88±4,66 % to 81,09±7,67 % and 92,6±2,19 % to 88,53±2,16 % respectively. However, viability of islet cells invildagliptin group was significantly higher than control group in 300 µl H_2O_2 (P<0,05). Hydrogen peroxideinduced lipid peroxidation and protein oxidation on islets in both groups (P<0,05). However, vildagliptin treated group was significantly decreased lipid peroxidation and protein oxidation in comparison with control group in 300 µl H_2O_2 medium (P<0,05). The SOD activities of both groups in both medium were not significantly different (P>0.05).
Conclusion: These results have suggested that, 100 mg/kg/day orally administered vildagliptin might be protective for islets against H_2O_2 induced lipid peroxidation and protein oxidation. In order to prevent the islet cells from the damage that may occur in the early stages of isolation, it may be appropriate to give vildagliptin to the donor priorto transplantation. However, far more investigation needs to be done in this field to improve the cell viability.
Keywords: pancreatic islet cell isolation, oxidative stress, vildagliptin

P049
Predictive factors associated with successful nonhuman primate islet isolation

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In order to identify factors associated with successful nonhuman primate islet isolation, we retrospectively analyzed the relationship between the characteristics of pancreas donors and enzymes used to islet isolation outcomes from cynomolgus monkey pancreata (n=192 isolations). A semi-automated method with Liberase HI was used (0.033g/ml). Factors predictive for higher islet yield (Islet Equivalents (IEQ)) in our linear regression analysis (R2=.273, p=.001) included donor weight, pancreas weight, enzyme endotoxin levels and cold ischemia time (CIT). T-Test analysis performed to evaluate differences between successful (≥ 5,370 IEQ/g) versus non-successful (< 5,370 IEQ/g) isolations revealed that Liberase activity levels (p=.006), Liberase endotoxin levels (p=.004), distention time (p=.017) and CIT up to 103 minutes (p=.003) were statistically significant factors. Distention time displayed an inverse relationship to islet yield with our linear (p=.001), T-test (p= .017) and ANOVA (p= .032) analysis. A new index (termed 'PANDIS') was created that resulted in a significant quadratic relationship (R2= .194, p < .001) to islet yield, thereby suggesting two isolation parameters (pancreas size divided by distention time) play an integral role in improving the procedure. In our perifusion analysis to assess islet function, we observed a significant logarithmic relationship (R2= .483, p < .0001) between insulin release at 3 mM glucose and insulin release values at 11 mM glucose stimulation. Our results indicate the benefits of increased CIT (up to 103 minutes) for local NHP pancreata and the detrimental effects of prolonged digestion time. Regarding our analysis of perifusion assays, delta insulin release, as opposed to the commonly used stimulation index may be the appropriate parameter for assessing islet function, with 3mM insulin release used as a predictive factor for future islet insulin response.

P050
Purified tissue dissociating enzyme performance in isolating mouse islets

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Isolated rodent islets continue to serve as the most practical model to study β cell biology in vitro. Primary islets offer the distinct advantage that they more faithfully reflect the biology of intracellular signaling pathways and secretory responses. Crude or partially purified tissue dissociating enzymes (TDE) continue to be used by most laboratories isolating rodent islets. However, inconsistencies in islet yield and quality often limit the extent and feasibility of primary islet studies. Variations often occur as a result of the crude partially purified TDEs used in the islet isolation procedure, which frequently exhibit lot-to-lot variations in activity and often require protocol modifications upon acquiring a new lot. While reports exist of purified TDEs used to isolate rodent islets, the practice is still not widespread and has not been fully optimized. We developed and evaluated a purified, defined TDE composition to isolate islets from CD1 mice using the method described by Gotoh where TDEs are directly infused into the main pancreatic duct *in situ*. Once a favorable enzyme composition was identified, a number of isolations were performed and compared to a partially purified collagenase. The purified TDEs gave islet yields of 215 ± 42 islets per mouse (n=18) versus 206 ± 77 islets per mouse (n=13) using the partially purified collagenase. A total of two lots of purified collagenase and three lots of purified protease were used in the 18 isolations reported above suggesting the consistency in performance of using purified TDE. Use of purified TDEs eliminate the need for labor intensive lot qualification process and allow for optimization of enzyme activity for specific applications or to address differences between mice strains. Defined and purified TDE also enable concise reporting of optimal TDE compositions which lead to improved productivity and confident comparing of results between laboratories.

P051
Two-step implantation device for cellular grafts: kinetics of subcutaneous pre-vascularization in rats

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We sought to evaluate the kinetics of neo-vascularization of an implantable device in the subcutaneous space prior to islet transplantation using imaging techniques. Devices (Converge Biotech) were pre-implanted subcutaneously in

Lewis rats 3 days, 1-, 2- and 3-wks prior to assessment of vascularization. Live microscopy of intraluminal device vasculature was evaluated using one-channel fluorescent fiber-optic microscope probes (Cellvizio®) following intravenous bolus of green fluorescence-labeled dextran. Confocal fluorescent microscopy was done on sections of devices explanted after intravenous administration of the lipophilic dye DiI (red fluorescence). Reversal of diabetes after transplantation of a suboptimal syngeneic islet mass in devices prevascularized for 3 days, 1, 2, 3, and 6 weeks was assessed in streptozotocin-induced diabetic rats.
Live microscopy revealed vascular structures on the inner walls of the devices starting 2 and 3 wks after implantation, with both number and complexity of vascular structures increasing with time. Statistically significantly increased vessel length ($p<<0.01$) and vessel area ($p<<0.01$) were observed when comparing 3-wks to 2-wks devices. Evaluation of tissue sections showed vascular structures in the surrounding of the devices at 2 and 3 wks post-implantation, while the degree of vascularization was minimal or absent at earlier time points. After transplantation of a suboptimal syngeneic islet mass, primary non-function was observed in all rats receiving islets 3 days after device implantation, whereas diabetes was corrected in a proportion of rats receiving islets after at least a week from device implantation.
Our studies suggest that neo-vascularization of the device wall starts early after implant, but the development of new vascular structures that are sufficiently complex to be functional may require ~2-wks. Furthermore, islet transplant outcomes indicate that at least a week of device pre-vascularization is needed to create a favorable environment for islet engraftment using a two-step device.
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P052
Lower -cell levels of antioxidant enzymes results in -cell loss

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Background: Reactive oxygen species (ROS) play important roles to cause deleterious effects of human tissue in several diseases. Antioxidant is ubiquitous enzymes which scavenge ROS that induce cell death of islet. There are few reports of comparison of antioxidants in human islet subsets. In this study, we examined the expression of these antioxidant enzymes in cell subsets from human islet preparations, in relation to the change of cellular composition observed during islet cell processing and following transplantation.
Materials and Methods: Single cell preparations from human islet preparations were double-stained with anti-catalase, anti-SOD2 antibody, or anti-glutathione peroxidase(GPX) with either anti-C-peptide, anti-Glucagon anti-Somatostatin antibody, to quantify the expression of antioxidant. Human islets cultured with or without H2O2 (50μM) for 24 hours were assessed the ROS-mediated vulnerability. DNA damage was assessed by anti 8OHdG antibody. Cellular composition of pre-isolation pancreatic tissue and renal sub-capsular islet grafts of mice were analyzed by confocal microscopy, while cell subpopulation analysis of post-isolation and diabetes patient using dissociated islets was performed using an iCys/LSC.
Results: There are great differences of catalase and GPX expression between b-cell and a-cell, which demonstrated that b-cell is more feasible than a-cell with oxidative stress induced by H2O2 and SNAP that cause DNA damage assessed by 8OHdG. H2O2 significantly reduced the β / α cell ratio compared to islet without H2O2. The ratio of β and α-cells significantly decreased following each step of islet cell processing and diabetes.
Conclusion: Catalase and GPX expression of β-cell is much lower thanα-cell in human islets leading to DNA damage by oxidative stress. These results suggest that antioxidant expression of islet subset account for the α-cell feasibility by oxidative stress and control of oxidative stress in diabetes and human islet transplantation could be of assistance in preventing progress of diabetes and improving clinical transplant outcomes.

P053
A novel static incubation method for the potency assessment of islets

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Despite the recent advances in islet cell transplantation, there still remains the need for a reproducible and easy potency assessment. Here we introduce a novel method for performing glucose stimulated insulin release (GSIR) of

islets of Langerhans by embedding islets in a slurry of sepharose beads to minimize mechanical perturbations. The method and data analysis was performed on 36 consecutive islet preparations.
Our results demonstrated that simple comparison of potency metrics to reversal of hyperglycemia in full mass sub-renal capsular transplants in athymic nude mice underestimated the predictive ability of potency tests. A more discriminating method was to compare rapid reversal of hyperglycemia (<= 5 days) of islet grafts to measured potency values. In our hands, the stimulation index was not the best metric for determining islet potency (ROC area under the curve = 0.77). The delta insulin (total produced in high – total produced in low 1) was more predictive of reversal time than the stimulation index (ROC area under the curve 0.932 vs. 0.778). Further, in an analysis of a subpopulation of 24 islet preparations, where an additional potency metric, fractional beta cell viability, was performed, we found that a combination of the two metrics enhanced predictive ability (ROC area under the curve 0.951 vs. 0.938 and 0.951 vs. 0.776, respectively), indicating that a multiple test platform is more predictive of islet graft outcome than a single potency metric.
Finally, we examined the importance of data normalization, comparing results normalized by IEQ enumeration versus assumed aliquot IEQ number. There was no significant difference between either enumeration or aliquot potency results in the hands of a single test performer. This indicates that as long as the potency assessment is performed by a single operator, results are independent of counts, as all preparations are exposed to the same source of potential enumeration/aliquotting errors.

P054
Incorporation of rat vascular progenitor cells into mosaic islet clusters to promote engraftment of transplanted pancreatic islets

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Pancreatic islet transplantation is an emerging cure for Type 1 Diabetes. Success is limited by death of the insulin-producing β-cells within the islet, from insufficient provision of oxygen and nutrients via the vasculature. Vasculogenic endothelial progenitor cells (EPCs) have the potential to improve islet engraftment.
Objective: To incorporate EPCs and islet cells into functional mosaic clusters in vitro.
Methods: Rat bone marrow-derived EPC were enriched by culture with endothelial growth factors on fibronectin. Phenotype was confirmed by flow cytometry and MatrigelTM assay. An embryoid body-forming medium enhanced the aggregation of dispersed rat islet cells and DiI-Acetylated Low Density Lipoprotein (DiI)-labeled EPCs over 3 days (d3) following centrifugation (400g/2min). Composition of clusters was examined via the β-cell marker Newport Green (NG) and analysed by flow cytometry and confocal microscopy. In vitro function was measured by static glucose-stimulated insulin release in response to high (25mM) and low (2.8mM) glucose.
Results: In culture, EPCs upregulated endothelial markers VEGFR2 and VCAM1 by d14 and formed tube-like structures in MatrigelTM. Mosaic clusters (diameter=142µm±32; n=20) contained more NG+ cells by d3 (76%) compared to clusters with islet cells alone (107µm±13; n=20; 49% NG+). DiI+EPC were distributed throughout mosaic clusters by confocal microscopy and comprised 41% of cells by d3. Mosaic clusters produced more insulin in high glucose (11347±1134pg/L) compared to clusters without EPC (6719±246pg/L).
Conclusion: Mosaic clusters were functional in vitro and will be tested for their ability to enhance islet engraftment in vivo.

P055
Expression of microRNAs in islet inflammation

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Inflammation greatly contributes to beta cell dysfunction/destruction through recruitment of immune cells to pancreatic islets and induction of pro-inflammatory cytokines in diabetes and post-transplant islet graft loss. Recent reports indicate that microRNAs (miRNAs) have a significant role in the mechanism of inflammation in vascular and chronic inflammatory diseases and diabetes. MiRNAs are non-coding gene products acting mostly as

translational repressors. We have studied expression of miRNAs in rat islets under inflammatory conditions: a) *in vitro* by utilizing pro-inflammatory cytokines critical to lesion formation following insulitis in T1D; b) *in vivo* in islets transplanted under the renal capsule of a syngeneic recipient. In the immediate post-implantation period, islet cells are exposed to multiple noxious stimuli through the stress-activated signal transduction pathways and the generation of inflammatory mediators, all contributing to loss of function and islet death.
The expression of miRNAs was examined using the Exiqon LNA based miRNA array platform and confirmed by RT-PCR. Out of 26 islet microRNAs commonly affected by *in vivo* and *in vitro* inflammatory conditions, 8 were confirmed by RT-PCR.
To identify potential microRNA gene targets, we looked for inverse association between the expression profile of miRNAs and corresponding mRNA targets identified by bioinformatics in genome-wide RNA microarray studies regarding inflammation of pancreatic beta cells. The miRNA target predictive algorithm Pictar identified 149 inversely correlated mRNAs. The same miRNA can target many genes. Thirty-five targeted genes are known to be ontologically associated with islet physiology, regulation of insulin signaling and secretion and diabetes. These studies may lead to identification of therapeutic targets to modulate miRNA expression and influence selected pathways associated with beta cell death and occurrence of diabetes.

P056
Impact of coculture with ischemic preconditioned Hep-G2 on insulin secreting function of RIN-5F

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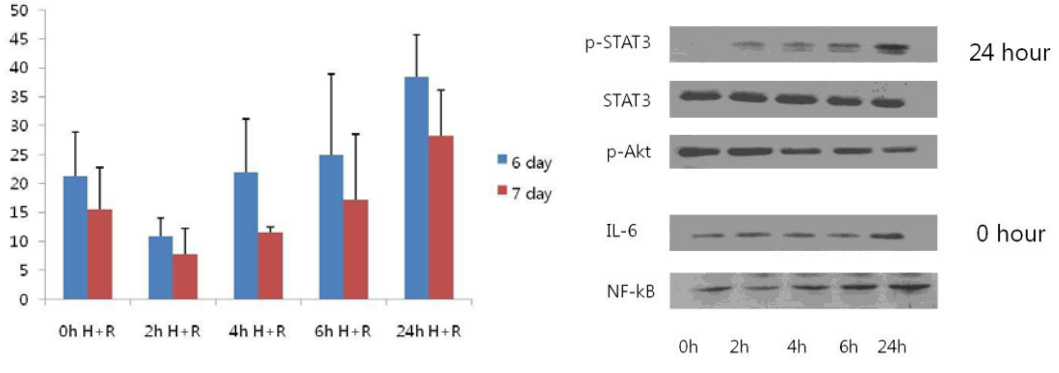
Introduction: The technique of islet cell isolation and culture considerably developed but the methods for prolongation of the survival of the transplanted islets is at a standstill. Hepatic ischemia and insufficient neovascularization of the islet were considered as the common barriers against long-term survival of islet. Survival hepatocytes from ischemic injury were reported to make a protective and regenerative effect on the hepatocytes with IL-6, STAT3 pathway.
Materials and Methods: Hep-G2 cell line was preconditioned in the hypoxic chamber for 0, 2, 4, 6 and 24 hrs, respectively and cocultured with RIN-5F. Cell viability, secreting insulin level, p-STAT3, IL-6, and NF-kB were measured in each group.
Results: Cocultured Hep-G2 and RIN-5F cell was aggregated and formed spheroid. Insulin secretion was increased by time-dependent method in ischemia-preconditioned group (4, 6, and 24 hrs; Fig. 1). p-STAT3, NF-kB and IL-6 also presented more in Western blotting according to the hypoxic time (Fig. 2). Cell viability of Hep-G2 was not different from each ischemic time group.
Conclusion: IL-6, STAT3 pathway known as a beneficial effect on the hepatocyte after ischemic injury also might have a good effect on insulin secreting function of islet cocultured with hepatocyte.



P057
Cafeic acid phenethyl ester protects cell viability of pancreatic islet cells against H2O2 induced injury

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The process of islets isolation results in pro-inflammatory and oxidative stress states means that loss of cellular viability and apoptosis. Caffeic acid phenethyl ester (CAPE), an active component of honeybee propolis extracts, has strong antioxidant activity together with anti-inflammatory action. The aim of this study was to improve the viability of isolated islets by CAPE treatment to donor rats.
Male Wistar Albino rats were divided into: control, ethanol (10%) and CAPE (10 μmol/kg for 4 days) groups. The isolated islets with more than 90% purity were placed in 0 or 300 μM H_2O_2containing media for 15 min. After H_2O_2 procedure, the islets were examined by using florescein diacetate and propidium iodine staining under florescence microscope. Additionally, the lipid peroxidation, protein oxidation and superoxide dismutase (SOD) activity were measured.
The H_2O_2treatment to islets induced lipid peroxidation and protein oxidation in control and ethanol groups. There was no significant difference on both lipid peroxidation and protein oxidation between with and without H_2O_2treatments in CAPE groups. The lipid peroxidation and protein oxidation were lower in H_2O_2treated CAPE group in comparison with H_2O_2treated control and ethanol groups. The SOD activity of H_2O_2treated groups had lower activity than groups without H_2O_2treatment in all groups. However, the CAPE treated group with H_2O_2treatment had higher SOD activity than control and ethanol groups with H_2O_2treatment. The islets viabilities of 300 μM H_2O_2treated groups were lower than that of 0 μM H_2O_2treated groups in regards to all three groups. On the other hand, the CAPE treated group had higher islet viability than control and ethanol groups in regards to 300 μM H_2O_2treated groups.
The treatment with CAPE to donor rats may help to increase islet defensive condition against all stress factors that induce early cell injury. Induction of antioxidant defense system and prevention of pro-inflammatory cytokines and mediators by CAPE in pancreas islets might play role to increase defensive state of islets.

P058
Comparative study, on enzymatic activity and molecules stability, between collagenases obtained by recombinant DNA technique and by extractive procedures

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In pancreatic islets purification the main enzymes used to disrupt the extracellular matrix are extracted from Clostridium hystoliticum, class I and class II collagenases (Coll-G and Coll-H). They are used together with neutral protease (Dispase) or thermolysin (Thermostable Neutral Protease) in extraction pancreas procedures. The quality of these enzyme mixtures are largely responsible for the success of the Edmonton protocol; however, operators working in the islets purification procedure found disagreement in efficiency when changing batch of enzyme production. This variability seems to be due to the different enzyme blends composition used, such as to endotoxines presence or enzymatic isoforms. Using biochemical approaches, we compared, composition, enzymatic activity and auto-digestion processes, of the C. hystoliticum collagenases, the neutral protease and the thermolysin obtained by extraction protocols with recombinant ones. Electrophoresis and gelatin-zymography pattern analyses shown heterogeneity composition in different mix of extractive collagenases; several more proteins and/or fragments respect the HPLC profiles publish, by vendors, were observed. Gelatin zymographyes shown a very high complex degradative patterns of extractive enzyme compared to recombinants. Additionally, the neutral protease shows contaminants having gelatinolytic activities. Inactivation/auto-digestive processes were analyzed at different working temperatures, showing more stability to 25°C than to 37°C. On the other hand, recombinant collagenases shown to be active to 30 °C, temperature at which mammalian matrix metalloproteases are slightly active. Heterogeneity in extractive enzymes can be due either to variability in the production process or to instability of mixture produced. In any case, the variable composition of the resulting enzyme blends is deleterious in the islet

purification protocols. These data suggest a not controlled digestive processes by extractive enzymes, probably due both to present contaminants in the blends, than to autocatalytic processes. Moreover, the presence of low molecular weight gelatine/degradative activities strongly suggest an action of them on the morpho/structural organization in islets of Langenhans, reducing their functionality. On the other hand, recombinant collagenases, developed individually, one by one and storage separately shown low auto-degenerative processes and to be highly stable.

P059
The effect of Nrf2 (nuclear factor erythroid2-related factor1) inducer on islet isolation

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Objective: To improve islet transplantation outcomes, it is critical to protect the islets against various insults during the whole isolation process. Nrf2 (nuclear factor erythroid2-related factor1)-Keap1(Kelch ECH Associating protein1) signaling pathway plays a major role in protecting the cells against oxidants, carcinogens and environmental and cytotoxic agents by mediating expression of numerous genes encoding anti-oxidant and cytoprotective molecules. The aim of this study was to investigate the effects of pretreatment with Nrf2 inducer on the quality of the islet cells following islet isolation.
Methods: Lewis rats (7wks) were fed with diets containing Nrf2-Keap1 inducer or regular diet for 72 hours before islet isolation. Islet yields, beta-cell content and insulin secretion were compared. Beta cell viability (apoptosis, necrosis) was assessed by Flow cytometry using three dyes (Newport green, TMRE, 7AAD). Additionally, viable beta-cell mass was calculated using the formula (IEQ x % beta cell X % beta cell viability).
Results: The Islet yield was significantly greater in the treated compared to the control group (2078±256 vs. 1709±85 IEQ, $P<0.05$). The stimulation index in the treated group was significantly higher when compared with that in the control group (5.7±1.4 vs. .2.6±0.6, $P<0.05$, respectively). In addition, islets harvested from the treated animals contained higher amount of beta cells when compared to the control group (86.4±3.6%, vs. 75.4±6.7, $P<0.05$, respectively). Flow cytometry analysis showed higher beta cell viability (72.0±6.5 vs. 70.8±3.6), lower cell death (18.2±5.3 vs. 21.9±5.7) and viable beta cell mass in the treated group compared to the Control group
Conclusions: Our study indicated that administration of Nrf2 inducer significantly improved islet yield, beta cell content, viability and function in rat isolation model. The results suggest that treatment with Nrf2 inducer may enhance the quality and quantity of the islets and as such may improve the islet transplant outcomes.

P060
Activation of peroxisome proliferator-activated receptor gamma prolongs islet allograft survival

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Exposing donor mice to carbon monoxide (CO) protects transplanted islet allografts from immune rejection after transplantation (referred as the “donor” effect). In an attempt to understand the mechanisms of the donor effect of CO, we found that donor treatment with CO upregulates expression of peroxisome proliferator-activated receptor gamma (PPARg), a transcriptional regulator, in isolated islets. In this study, we evaluated whether PPARg contributes to the survival and function of transplanted islets and whether PPARg mediates the protective effect of CO in a major mismatch islet allogeneic transplantation model.BALB/c (H-2d) islets in which PPARg activity was induced by its agonists, 15-Deoxy-D-12, 14-prostaglandin J2 (15d-PGJ2) or troglitazone were transplanted into C57BL/6 (H-2b) recipients that had been rendered diabetic by streptozotocin. Blood glucose levels of recipients were monitored to determine the function of transplanted islets. Our data indicated that PPARg activation in islets led to a high percentage of BALB/c islets survived long-term in C57BL/6 recipients. Activation of PPARg in the donor suppressed expressions of proinflammatory cytokines including tumor necrosis factor alpha (TNF-a) and inducible nitric oxide synthase (iNOS) in transplanted islets. Blocking PPARg activity by its antagonist, GW9662, abrogated the donor effect of CO in the islet transplantation model, and the anti-apoptotic effect of CO in vitro in an insulinoma cell line, beta TC3 cells. Our data demonstrate that PPARg plays a critical role in the survival and function of transplanted islets after transplantation in the recipient. The protective effects of CO are at least in part mediated by PPARg.

P061

A quantitative indicator of functioning islet mass based on MRI imaging of intraportally transplanted islets

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Although the clinical use of magnetic resonance (MR) imaging to assess transplanted islets is promising, no algorithms are available to quantitatively interpret MR images of transplanted islets. We derived a quantitative indicator of functional islet mass based on MR imaging of intraportally transplanted islets.We evaluated the effect of ferucarbotran-labeling of islets on the in vitro and in vivo function of islets. Using a rat syngeneic intraportal islet transplantation model, we quantitatively assessed the relationships between total area, number of hypointense spots on MR imaging and glycemic control of the recipients.

Ferucarbotran-labeling of islets does not affect the glucose-stimulated insulin secretion of islets or the post-transplantation outcomes of mouse syngeneic islet transplantation into the renal subcapsular space. In the rat syngeneic intraportal islet transplantation model, the total area of hypointense spots on MR imaging was greater in the recipients that achieved diabetes reversal ($p = 0.002$), whereas the total number of hypointense spots was not different ($p = 0.757$). Further quantification according to the area, size, and number of hypointense spots revealed that the number of hypointense spots per recipient that belong to the largest quartile at one week post-transplantation was greater in the diabetes reversal group ($p < 0.001$). The total area of hypointense spots that belong to the largest quartile correlated with blood glucose level at 3 weeks post-transplantation ($p = 0.0002$), with no overlap in the range between recipients with and without diabetes reversal.

In conclusion, the area and number of hypointense spots that belong to the largest quartile reflected the functional islet mass, but the number of hypointense spots did not. This finding may explain the discrepancy between transplanted islet mass and total number of hypointense spots observed in recent studies.

P062

Improvement of graft survival time by surface modification with 6-armed PEG and immunosuppressive drugs in islet transplantation

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Background: Surface modification using poly(ethylene glycol) (PEG) onto islet is a promising approach for protecting transplanted islets from host immune cells. However, the surface modification alone is not sufficient to completely prevent the attack from the host immune reactions. This study proposed a new combination therapy using multi-branched 6-arm-PEG-catechol in combination with low dose of FK506 and anti-CD4 mAb for improving graft survival time in xenotransplantation.

Methods: The end groups of 6-armed PEG were replaced with a catechol and were grafted onto the surface of rodent pancreatic islet. The surface coverage profile, the viability and the functionality of islets were evaluated *in vitro*. Then, the graft survival time of surface modified islets was evaluated in diabetic C57BL/6 mice. To evaluate the synergistic effect, low dose of immunosuppressive drugs (FK506 and anti-CD4 mAb) were co-administered into diabetic mice transplanted with surface modified islets.

Results: Suspension of pancreatic islets in 1% (w/v) of PEG solution (reaction time: 1 h) didn't affect the cell viability and the functional activity. The median graft survival time (MST) of 6-arm-PEG-catechol grafted islets (12.0 ± 1.1 days) was slightly increased, when compared to unmodified islets (10.5 ± 1.3 days). However, when 0.2 mg/kg of FK506 was daily administered, the MST of 6-arm-PEG-catechol grafted islet (21.0 ± 1.9 days) was significantly increased, when compared to those of unmodified islets treated with 0.2 mg/kg of FK506 (10.0 ± 0.9 days). Interestingly, the recipients of 6-arm-PEG-catechol grafted islets treated with 0.2 mg/kg of FK506 and 0.1

mg/mouse of anti-CD4 mAb maintained their normoglycemia for up to 50 days of transplantation without glucose level fluctuation.
Conclusion: Newly developed protocol using 6-arm-PEG catechol in combination with FK506 and anti-CD4 mAb would certainly be an effective combination therapy for the treatment of type 1 diabetes.

P064
Bioengineering of functional islet tissues in subcutaneous site

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Background: Pancreatic islet transplantation has been established as a promising new approach for treating insulin-dependent diabetes mellitus (DM). However, many issues remained to be resolved that associated with cell infusion into blood stream. Here we demonstrate our novel and scaffold-free based islet tissue bioengineering approach in subcutaneous site utilizing dissociated islet cells. Engineered neo-islet tissues in subcutaneous site successfully reverted to steady normoglycemic level in diabetic mice.
Creation of islet cell sheet: We first prepared temperature-responsive culture dish specific for islet cell culturing and subsequent harvest as a monolayered cell configuration, cell sheet. In detail, specific amount of temperature-responsive polymer, poly(N-isopropylacrylamide) (PIPAAm) was grafted on plastic culture dish followed by coating with rat laminin-332. Dispersed rat islet cells were plated onto the special PIPAAm dishes. After the cells reached confluency, islet cells were successfully harvested as a monolayered cell sheet by lowering the culture temperature to 20°C for 20 min. The functional activity of the islet cell sheets was confirmed by histological, biochemical, and ultrastructural examinations.
Subcutaneous islet tissue bioengineering: Harvested islet cell sheets were transplanted into subcutaneous spaceof diabetic SCID mice. Histological assessments revealed that highly vascularized thin-layer neo-islet tissues were formed. All diabetic recipients achieved normoglycemia that persist long-term. Normal responses to glucose tolerance test were observed.
Conclusions: A novel and simple islet tissue bioengineering approach was described. This islet cell sheet-based approach provided therapeutic values in reverting the hyperglycemic state of diabetic mice by *de novo* engineering islet tissues in an ectopic subcutaneous site.

P065
Anesthetic preconditioning protects beta-cells from oxygen free radical-induced cell death via p38MAPK

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Objectives: Islet transplantation is a promising therapeutic modality for the treatment of insulin-dependent diabetes. The susceptibility ofislets to oxidative stress during isolation and after transplant significantly reduces the number of viable islets. Volatile anesthetics are cytoprotective against ischemia/reperfusion injury (anesthetic pre-conditioning or APC). In this study we investigated the effectiveness of Isoflurane (Iso) to improve islet cell survival after induction of oxidative stress with hydrogen peroxide (H_2O_2).
Methods: Murine insulinoma beta-TC3 cells were used as a surrogate source of beta-cells in vitro, incubated for 48h then subdivided into 4 groups: Group 1 were sham cultured beta-TC3 cells; Group 2 cells were subdivided into those pre-treated with 0.5 mM Iso for 15 min or 60 min without H_2O_2, followed by washout of Iso; Group 3 were exposed only to 0.3mM H_2O_2 for 3h; Group 4 were pre-treated with Iso for 15min or 60min, followed by Iso washout and then exposure to 0.3mM H_2O_2 for 3h. Cells were harvested and assayed for apoptosis and viability via FACS analysis. Total and phosphorylated stress-activated protein kinases profile were evaluated by Luminex technology. Data was analyzed using ANOVA with post hoc Tukey for multiple comparisons.
Results: H_2O_2 decreased beta- cell survival to 44±13% compared to controls ($p<0.01$). Pretreatment with Iso significantly improved beta-TC3 cell survival compared to cells exposed to H_2O_2 alone [98±2% vs. 44±13% after 15min ($p<0.01$), and 105±0% vs. 44±13%, $p<0.01$ after 60min)]. p38MAPK activity (measured as phosphorylated/total protein) was significantly higher in H_2O_2 ($p=0.008$) and significantly suppressed by Iso pretreatment ($p=0.006$).

Conclusions: Our findings suggest that in this experimental model of APC, Iso protects beta-cells against H_2O_2-induced apoptosis via p38MAPK. Iso pre-treatment may allow better preservation of islet cells in culture thereby increasing the success of islet cell transplantation.

P066
Technique of endoscopic biopsy of islets transplanted into the gastric submucosal space in pigs

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Background & Aims: Clinical islet transplantation (Tx) is generally performed by infusion islets into the portal vein. One disadvantage of this approach is that it is not possible to adequately biopsy the islets in the liver. Islet Tx into the gastric submucosal space (GSMS) can be carried out by minimally invasive endoscopic injection, and has the added advantage that it should be possible to successfully undertake endoscopic biopsy of the islets in this site. The aim of this study was to determine feasibility and accuracy of endoscopic biopsy of islets transplanted into the GSMS.
Methods: We performed islet Tx into the GSMS in non-immunosuppressed pigs using an endoscope, under full inhalational anesthesia. Islets were transplanted at 4 sites in the GSMS of each pig. The observation of islet Tx site by Endoscopic ultrasonography (EUS) and biopsy at 2 transolanted sites by modified endoscopic submucosal dissection (ESD) using IT-2 knife were carried out successfully and uncomplicated in all pigs 5 days after islet Tx. (modified ESD technique; the graft was taken by snaring after cutting around the islet transplanted site by IT2 knife, A video of the technique will be shown.) Tissues obtained at biopsy were compared to those obtained after necropsy (full-thickness sections of the gastric wall around the sites of the remaining islets and biopsies), and examined by histology and immunohistochemistry to confirm the presence of the islet grafts and any features of rejection.
Result: EUS was effective to observe islet Tx site, and ESD was found to be safe and provided biopsies which allowed for good histopathological assessment, comparable to full-thickness sections. All biopsies included islets with insulin-positive staining. There was significant CD3+ and CD68+ cell infiltration in the islet masses obtained from both biopsy and sections taken at necropsy, with similar histopathological features.
Conclusion: ndoscopic biopsy of islet allografts in the GSMS is safe and provides accurate histopathological data, and would provide a significant advance if translated into clinical practice.

P067
Treatment with PKC epsilon agonist improves islet graft function in a syngeneic diabetic mouse transplant model

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Islet cell transplantation after isolation from deceased donor pancreata is an attractive method of beta cell replacement. However the loss of islet cell viability during and after recovery is a limiting factor to the optimization of graft utilization. Previously we demonstrated that translocation of the isoenzyme PKCε to the cell membrane through its specific activator peptide protected islet cells during isolation from apoptosis and increased cell survival *in-vitro*. In this study we assessed PKCε treated islet graft survival and function during the peritransplant period in a syngeneic mouse streptozotocin-induced diabetic transplant model. Islet cells were isolated from wild-type BALB/c mice preconditioned with either a PKCε activator (yeRACK) or a TAT carrier control peptide and further treated with the same agents during isolation, purification and *in vitro* post-isolation. A marginal functional islet cell mass was transplanted under the kidney capsule of diabetic mice. The diabetes reversal rate (day 11 post transplant) for the grafts treated with PKCε activator (n=3) was not different than for the TAT treated grafts (n=3). However, the post transplant intraperitoneal glucose tolerance testing (IPGTT) showed improved insulin response in mice transplanted with PKCε treated islets as demonstrated by the 50% reduction of the IPGTT area under the curve compared to mice transplanted with TAT treated islets. In conclusion, a conditioning regimen using PKCε agonist

during pancreatic recovery and islet isolation may improve islet graft function and resistance to high glucose stress after transplantation.
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P068
The availability of pituitary adenylate cyclase-activating polypeptide (PACAP) in islet transplantation

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is an islet substance serving as an intra-islet amplifier of glucose induced insulin secretion. It has been reported that administration of PACAP maintained beta-cell mass and resulted in cyto-protection from glucose toxicity. Moreover, PACAP increases glucose-stimulated insulin release in vitro. In this study, the the role of the PACAP was analyzed using transgenic mice islet, and the availability of PACAP was tested in human islet transplantaion.
Methods: Single islets of pancreatic beta-cell specific PACAP trangenic mice were havested and culetured in 5G or 22G. The amplitude of Ca^{2+} response and the insulin release were anlyzed. Human islets were cultured in the presence or absence of PACAP (10^{-12}M) for 48 hours. Beta cell viability was assessed by FACS, cellular composition analysis was performed by iCys/LSC and glucose stimulated insulin secretion was tested.
Results: In transgenic mice islets, inslin secretion and resistance to glucose toxicity were improved. In human islet, there were significant improvements in terms of beta cell viability and cellular composition in between islets cultured with or without PACAP. Moreover, glucose stimulated insulin secretion significantly improved in islets cultured with PACAP, compared to controls.
Conclusions: Our study indicated that PACAP maintained islet viability and beta cell content during culture, and improved insulin secretion in vitro.

P069
Validation of growth based rapid microbiology method using the BacT/ALERT® 3D system for cellular therapy products

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Sterility testing is an essential part of in-process and release testing for cellular therapy products. This study validated the BacT/Alert® 3D System (BTS) for use in testing cellular products. The FDA recommends that sterility testing be performed as outlined in 21 CFR 610.12. We have developed a validation protocol comparing the CFR method with the BTS. The purpose of study was to test that BTS is capable of detecting a wide variety of microorganisms in a variety of in-process, final product and raw materials.
Methods: Three different cellular products (islet, hematopoietic and mesenchymal cells) were inoculated with selected microbial challenge organisms (4 strains). These strains include gram-positive, gram-negative and spore-forming fungus. Each challenge organism was prepared as high and low inoculums. In the second phase, media was tested to prepare in-process and final product samples as well as raw material samples. Each sample was inoculated with each of the microbial challenge organisms. All samples were incubated for a period of 14 days and cultured at 35°C. Every sample was run in triplicate. The third phase was a comparison between BTS and CFR methods. In-process and final product samples were collected from all three cell products. All samples were divided into two aliquots: one was analyzed using the BTS; the other one was be sent to the reference laboratory and tested according to the CFR method.
Results: Challenge cultures were detected in a mean of 95 %. All three tested products detected the organisms 100%. With each bacterium and fungus, the BTS was found to be more rapid and sensitive for detection.
Conclusion: This study validates the use of the BTS for the detection of bacteria in cellular products. This study will not only permit us to achieve a more cost-effective process, but also deliver consistent results.

P070
Improved human islet isolation outcomes using a mammalian tissue-free enzyme blend

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Lot to lot variability and changes in enzyme blends performance over time have traditionally imposed challenges to standardization of islet isolation methods, reproducibility and consistency in islet isolation outcomes. In addition, the use of mammalian derived reagents in the enzyme manufacturing process introduced regulatory concerns for their use in clinical trials. To address these challenges, a novel mammalian tissue-free collagenase blend product was developed (Liberase MTF, Roche, Indianapolis, IN). Human islet isolation outcomes using this novel enzyme blend were compared to those obtained using the previously developed enzyme blend, Liberase HI.
Methods: Islets were isolated from pancreata using MTF enzyme (n=46) and Liberase HI enzyme (n=75). Deceased human donor pancreata were processed using a modified automated method, following established protocols and except for the enzyme preparation used, all steps were similar. Isolations using Liberase HI were from 2005 to 2006 compared to Liberase MTF from 2009 to current.
Results: No significant differences in donor age, cold ischemia time, digestion time, or weight of the pancreases were observed between the two groups. All examined islet cell product parameters were significantly improved with Liberase MTF compared to Liberase HI. Islet yield before purification was 457±184 103 islet equivalents (IEQ) for Liberase MTF compared to 373±187 103 IEQ for Liberase HI (p=0.018). Post purification IEQ were 361±179 103 Liberase MTF versus 290±181 103 with Liberase HI (p=0.038). The post purification purity was 74±17 % when Liberase MTF was used, compared to 51±19 % for Liberase HI (p< 0.001). Finally, insulin stimulation index of islet cell products obtained using Liberase MTF were significantly higher compared to the Liberase HI (3.6±3.3 vs. 2.14±1.9, respectively; p=0.009).
Conclusion: We conclude that the performance of Liberase MTF was significantly better compared to Liberase HI. This improved enzyme blend can therefore be effectively utilized for experimental and clinical islet cell processing.

P071
Writing an investigational new drug (IND) application in an academia: step by step approach

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Regulatory submissions are the most critical component in clinical research as they accelerate time to help in bringing the benefits to patients sooner. This study describes step by step approach of writing IND and how to overcome hurdles faced during the process in an academic set up.Research and development activities in academia lead to New Drug Application (IND). A sequential approach commonly used today increases the difficulty of preparing an IND. We have found that by adhering to key principles identified in this paper, a timely and successful IND submission can be achieved. Even before the start of the research, investigators should study the relevant FDA regulations and review the forms for the IND submission to determine how the organization can best meet FDA requirements. The forms are available from the FDA web. Institutes that devote disciplined and systematic attention to details from the start, progress far more efficiently towards successful start of trials than those institutes that delay the ground work.The scientific research behind the drug should be comprehensive with rigorous emphasis on the documentation of research records. The failure to design, execute, and generate right data from the onset can come back to hurt the entire effort due to unnecessary and costly delays. In broad terms, the IND requirements are divided into 11-12 sections. The ultimate goal is to demonstrate safety of the new drug through pharmacology, pharmacokinetic and toxicology studies. In addition, the safety of the new drug should also be demonstrated through the judicious use in initial human studies via a well vetted study protocol.

P072
Immunological assessment of islet allograft recipients treated with steroid free immune suppression

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We monitored immunological changes in sixteen islet cell recipients, who had long-term type 1 diabetes and were treated with steroid-free immunosuppression and given sequential islet cell infusions. Cytotoxic lymphocyte (CL) gene expression and immunophenotypic analyses of lymphocyte surface antigens in peripheral blood were performed by RT-PCR and flow cytometry, respectively. We observed, for most patients, that CL gene expression can be divided into four phases in relation to graft status: CS/M- (clinically stable/molecular negative, defined as stable glycemia, insulin independent, and no CL gene elevation); CS/M+ (clinically stable/molecular positive, defined as stable glycemia, insulin independent, and elevation of CL genes); CUS/M- (clinically unstable/molecular negative, defined as frequent increase in blood glucose and/or return to insulin therapy, and no CL gene elevation); and GL/M+ (graft loss/molecular positive, defined as increased exogenous insulin, further reduction in c-peptide, and elevation of CL genes). We utilized CL gene expression as a molecular flag that signals initiation of immune mediated islet destruction, to subdivide and analyze the immunophenotypic data in reference to the individual's graft status. Patients were divided into short-term (ST) and long-term (LT) graft function groups, based on the length of stable glycemia and insulin independence post-transplant. B lymphocytes were significantly higher at baseline and in all four phases for ST patients as compared to LT patients, implying a role for B cells in graft dysfunction. In addition, we found that elevation of perforin mRNA correlated well with an increase of autoantibodies, after the first year post-transplant, suggesting a possible involvement of perforin in the reoccurrence of autoimmunity. Here we utilized molecular assays to "flag" time points in which significant changes in anti-graft immunity can be detected in the periphery.

P074

Replacement of thermolysin in human islet isolation using Bacillus polymyxa protease (Dispase)

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Consistent and effective enzymatic dissociation remains an issue in human islet transplantation. Collagenase/Thermolysin mixtures are commonly used but some results indicate that Thermolysin may adversely affect islet survival and function; additionally lots of thermolysin have shown to lose function over time. This study compares enzymatic dissociation utilizing collagenase supplemented with either Thermolysin or BP Protease (BPP). BPP, dispase, is a gentler proteolytic enzyme and remains stable during low temperature storage and has been used successfully in porcine islet isolation.

Human islets were isolated at the University of California, Irvine from cadaveric organ donors, with research consent, using purified Collagenase HA mixed with either thermolysin (6mg) or BPP (1.1mil NPU) followed by continuous ficoll purification. After culture, at 37^{o}C and 5% CO_2, islets were assessed for yield, viability using fluorescein diacetate and propidium iodide (FDA/PI) and function in response to glucose challenge.

Donor related variables (donor age, BMI, cold pancreas ischemia) were equivalent. Enzymatic digestion time at 37°C was statistically equivalent between the two groups, 15±1 minutes (mean±SEM) for thermolysin and 17±1 min for BPP. Islet yield (IE/gm processed pancreas) were 3517±156 in the thermolysin group and 3824±202 in the BPP group. Viability, after 48 hours in tissue culture (37^{o}C, 5%CO^2) was 74±5% and 86±2%, respectively ($p<0.05$, test). In vitro islet function after overnight tissue culture as measured using the stimulation index (SI, ratio of high (28mM) over low (2.8mM) glucose) was 2.4±0.5 in the thermolysin group and 2.8±0.8 in the BPP group (p=ns).

Preliminary results suggest that dispase can be utilized in place of thermolysin to recover islets after enzymatic dissociation of the cadaveric pancreas. Further in vitro analysis of the islets and clinical transplant studies will validate this new enzymatic dissociation strategy

P075

Exercise in a islet-transplanted nonpro marathon-runner: Effects on training, autoimmunity and metabolic profile

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Type 1 diabetes mellitus (T1DM) is a chronically progressive autoimmune disease in which the adverse immune response is induced and promoted by the interaction of genetic and environmental factors. Recent studies have suggested that physical exercise may interfere with immune system function even at low intensity and duration. Here, an islet-transplanted T1DM patient (M, 44yrs) has been longitudinally monitored for autoimmunity markers, metabolic profile and physical performance, being himself a nonpro marathon runner, over a 7-year period from the islet allograft. Given his discontinuous story of training (because of injuries, medical issues etc), we identified 4 phases throughout this period, lasting ca 2 years each, characterized by rest and training, alternatively. Administration of an ad hoc regime of training (supervised interval training) showed an improvement in glycolsylated hemoglobin (HbA1c -9.2%, $p<.05$), C-reactive protein (-16.6%, $p<.05$) and a decrease of insulin treatment (from 4-8 to 4-6 U/die) during the 2nd phase with respect to the 1st phase of rest (recovery after allograft). In the 3rd phase (post-injury resting), Hb1Ac increased by 13.3% ($p<.05$ vs 2nd phase). In the 4th phase, exercise training was accompanied by an amelioration of Hb1Ac of 22% respect to the 3rd phase. Insulin units diminished dramatically with respect to the 1st phase (2-3 U twice a week), and so did the autoimmunity markers (antiGAD Ab from 0.5 to 0.0 AU; antiINS Ab from 6.6 to 1.6 AU). Race time during competition improved by 10.5% as compared with the 2nd phase of training ($p<.05$). Also in the 4th phase, aerobic-, anaerobic thresholds and heart rates resulted significantly higher than the previous phases ($p<.05$). Altogether, these data suggest that the alternation of detrimental metabolic and autoimmunity profiles may be associated with the interleaving of training/resting periods, evoking a potential role for exercise in a positive immunomodulation of system functions against T1DM progression and inflammation.

P076
Impact of donor age on islet isolation for transplantation

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Precise assessment of the islet quality is an important goal of human islet transplantation. Currently, there is no optimal tool to assess islet potency prior to transplantation. We have recently shown through the evaluation of transplanted human islets in diabetic immuno-compromised mice (n=175) that none of the routine tests predicted the potency of the transplant. Furthermore, among many factors tested, only donor age was correlated with the reversal of diabetes in the mouse bioassay. In the present study, to explore underlying mechanisms, we first examined islet size distribution and cellular composition using autopsy pancreas specimens and compared between donors older than 50 yr (n=8; 51-81 yr) and younger ones (n=6; 15-47 yr). There was no difference in total islet area per pancreas (1.31 ±0.34 % and 1.42 ±0.35 %, respectively) or cellular compositions (beta-cells: 0.56 ±0.15 % and 0.65 ±0.13 %; alpha-cells: 0.43 ±0.11 % and 0.38 ±0.11 %; and delta-cells: 0.32 ±0.01 and 0.39 ±0.14, respectively). Note that significant pathological changes are observed in patients with type 2 diabetes (n=10; 65-81 yr); islet area 0.50 ±0.01 % (P<0.004), beta-cells 0.29 ±0.05 (P<0.005), alpha-cells 0.16 ±0.06 %, (P=0.058) and delta-cells 0.50 ±0.01 % (P<0.004). Collectively, these results suggest that aging per se does not affect islet mass or cellular composition. Next we studied effects of aging on islet isolation between donors older than 47 yr (n=68) and younger ones (n=107). Beta- and delta-cell mass was significantly lost from older donors (136235±11697 and 175962±9706 IEQ, P<0.05; 32477±2507 and 51387±3834 IEQ, P<0.01, respectively). The result suggests that islets from young donors are more resistant to the isolation procedure. On-going studies include molecular markers associated with aging such as glucokinase, cell proliferation, apoptosis and key transcription factors.

P077
New strategy of cell transplantation: Hepatocyte transplantaion using after hepatectomy organ of hepatocellular carcinoma

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Introduction: The patient who underwent large hepatectomy sometime easily became liver failure. Recently large hepatectomy is very popular for hepatocellular carcinoma (HCC) and cholangiocarcinoma(CCC).

This time, we paid attention to this rececte liver. This rececte liver have a large quantity of normal hepatocytes with carcinoma cell. If completely divited two group using FACS for tumor Ab, thrse cell were able to transplant. This transplant became the singenic transplant and thought that it was possible for the hepatocytes transplant that future clinical which was effective for liver failure treatment was near after the art without the immunosuppressive drug.

Material and method: Immortalization rat hepatocytes having SV40 largeT antigen as the artificial chromosome vector which we already developed in our laboratory this time. It is this cell that is cancer cell.

At first I marked this cancer cell (I use the immortalization hepatocytes which I introduced GFP into) and mixed this cell and normal hepatocytes and cultured it and I divided cancer cell group using FACS afterwards and examined it whether the next normalcy hepatocytes were implantable cells.

G1 Cancer cell group: Artificial chromosome (hepatocytes having SV40 largeT antigen +GFP): 1X106), G2 Normal hepatocytes group: normal hepatocytes (Lewis Rat cell separation; 1X106). Mix these both counties at 1:1 (I do each cell) and divide into two groups using FACS again immediately afterwards.

I confirm that I culture it and take effect, and GFP does not get mixed with a fluorescent microscope and confirm the yes or no of the SV40 largeT antigen in PCR again afterwards. Examined it afterwards whether it was the quantity of having early instruction or not of a survival rate and the apoptosis of this cell and this cell that is the implantable cell which took my eyes off it for quantity and was implantable enough.

Conclusion: The normal hepatocytes and cancer cell were able to prove that they were separable by this method. However, it is this experiment problems whether I can isolate it surely and the cell count can extract a large quantity of implantable cells with a small quantity in the real clinical place.

P078

Hepatocyte transplantation in rats with acute liver failure using cells labeled with a clinical grade MRI contrast agent

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Aims: The fate of hepatocytes after cell transplantation in man is not well defined. The aims of the study were to label hepatocytes using superparamagnetic iron oxide (SPIO) nanoparticles and perform *in vivo* experiments on tracking labeled cells by MRI.

Methods: Human and rat hepatocytes were labeled in culture for 16 h with clinical SPIO nanoparticles (12.5 μg Fe/ml) using protamine sulphate (3 μg/ml) as a transfection agent. Cellular iron uptake was detected by Prussian blue staining, and quantified by a ferrozine–based assay. Cell viability and function were assessed using LDH leakage, mitochondrial dehydrogenase activity, albumin and urea assays. Intrasplenic transplantation of $2x10^7$ male rat hepatocytes labeled with SPIO nanoparticles (n=4) or non-labeled (n=4) was performed in female recipients 28-30h after acute liver failure induction by intraperitoneal injection of D-galactosamine. SPIO-labeled and non-labeled hepatocytes were also marked with the fluorescent dye CM-DiI. T2*-weighted gradient-echo images at 7-T MRI were acquired at day 7. Transplanted cells were detected in liver by PCR for the Y-chromosome (Sry-2 gene) and histological analysis.

Results: Mean intracellular iron concentrations were 11.4±SE1.1 pg/cell in human and 8.6±0.3 pg/cell in rat hepatocytes. Cell viability and metabolic function were not significantly affected at these SPIO concentrations. SPIO nanoparticles could be detected by MRI in rat liver 6 days after transplantation in the 3 survivors. On histology, most of the SPIO particles were located in Kupffer cells, indicating the loss of iron oxide particles from hepatocytes. In keeping with this, SPIO-labeled cells could not be detected in the liver by the fluorescent dye or PCR for Sry-2 gene.

Conclusions: Optimum conditions to label human hepatocytes with SPIO nanoparticles were established. Clearance of hepatocytes after transplantation limits the value of MRI using SPIO nanoparticles for assessing long-term cell engraftment.

P079

The radical scavenger edaravone counteracts for acute transplant hepatocyte rejection in rat

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Introduction: It has already been established that hepatocyte transplantation (HTx) in animal models, such as both chemically and surgically induced acute liver failure, liver-based metabolic disease,resulted in significant improvement of liver function and survival.
However, the transplant effects are not enough, and it is thought that the transplant cell by the hepatocyte transplant wears the life of the cell by a large quantity of NO by the stoppage of the organization, and an obstruction is angry at the one of the reason. Fine weather I administered edaravone with transplant this time and examined the effect.
Material and Method: The edaravone method, It takes effect in edaravone (3mg/kg. iv.) to a no albumin rat (NARs) just before 24 hours Hepatocyte transplant.
The hepatocyte isolated with cell separation method and mixes a 30X106 cell with solution of 0.5ml and injects it in 25G to the spleen directly. (Lewis rat (150-250g) entirely.) Collected blood sample on 0, 3, 5, 7, the 14th day and measured blood albumin value by ELISA. G1 Control (medium injection),G2 Normal hepatocyte transplant, G3 Normal hepatocyte transplant + edaravone, G4 edaravone without Tx
Result: The albumin value accepted increase with the edaravone treated group significantly. At the same time the spleen which transplanted these hepatocytes could observe a hepatocyte entirely, and, transplant two weeks later, hepatocytes increased with the edaravone treated group significantly again.
Consideration: It was proved that the edaravone was effective for a transplant cell in this study, and clinical application would be possible in future, and it was thought that I would be useful in the future.

P080
MRI monitoring during liver cell transplantation to the spleen in a porcine model

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Objectives: Liver cell transplantation (LCT) is a promising approach for the treatment of metabolic liver disorders. However, a method for non-invasive monitoring during LCT is not available clinically, and thus little is known about the processes during and following LCT. Labeling of liver cells with superparamagnetic iron oxide particles as intracellular contrast agent can enable the visualization of transplanted cells by magnetic resonance imaging (MRI). The aim of this study was to investigate the feasibility of MRI monitoring during liver cell infusion to the spleen, which is considered an ectopic implantation site for LCT.
Methods: Male porcine liver cells were labeled with micron-sized iron oxide particles (MPIO) and infused to the spleen of female fully-grown pigs (n=5) through a catheter placed in the lineal artery. MRI monitoring was performed using a conventional 3.0 Tesla MR scanner. Initially, T1- and T2-weighted pulse sequences were tested for the detection of MPIO-labeled cells in the spleen. Thereafter, fast dynamic MRI was performed during cell infusion. MR findings were verified by histological and immunohistological examination.
Results: Images from static MRI (repetition time / echo time: 2,500/105.2 ms) showed significantly lower signal intensity and signal-to-noise ratio after cell infusion compared to pretransplant images. T2-weighted fast dynamic MRI enabled visualization of continuous signal decrease of the spleen during cell infusion. T1-weighted sequences did not show signal decrease at the same time. When cells were infused systematically, no signal changes in the spleen were observed. After successful cell delivery, the arterial vessels of the spleen and the surrounding parenchyma contained large numbers of CK18-positive and Fish-positive male liver cells. Cell translocation to the liver was not observed by MRI during cell infusion to the spleen.
Conclusions: This study shows that fast dynamic MRI can enable non-invasive visualization of liver cell distribution in the spleen and verification of the success of cell delivery. MRI monitoring enabled visualization of cell arrival in the spleen, indicating that the spleen may act as an temporal reservoir for liver cells during LCT. Thus, MRI monitoring could be useful for preclinical studies and for quality control of LCT in the clinical setting.

P081
Engineering of liver tissues containing liver-specific non-parenchymal cells at the ectopic site

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Introduction: Liver tissue engineering is an emerging field in which a functional liver system is created in vivo using isolated hepatocytes. Here we investigate whether primary hepatocytes transplanted into the subrenal capsule space could proliferate and construct liver tissues in response to the continuous liver regeneration stimulus derived from chronic hepatic injury of the native liver. We further discuss the process of liver tissue construction in terms of non-parenchymal recruitment.
Materials and methods: Donor hepatocytes were isolated from humanα1-antitrypsin (hAAT) transgenic mice. Recipient mice were inoculated twice withmonocrotaline (MCT) at an interval of 2 weeks. Two weeks after the second MCT injection, mice were subjected to 70% partial hepatectomy (PH) and transplantation of 1×10^6hepatocytes into the subrenal capsule space. Tissue volume of the engineered liver was assessed by measuring the serum hAAT levels.
Results: The engineered liver tissue volume showed continuous increase in MCT/PH treated mice throughout 150 days experimental period. H&E staining at day 150 revealed that the engineered liver tissues were significantly thicker in MCT-PH group (~300 μm) (Fig. 1) than that in the control group (~30 μm). Although the liver tissues were initially made by purified hepatocytes, numerous liver-specific non-parenchymal cells (stellate cells, sinusoidal endothelial cells, kupffer cells) emerged in the engineered livers. Detailed investigation revealed that the non-parenchymal cells were derived from recipients. Moreover, liver zonation (zones 1, 2, and 3) was formed in engineered livers.
Conclusion: The presented approach using primary hepatocytes enabled us to create 3-dimensional thick liver tissues composed of parenchymal and liver-specific non-parechymal cells under the kidney capsule, in response to the chronic hepatic injury. The constructed liver tissue possessed complex liver structures along with the liver zonation. This finding paves the way for neo-organogenesis of liver at the ectopic site.

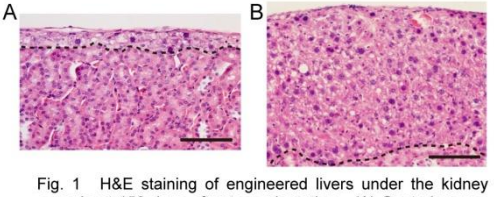

Fig. 1 H&E staining of engineered livers under the kidney capsule at 150 days after transplantation. (A) Control mouse (B) MCT/PH treated mouse. Dot lines represent the boundary between engineered liver and renal parenchyma. Bars: 100 μm

P082
Therapeutic effects of adipose-derived cells on chronic liver injury in mice

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Background: Chronic liver injury (CLI) is a serious disease that could lead to liver cirrhosis and hepatocellular carcinoma, and the establishment of new therapeutic modes including regenerative medicine is highly desired. Adipose-derived cells (ADCs) could be a promising candidate cells for various inflammatory disorders because of their anti-inflammatory property. Here we investigated the therapeutic potential of ADCs on mice with CLI.
Materials & Methods: CLI model mice were created by repeating the subcutaneous injection of carbon tetrachloride (CCl_4) to female C57Bl/6 mice (1 mL/kg, twice a week for 4 weeks). After being isolated from inguinal adipose tissues of male C57Bl/6 mice, ADCs were transplanted to the recipient CLI mice via tail veins. Three different numbers of ADCs were transplanted (low: 1.5×10^3, middle: 1.5×10^4, and high: 1.5×10^5 ADCs/mice). CCl_4 injections were continued after the procedure. Seven days after the ADC transplantation, mice were subjected to blood biochemical tests, histological examinations, and gene expression analyses.
Results: None of the recipient mice showed any observable adverse event after ADC transplantation. Serum AST, ALT, and LDH values of the CLI mice with high-ADCs were significantly lower than those in no-ADCs control group (AST: 1736 ± 495 vs 5092 ± 3113, ALT: 3132 ± 1015 vs 6846 ± 2791, and LDH: 4530 ± 1936 vs 16492 ± 951 IU/L). Prothrombin times of CLI mice with high-ADCs were significantly shorter than those of the control mice (11.5 ± 0.6 vs 14.6 ± 2.4 sec). Histological analyses revealed the significant attenuation of liver injury in the CLI mice with high-ADCs. Liver samples of high-ADCs group showed the down-regulation of inflammation-related gene expressions (TNFαand IL-6).

Conclusions: The present study demonstrated that ADCs-based therapy could provide therapeutic potentials for CLI at least in part by attenuating the inflammatory status of the liver.

P083
In vivo propagation and genetic modification of hepatocytes toward gene and cell therapy

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Background: Hepatocyte-based therapies have attracted attentions for many forms of liver diseases including liver-based inherited diseases as a new generation therapy. However, present cell culture condition is unable to give sufficient proliferation of hepatocytes. If hepatocyte proliferation systems that can be also used for efficient gene transduction are established, the system will offer a great advance for developing a valuable therapy. The present study aimed at developing the system using hepatocytes from hemophilia, as one example of liver-based inherited diseases.
Methods: Hepatocytes were isolated from the liver of coagulation factor IX-KO (FIX-KO) mice, a mouse model of hemophlia B. A total of $5x10^5$ hepatocytes were transplanted into the liver of uPA/SCID mice, and the liver repopulation status with FIX-KO hepatocytes was assessed by clotting assay and genomic analysis. After the complete repopulation of uPA/SCID liver with FIX-KO hepatocytes was achieved, AAV vector (AAV8-hF.IX16) was injected through the tail vein. Blood samples were periodically collected for assessing of FIX activity and antigen levels.
Results: The plasma FIX activity of recipient uPA/SCID mice showed a progressive decrease after FIX-KO hepatocyte transplantation. At 8 weeks, FIX activity became undetectable (< 0.5% normal mouse plasma), and the genomic analyses showed FIX-KO hepatocytes occupied more than 99.5 % hepatocytes of the recipient uPA/SCID, indicating that the transplanted FIX-KO hepatocytes actively proliferated to fully reconstitute the uPA/SCID livers. After AAV infusion, FIX-KO hepatocyte-repopulated uPA/SCID mice showed a persistent high plasma hFIX levels (> 50,000 ng/mL) that was similar to hFIX levels observed in AAV-injected naïve FIX-KO mice.
Conclusions: The present studies demonstrated that fully-reconstituted-hemophilic livers can be made in uPA/SCID mice, and the livers can be also efficiently gene-modified with AAV vector. The cell transplantation experiments using the propagated and gene-corrected hepatocytes to FIX-KO mice are in progress.

P084
Intrasplenic transplanted liver parehchymatous cells form bile canaliculi

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Isolated liver parenchymatous cells contain hepatocytes (HC), cholangiocytes and most likely hepatic stem cells. We worked out a method for successful intrasplenic transplantation of large numbers of hepatocytes not undergoing elimination by scavenger cells. Recipients of HC were irradiated with 8 Gy, received I.V. 0.1mg/kg b.w. of AAGM1 antiserum on day 2, were reconstituted with 10^7 syngeneic BMC on day 3 and grafted on the same day with 10^7 of syngeneic HC into the spleen. Six months later, trabeculae-forming glycogen-rich hepatocytes could be seen. Interestingly, we found that recipients with ligated common bile duct also formed canaliculi, whereas, those with free bile flow did not. Moreover, transplanted hepatocytes remained at a distance from canaliculi.
Aim: To prove that the cells forming canaliculi are cholangiocytes, by studying their phenotypes and compare them with those from recipient liver parenchyma.
Methods: HC were transplanted as described above. Common bile duct was ligated. Three months later spleens were harvested, snap-frozen, and slightly stained with hematoxilin. Cholangiocytes forming canaliculi were harvested using laser capture microscope. Liver cholangiocytes were isolated using routine technique. Both cholangiocyte populations were stained for keratin 19 and gamma- glutamyl transpeptidase.
Results: Cholangiocytes from both sources were of various sizes. They were keratin 19 and gamma- glutamyl transpeptidase positive and expressed genes for these proteins.

Conclusions: Newly formed cholangiocyte phenotypes didn't differ from those of recipient liver. Open questions remain why hepatocytes and cholangiocytes did not form lobules but grew separately and which humoral factors were produced in liver with bile stasis responsible for formation of canaliculi.

P085
Transplanted hepatocytes are destructed in the process of innate immunity-methods of prevention

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Hepatocyte (HC) transplantation is a therapeutic modality striving for establishing its place in clinical transplantology. The transplanted (tx) hepatocytes need proper environment niche at the site of grafting in order to resume their function. These conditions are: supply of nutrients (blood perfusion), prevention from attack of scavenging cells as granulocytes and macrophages, contact with stromal cells and supply of specific growth factors.
Aim: To protect the tx HC by elimination of the recipient scavenger and NK cells, stimulation of tx HC function by partial hepatectomy and bile duct ligation.
Methods: LEW rat recipients of HC were irradiated with 8 Gy, received I.V. 0.1mg/kg b.w. of AAGM1 antiserum on day 2, were reconstituted with 10^7 syngeneic BMC on day 3 and grafted on the same day with 10^7 of syngeneic HC into the spleen.
Results: Successful 6 month intrasplenic survival of transplantated HC was obtained. On histology, glycogen-rich but lacking albumine hepatocytes were seen. Many HC have lost their rectangular shape, became disfigured being sqeezed between fibroblasts.
Conclusions: Attenuation of the innate response bytemporary elimination of scavenging and NK cells from the spleen and stimulation of hepatocyte proliferation by partial hepatectomy and bile duct ligation resulted in protection of the tx HC and their formation of trabeculae and bile ducts. Fibroblast and collagen accumulated along the HC trabeculae but not in other parts of spleen. It is possible, that stellate cells transplanted together with HC are responsible for fibrous tissue formation at the site of HC implantation.

P086
Searching for keratinocyte spore-stem cells

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Rationale. Keratinocyte (KC) stem cells are located in the basic epidermal layer and hair follicle bulge. They express the p63 and CD29 antigens and proliferate slowly retaining DNA label. Moreover, they initiate the transient daughter cell proliferation. This has been observed under normal conditions as well as in hyperkeratoses. However, it remains unclear how can basic layer KC crawl upon epidermis-deprived surfaces as wounds and ulcers. We noticed that KC covering edges of ulcers originate not from basic layers but from stratum spinosum. The question arouse as to whether these cells are not another form of KC stem cells, the so called spore-stem cells. Aim. To study which KC population covers healing ulcers. Methods. Study was carried out on 15 patients with long lasting leg venous ulcers. Microscopical glass was laid upon ulcer surface and its edge and kept for 24h. Cells from granulation tissue and ulcer edge adhered to glass. This procedure was repeated every other day for 10 days. Adherent cells were stained for p63, CD29, PCNA, Ki67 and keratin 6.16 and 17. Viability test based on KC enzymatic activity was done. Results. Among the whole population of infiltrating granulocytes and macrophages single large cells of a diameter of 20-30 microns with small nucleus resembling by shape those from stratum spinosum and granulosum, revealing full enzymatic activity were identified. They were more numerous close to the ulcer edge. They were p63 and CD29-negative. Some of them underwent mitoses, others had two small nuclei. No other type keratin-containing KC could be seen. Conclusions. Large nucleated KC colonize ulcer surface close to its edge but some could also be seen dispersed on ulcer surface far from edge forming small colonies. The phenotype of these cells was different from that of epidermal basic layer KC.

P087

Mitogenic effects of human tissue fluid/lymph containing cytokines on keratinocyte proliferation

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Background: Cultured keratinocytes (KC) are needed for covering large burn wounds and ulcers. They are cultured in artificial media, however, the yield is always low and viability is limited. We found previously that human skin tissue fluid and lymph (TF/L) contain high levels of growth factors and cytokines. TF/L may then serve as the most physiological medium for KC proliferation.
Aim: To study the effect of human TF/L containing IL-1β, IL-6, TNF-α, KGF, TGF-βon human KC from lower extremity skin and to show which cytokines and growth factors of human skin TF/L have influence on KC: proliferation, differentiation and expression of epidermal stem cell markers
Material and methods: Isolated KC were cultured for 1 to 14 days in TF/L and standard medium as a control. Neutralization of IL-1β, IL-6, TNF-α, KGF, TGF-β in TF/Land blocking their receptors on KC helped to define which cytokine stimulated KC proliferation and differentiation.
Results: KC cultured in TF/L showed higher percentage of mitotic and basal layer cells as well as lower percentage of differentiated cells from upper layers vs control. Higher percentage of p63(48 vs 8), CD29(52.4 vs 41,4), Ki67(57 vs 23,8), PCNA(63 vs 38), CK6(15,5 vs 4,4), CK17(10,6 vs 5,5), CK16(26,4 vs 15,3) and decrease in percentage of CK 10(52 vs 77,5), filagrin(19,6 vs 48,5) and involucrin(18,8 vs 45,3) positive KC was observed vs control (all $p<0.05$). Neutralization of IL-1β, IL-6, TNF-α, KGF and blocking their receptors on KC caused decrease in percentage of mitotic cells. Quantitative evaluation of KC growth revealed higher proliferation rate ratio after KC culture in TF/L than control medium. Neutralization of studied cytokines and growth factors, except of TGF-β, revealed decreased proliferation of KC.
Conclusion: TF/L containing natural cytokines have a stimulating effect on KC proliferation and expression of KC stem cell markers.

P088
Cold-preservation of freshly isolated human adult hepatocytes for liver cell therapy

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Currently, liver organ transplantation is the gold standard treatment of hepatic failure, hepatocellular deficiency and genetic metabolic disorders. Liver cell therapy based on adult human hepatocyte infusion could be an alternative. During the last 15 years only 100 patients around the world have been treated with this procedure due to lack of viable cells and problems in cell conservation (freshly isolated cells and cryopreservation). In this context, and to start a liver cell therapy program in France, we evaluated the cold-preservation of freshly isolated hepatocytes in three different solutions commonly used for organ conservation: University of Wisconsin (UW), Institute George Lopez (IGL1) and hypothermosol-FRS (HT-FRS). Hepatocytes isolated from liver resections or donor livers were stored at 2-8°C in cryopreservation bags at 10e6cells/ml for 12 to 96h. In all cases, cell viability decreased with time. However after 12 and 24h (for IGL1 and HT-FRS) more than 60% hepatocytes were still viable (acceptable limit for human cell infusion). Following the 12-24h cold-preservation period, hepatocytes were cultured on collagen coated dishes and their phenotype was analysed. Urea synthesis, plasmatic protein production and CYP450 induction were close to that observed in primary culture of freshly isolated hepatocytes. Preliminary analysis of transplantation experiments in mice indicated that cold-preserved human hepatocytes were able to engraft and were functional as demonstrated by the presence of human albumin in mice sera. In conclusion, cold-preservation of human hepatocytes in suspension allows the viability and functionality for 12 h after isolation. This time window could be usefull for liver biotherapy development as it allows cell transport, patient preparation and repeated infusions.

P089

HEPG2C3A and primary human hepatocyte encapsulation for liver implantation

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The liver is an organ with a high ability of regeneration, but some factors such as diseases, drugs or alcoholism lead to cirrhosis. Up to now, liver transplant is the only treatment available in the most severe cases and many patients die waiting for an organ. Several artificial and bioartificial systems are under study, aiming at replacing either detoxication or whole liver functions in an extracorporeal circuit. Such systems are of extreme interest for the patient's recovery during acute phases. In parallel, injection of hepatocyte encapsulated in porous biomaterials can be proposed to supply liver function on longer term basis. The UTC laboratory has a strong expertise in the area of hepatocyte encapsulation in alginate beads, which led to the design of a fluidized bed bioartificial liver. In the present study, our objectives consist in screening different types of biomaterials to optimize implantation of cells in the host. Cell encapsulation will prevent them from immune rejection and act as a niche in the liver. In a first step, several materials such as collagen or fibrinogen, proteins often used in hepatocyte cultures, were combined with alginate or directly composed the gel. Beads were produced using either a co-axial air flow extruder (home made design). In a second step, hepatic cells (human cell line HepG2C3A and primary human hepatocytes) were mixed with the most promising biomaterials. The viability of encapsulated cells and their functionalities were compared to those observed in our "basic" alginate beads. In association with the INSERM group in Montpellier (France), several configurations will be implanted in a rodent model, in order to reinforce the feasibility of the approach. Specific experiments will be developed to localize the position of the cells hosting beads.

P092
Improved function and proliferation of INS-1 cells cultured on low-fouling surfaces bearing CDPGYIGSR and RGD peptides, and fibronectin

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Rat insulinoma cells (INS-1), an immortalized pancreatic beta cell line, were cultured on substrates bearing low-fouling carboxymethyl dextran (CMD) on which fibronectin and two peptides derived from the extracellular matrix (ECM) were covalently linked to study the function and proliferation of INS-1 cells in respect to the ECM-cell interaction. The ECM peptides used were RGD (Arg-Gly-Asp) and CDPGYIGSR (a synthetic laminin nonapeptide). INS-1 cells were non-adherent on CMD and RGE (Arg-Gly-Glu)-CMD, with some unspecific binding on RGE-CMD, but adhered tightly to fibronectin, the two peptides and to tissue culture polystyrene (TCPS) surfaces. When cultured on fibronectin, RGD and CDPGYIGSR, INS-1 cells showed higher insulin secretion, when stimulated with glucose, compared to TCPS, CMD, and RGE (negative control). The cell number was measured with a CyQUANT® Assay estimating the DNA content of the samples. The INS-1 cell number increased in all conditions with the least cell number on CMD and the highest on fibronectin. Immunostaining for E-cadherin and the integrins αvß3 and α5 showed no convincing differences between the conditions but proved that cell-cell contacts supported cell proliferation in the non-adherent CMD and RGE-CMD systems, and confirmed the integrin-mediated cell binding to the ECM-covered surfaces. Overall, INS-1 cells exposed to fibronectin-, CDPGYIGSR-, and RGD-modified CMD surfaces showed elevated insulin secretion and increased cell numbers and therefore better function.

P093
Porcine pancreatic islets cultured in fibrin show improved resistance towards hydrogen

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In diabetes, the functional loss and destruction of ß-cells is mediated in part by the secretion of pro-inflammatory cytokines and cytotoxic reactive oxygen species. This study hypothesizes that cell-extracellular matrix interactions improve islet function and survival. 1,000 islet equivalents (IEQ) from porcine origin were embedded in fibrin or, as

a control, seeded on tissue culture polystyrene (TCPS) well plates to study the islets ability to resist cytotoxic reactive oxygen species i.e., hydrogen peroxide (H_2O_2) used in two different concentrations (100 μM, and 500 μM). Fibrin-embedded islets exerted sprouting activity if cultured without or with 100 μM H_2O_2, whereas islets in fibrin incubated with 500 μM H_2O_2 showed none or almost no sprouting activity. Islets in fibrin seemed to be protected against H_2O_2 compared to those on TCPS which were widely disaggregated with 500 μM H_2O_2. Paraffin-embedded islets were stained for insulin and glucagon. Insulin and glucagon expression was more pronounced in islets cultured in fibrin compared to those on TCPS. Overall, H_2O_2 incubation led to decreased expression in all conditions. A TUNEL assay was performed with the fibrin-embedded islets. Glucose-stimulated insulin secretion was carried out to compare culture conditions. This study reveals that pancreatic islets are better protected against reactive oxygen species if embedded in fibrin gels.

P094
Particulate oxygen generating substances (POGS) enhance the viability of encapsulated human islets

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Extended periods of hypoxia during processing prior to transplantation limits the viability of encapsulated islet grafts. In the present study, we explored the potential use of POGS to enhance the viability of encapsulated islets. We examined O_2 release profiles of two types of POGS, CaO_2 and MgO_2, from alginate microbeads, and selected MgO_2 for further studies. Islets were isolated from cadaveric human pancreas using the Ricordi technique, and following purification, were cultured for 24 hours in Memphis Serum Free Media (MSFM). Islet samples were then suspended in either 1.5% ultra-pure low-viscosity high mannuronic acid (LVM) alginate alone, or with 2.5% MgO_2 + 100 U/ml Catalase +1.5 mM Trolox. The islet suspensions were extruded through a microfluidic device set at a flow rate of 1.4 ml/min and an air pressure of 15 psi to form microcapsules with a mean ± SD diameter of 400 ±100 μM. After crosslinking in a solution of 1.1% $CaCl_2$ and 10 mM HEPES, microcapsules were cultured in MSFM for 24 hours. Live/Dead stains with carboxy-fluorescein diacetate (CFDA) and Propidium Iodide (PI) were performed on the islets after an additional 24 hours culture. Confocal microscopy was used to image islets and viability was quantified using NIH Image J software. The duration of O_2 release from POGS was significantly reduced by the presence of antioxidants. Using ANOVA, the %mean ± SD viability of naked (control) islets was lower (7.1 ± 6) than that measured in islets encapsulated alone 47.4 ± 5.6 ($p<0.01$, n=3). The viability of islets encapsulated with MgO_2+Catalase + Trolox was 76.2 ±10.9, which was greater than that measured in islets encapsulated alone (($p<0.05$). These data suggest that the microcapsule 3D environment enhances the viability of islets in culture and that O_2 delivery from POGS promotes encapsulated islet cell viability in the presence of antioxidants.

P095
INS-1 cell responses towards biomimetic surfaces bearing the CDPGYIGSR peptide following down regulation of the 67 kDa laminin receptor

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The 67 LR (67 kDa laminin receptor) is a non-integrin cell surface receptor that mediates high-affinity cell binding to laminin. The minimal sequence required for 67 LR recognition is the YIGSR peptide enclosed in the laminin beta 1 chain. In the present work, down regulation of the 67 LR in INS-1 cells (rat insulinoma cell line) was carried out via RNA interference to investigate the cell responses towards surfaces bearing CDPGYIGSR and the effects on cell adhesion and glucose-stimulated insulin secretion. INS-1 cells were transfected with 67 LR siRNA and the expression of 67 LR was evaluated 48 hours later by Western blotting and RT-PCR analysis. Bioactive surfaces were engineered to study cell-substrate interactions using a well-controlled environment. To limit non-specific cell adhesion, synthetic CDPGYIGSR laminin peptide or GRGDS peptide were covalently immobilized on carboxymethyl dextran (CMD) low-fouling surfaces. Transfected and non-transfected INS-1 cells were used to perform cell adhesion assay on CMD (negative control), GRGDS (positive control) and CDPGYIGSR. Cell

adhesion was evaluated after 3 hours. Also, changes in cell morphology were observed during the first 24 hours. Transfected and non-transfected INS-1 cells were plated on CDPGYIGSR surfaces, were glucose stimulated and then insulin secretion was quantitatively determined. INS-1 were transfected and the expression of 67 LR was successfully down regulated. The receptor-ligand interaction was compromised by knocking down the 67 LR, as evidenced by a high reduction of cell adhesion on CDPGYIGSR surfaces observed 3 hours after seeding.

P096
Effect of separation distance between INS-1 cell and endothelial cell monolayers in a 3D environment over INS-1 cell function

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Signals from endothelial cells are important for pancreatic beta cells to exert their function. Also, during development, pancreatic cells can produce VEGF to attract endothelial cells, resulting in the formation of a vascular network within the islets. In this study, a cell culture chamber was built using micro-fabrication techniques and validated to study interaction between monolayers constituted of INS-1 cells (rat insulinoma cell line) and endothelial cells separated by precise distances. Cell monolayers were first formed by culturing separately INS-1 cells and HUVEC (human umbilical vein endothelial cells) on biomimetic surfaces made of CMD (carboxymethyl dextran) layers on which the cell adhesive RGD peptide was grafted. Then, these flat surfaces (one bearing INS-1 cells and the other HUVEC) were positioned vertically to face each other in the cell culture chamber allowing to precisely vary the separation distance between these surfaces. The interstitial space between the two surfaces was filled with fibrin, allowing cells to migrate into it. The separation distance was adjusted from 100 to 200µm. Cell culture was carried out for 72 hours. To distinguish cell types, HUVEC were labeled with Qtracker® cell labeling kit and samples were imaged by microscopy. Cell migration was assessed as well as insulin secretion following glucose stimulation.

P097
Technological characterization of silk fibroin non-woven scaffolds for adipose-derived stem cells and pancreatic islet co-transplantation

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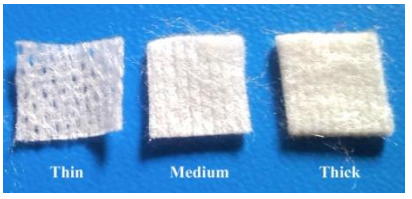
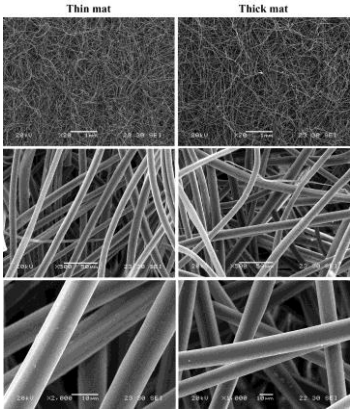
Allogeneic pancreatic islet transplantation in the liver is a therapeutic approach for the type I diabetes mellitus; alternative implant sites could improve long-term islet graft efficiency. Moreover, islet co-transplantation with mesenchymal stem cells have been proposed to promote the neo-vascularization, and to modulate the immune/inflammatory response, preventing the rejection of the graft. Such approach requires a medical device suitable to support islet and mesenchymal stem cell survival and function: in this work silk fibroin non-woven mats were designed as scaffolds for mesenchymal stem cell and pancreatic islet co-transplantation. Three different fibroin non-woven mats (thin, medium and thick) were obtained with a large scale water entanglement method: the scaffold thickness does not influence the fiber orientation and their distribution (Fig. 1). Mats were then sterilized by autoclaving or gamma rays, and characterized by scanning electron microscopy (SEM), energy dispersive X-ray (EDX) analysis, tensile strength and stretching, Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC). The SEM investigation of thin and thick scaffolds (Fig.2) indicates that the microstructure is similar: both scaffolds present evident porous, homogeneous aspect (Fig.2 top), paralleled fibers (Fig.2 center), with smooth surface, appreciable at higher magnification (Fig.2 bottom). The morphological investigation reflects an optimized mat production process to obtain scaffolds with very different macroscopical characteristics, but similar texture and porosity. EDX analysis underlines the high fibroin purity: carbon, oxygen and sulfur are the main chemical elements in the fibroin composition, and no contaminant heavy metal from water entanglement process were detected. The non-woven fiber orientation presents a significant effect on mechanical properties, making it suitable for use as scaffold for tissue engineering. FTIR and DSC analyses demonstrate that sterilization does not induce fibroin degradation. In conclusion, non-woven mats present high porosity and purity of

fibroin fibers, stability after sterilization process, and mechanical properties suitable for their use as scaffold for islet-mesenchymal stem cell co-transplantation. Technological resources are available for large scale and low cost silk fibroin non-woven production, intended for advanced therapies, and following GMP guidelines.





P098
Preparation and characterization of films using silk fibroin, pectin and glycerin for bioengineered skin

Marta Galuzzi[1], Theodora Chlapanidas[2], Marta Cecilia Tosca[1,2], Silvio Faragò[3], Giulia Lucconi[2], Barbara Antonioli[1], Daniele Vigo[4], Massimo Faustini[4], Mario Marazzi[1], Maria Luisa Torre[2]

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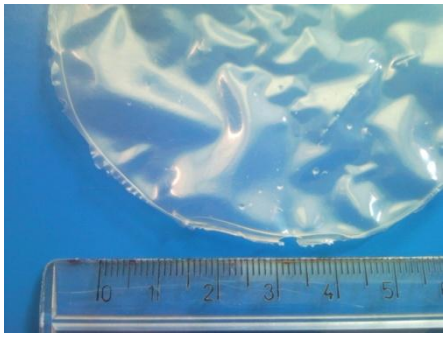
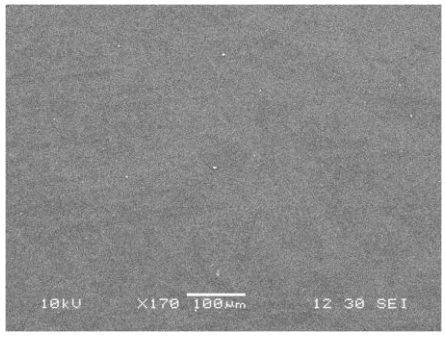
Silk fibroin, the main protein obtained from Bombyx mori cocoons, is an excellent material for tissue engineering. Fibroin fibers can be processed in different ways in order to obtain various scaffolds (for example films, gels, sponges or mats). Nowadays, for wound dressing, bioengineered skin is employed; often these products must be removed and replaced with new cell-enriched scaffolds: this technique could delay the skin tissue regeneration. The aim of this work is to prepare an adequate film based on silk fibroin, pectin and glycerin for skin tissue engineering. Bombyx mori cocoons were degummed in autoclave, washed and dried. Fibroin fibers were solubilized in calcium nitrate/methanol solution. Eighteen films were obtained using different fibroin-pectin-glycerin ratios with casting method. The first group of films was composed by fibroin and pectin only (pectin weight composition from 1 to 10 %): pectin was added to allow fibroin conformational transition. In the second and the third group of films, glycerin was added as a plasticizer: the second group of films was composed by pectin:glycerin in rate 1:1, while third group by pectin:glycerin in rate 1:2, using the concentration of pectin from 1 to 10 %. Films were then sterilized by autoclave, and characterized before/after sterilization by Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC) analyses. Morphological investigations were performed by scanning electron microscopy. A stable fibroin conformation was obtained using pectin in concentration of 6%, but films composed by fibroin and pectin only were inadequate for tissue engineering because of their stiffness and fragility. The best results were obtained using pectin:glycerin in rate 1:2 and the best formulation was 82% fibroin, 6% pectin and 12% glycerin (% weight composition) (Fig.1). The SEM investigation (Fig.2) indicates that films maintained their structural integrity after sterilization, with homogeneous and smooth surfaces. FTIR and DSC analyses demonstrate that sterilization does not induce fibroin degradation. In conclusion, silk fibroin films present highly smooth surface, stability after sterilization process, and mechanical properties suitable for their use as scaffold for wound dressing and regenerative medicine.



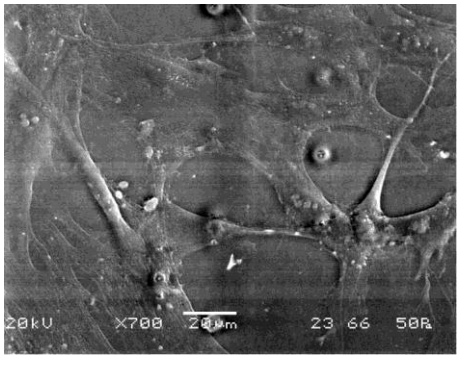


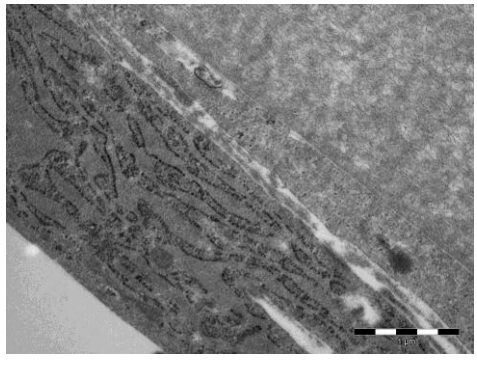
P099
Silk fibroin film and adipose-derived stem cells as feeder layer for skin advanced therapy

Marta Cecilia Tosca[1,2], Theodora Chlapanidas[2], Silvio Faragò[3], Barbara Antonioli[1], Marta Galuzzi[1], Giulia Lucconi[2], Daniele Vigo[4], Massimo Faustini[4], Mario Marazzi[1], Maria Luisa Torre[2]

[1]Struttura Semplice Terapia Tissutale, Azienda Ospedaliera Ospedale Niguarda Ca' Granda, Milano; [2]Dipartimento di Scienze del Farmaco, Università di Pavia, Pavia; [3]Laboratorio di Microscopia Elettronica, Stazione Sperimentale per la Seta, Milano; [4]Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università di Milano, Milano; Italy

Nowadays, for skin tissue engineering, keratinocytes are isolated, cultured in vitro and then seeded into a scaffold; this procedure presents essentially two limits: keratinocyte culture needs a feeder layer (generally animal cells) to grow, and the biodegradation of scaffold is too slow when implanted in vivo, and then it must be removed. Silk fibroin, extracted from Bombyx mori cocoons, is an optimal biomaterial for tissue engineering due to its high biocompatibility, biodegradability, elasticity and resistance. The aim of this work is to evaluate adipose-derived stem cell (ADSC) adhesion and proliferation on silk fibroin films as a feeder layer for regenerative medicine purposes. Films were prepared by casting method, using silk fibroin, pectin and glycerin, and sterilized in autoclave. Human adipose stromal vascular fraction was plated on plastic surface and adherent ADSC were expanded till the 3rd passage. Cells were then cultured on films (20,000 cells/cm2 of film) for 15 days at 37 °C, 5% CO2 and characterized by optical and electronic (SEM and TEM) microscopy. Silk fibroin films maintained their structural integrity till the end of cell culture. The morphological investigations showed that cells adhere to the support, with a simil-fibroblastic shape (fig.1) and reach confluence. The ultrastructural analysis indicated the presence of adhesion proteins that promote cell anchorage to the film, forming a multilayered cell structure; moreover, typical active-cell features as nuclei, mitochondria, rough endoplasmic reticulum, lysosomes and vacuoles were observed (fig. 2). In conclusion, silk fibroin films are promising devices for the culture of ADSC as an autologous feeder layer during the production of bioengineered skin.





P100
Adipose-derived stem cell encapsulation in alginate for advanced therapies

Theodora Chlapanidas[1], Marta Cecilia Tosca[1,2], Melissa Mantelli[3], Maria Antonietta Avanzini[3], Giulia Lucconi[1], Marta Galuzzi[2], Barbara Antonioli[2], Daniele Vigo[4], Massimo Faustini[4], Mario Marazzi[2], Maria Luisa Torre[1]

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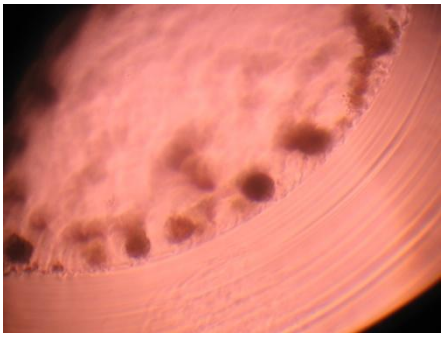
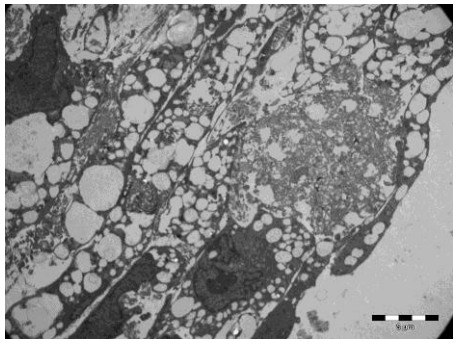
The use of adipose-derived stem cells (ADSC) has recently been proposed in tissue engineering and regenerative medicine to control several inflammatory and autoimmune pathologies, as well as graft-versus-host disease. Their therapeutic potential may be explained by the release of growth factors and cytokines mediating paracrine actions. ADSC can be thought of as a "site-regulated drug factory/store" and, for clinical purposes, different approaches may be implemented: cell administration by systemic intravascular perfusion or implantation of a drug delivery system. The aim of this study was to investigate the behavior of ADSC loaded in implantable microcapsules for advanced therapies. Human ADSC expanded until passage 3 were added to a saturated CaCl2 solution and the resulting suspension was added dropwise to a sodium alginate solution under stirring. Obtained capsules were cultured for 28 days, collecting supernatants once a week. Adherent cells were also cultured following the same conditions, as a control. ADSC characterization was performed by capsule size distribution and weight, optical and electronic (SEM and TEM) microscopy and flow cytometric analysis. Cytokine secretion was evaluated in supernatants by ELISA. Capsules exhibited structural integrity until the end of culture. ADSC maintained their viability, with a spherical shape, and aggregated into clusters migrating to the inner alginate membrane surface since the third day of culture (Fig.1,10X). After twenty-eight day of culture, TEM images indicated the presence of nuclei, endoplasmic reticulum, mitochondria and a large numbers of vacuoles, typical of active, vital cells (Fig.2). Low percentages of encapsulated ADSC expressed the surface antigens CD73, CD105 and CD13 compared to control ADSC, while they remained negative for HLA-I, CD33, CD34, CD45 expression. Lower amounts of IL-6, IL-7 and IL-8, were secreted compared to adherent ADSC. Very low levels of TNF-α and INF-γ were detected in supernatants of both encapsulated and adherent ADSC. In conclusion, differences in phenotype and cytokine secretion between encapsulated and adherent ADSC have been observed; whether these differences influence their in vitro and in vivo activity should be further investigated.





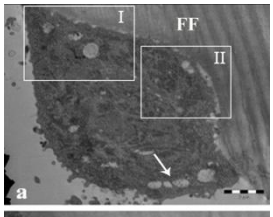
P101
Culture of adipose stem cells and pancreatic islets on fibroin non-woven scaffolds for co-transplantation in diabetic pathologies

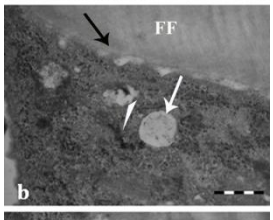
Barbara Antonioli[1], Marta Cecilia Tosca[1,2], Theodora Chlapanidas[2], Silvio Faragò[3], Marta Galuzzi[1], Giulia Lucconi[2], Daniele Vigo[4], Massimo Faustini[4], Mario Marazzi[1], Maria Luisa Torre[2]

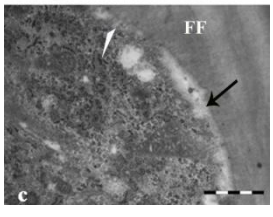
[1]Struttura Semplice Terapia Tissutale, Azienda Ospedaliera Ospedale Niguarda Ca' Granda, Milano; [2]Dipartimento di Scienze del Farmaco, Università di Pavia, Pavia; [3]Laboratorio di Microscopia Elettronica, Stazione Sperimentale per la Seta, Milano; [4]Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università di Milano, Milano; Italy

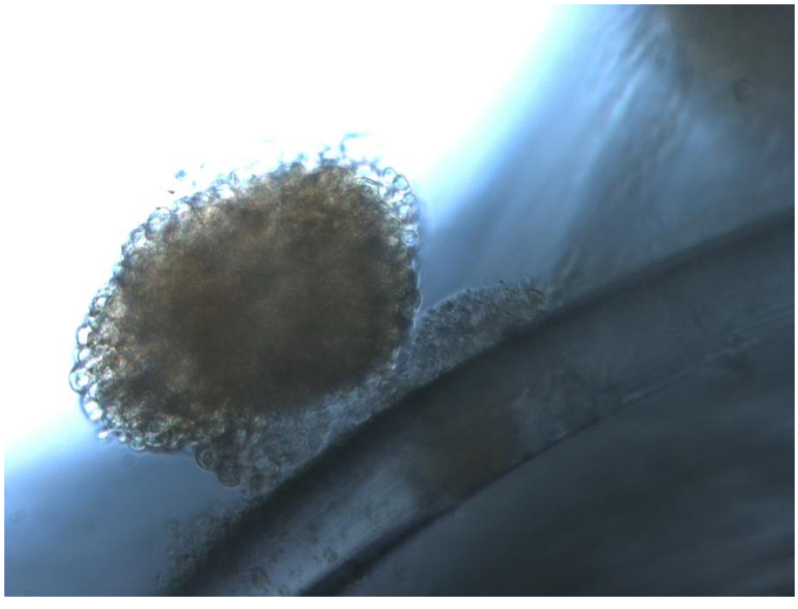
Allogeneic pancreatic islet (PI) transplantation aims to a constant physiological glycemic control in type I diabetes, although it presents some limits: the scarcity of donor pancreas, the need of two donors for a single transplant and the loss of PI function due to an immediate inflammatory reaction. Mesenchymal stem cells have been proposed to promote graft vascularization and modulation of immune response, preventing the rejection of the graft by reducing the inflammatory cytokine production. The aim of this work was to evaluate adipose derived-stem cells (ADSCs) and PI attachment on non-woven silk fibroin scaffolds as a novel therapeutic platform for co-transplantation. The idea is based on the separately culture of these two cell lines and the assemblage of the two modules at the time of transplantation; stem cells could be isolated from patient and cultured before PI availability. ADSCs and PI are isolated and separately cultured on scaffolds produced with the water entanglement method: ADSCs for 15 days, while PI for one day. Morphological (SEM and TEM) and immunohistochemical investigations were performed. Non-woven fibroin scaffold appears as a compact, tangled network; mat-forming fibers are smooth and

isodiametrical. When ADSCs are cultured for 15 days, both abundant extracellular matrix and adhered cells can be appreciated; the cells migrate inside the scaffold and colonize it, and the cytoplasmic pattern is typical for fully active cells (Fig.1 TEM of ADSCs after 15 days of culture (a). The insets I and II in (a) are magnified in (b) and (c), respectively. FF: fibroin fibers; White arrows: vesicles; black arrow: cell-fibroin fibers interface; white arrowheads: rough endoplasmic reticule. Bar in (a): 2 µm; bars in (b) and (c): 1 µm.). PI after a 1-day culture closely adhere to the fibres, and porosity allows the cell distribution inside the scaffold (Fig.2, 20X). The immunostaining of cultured PI shows a slight positivity to glucagon and a more marked one to insulin. In conclusion, fibroin non-woven scaffolds is a promising support for the culture both of ADSCs and PI; the feasibility of culturing separately cells and assembling them before transplantation may improve the follow-up of diabetes treatment, reducing the inflammatory response and the number of requested PI. The prototypical formulation represents a novel platform which may be modified to meet various clinical requirements.









P102

Assessment of pancreatic tissue oxygenation via fluorine magnetic resonance spectroscopy during culture on silicon rubber membrane

Thomas M. Suszynski[1], Samuel A. Stein[1], Efstathios S. Avgoustiniatos[1], Eric J. Falde[1], Jennifer P. Kitzmann[1], John R. Wilson[2], Bruce E. Hammer[3], Klearchos K. Papas[1]

[1]Surgery, University of Minnesota, Minneapolis; [2]Wilson Wolf Manufacturing Corporation, New Brighton; [3]Radiology, University of Minnesota, Minneapolis; MN, United States

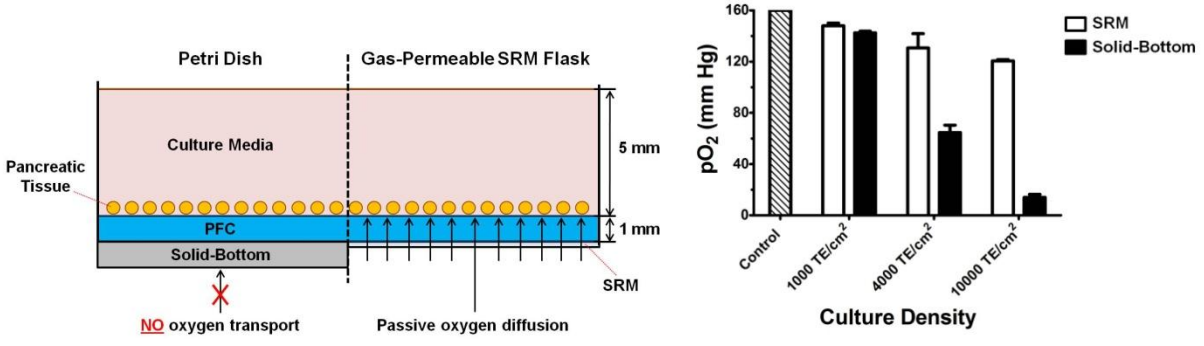
Introduction: Islet culture has become standard practice prior to clinical islet transplantation. Nevertheless, solid-bottom culture flasks are impermeable to oxygen and may not adequately oxygenate an islet preparation. It is believed that culture on silicone rubber membranes (SRM) will improve islet oxygenation, particularly when cultured at higher densities. In this preliminary study, we measured the average partial oxygen pressure (pO_2) underneath digested impure pancreatic tissue in both solid-bottom and SRM flasks using non-invasive fluorine magnetic resonance spectroscopy (^{19}F-MRS).

Methods: A human pancreas was isolated using standard protocol at the Schulze Diabetes Institute, and the post-purification impure fraction was cultured on both solid-bottom Petri dishes and SRM flasks at 1000, 4000, and 10000 tissue equivalents (TE, defined as the amount of impure tissue having the same DNA content as an islet equivalent) per cm^2 (Figure 1).

Figure 1: Schematic illustrating the solid-bottom Petri dish and SRM culture flask.

Run order was randomized and each condition was examined by ^{19}F-MRS at 14°C using a 5T spectrometer and centered within a volume (saddle) coil. A standard inversion recovery sequence was used to obtain a characteristic ^{19}F spin-lattice relaxation time (T1), which was converted to a steady-state average pO_2 estimate using a previously determined linear calibration (pO_2 (mm Hg) = $2.265 \cdot 10^6$/T1 (ms) – 881; R^2 = 1). Each condition was assessed in replicate (n = 3-4).

Results: Increasing the TE surface density (TE/cm^2) yielded a dose-dependent decrease in the measured pO_2 (Figure 2). Solid-bottom Petri dishes supplied less oxygen to islets than SRM flasks – as indicated by the significantly lower pO_2 measurements.
Figure 2: Mean pO_2 measurements (via ^{19}F-MRS) for all high density culture conditions in either solid-bottom Petri dishes or SRM flasks.
Conclusion: High-density culture of islets is possible on SRM due to adequate oxygenation. Non-invasive measurements of pO_2 using ^{19}F-MRS validate that tissue oxygenation during culture may be improved through the use of SRM.

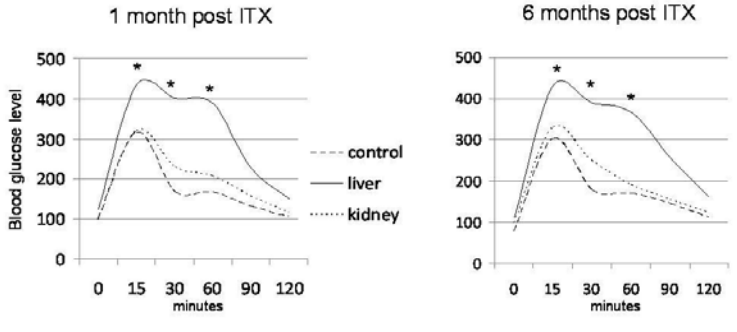


P103
Comparison study on functionality of pancreatic islets transplanted in the liver or under the kidney capsule of diabetic mice

Daria Zorzi, Tammy Phan, Boyd Carr, Luca Cicalese, Cristiana Rastellini

Deaprtment of Surgery, Texas Transplant Center, University of Texas Medical Branch, Galveston, TX, United States

Background: In the clinical setting, the liver is the site currently used for pancreatic islet transplant (ITX). Although results have improved overtime due to better immunosuppression and isolation techniques, the liver as implantation site may represent a limiting factor. The aim of this study is to investigate islet functionality at different post-operative time points when transplanted in the liver
or kidney as compared to native islets in control animals.
Material and Methods: Pancreatic islets where isolated from 9-12 weeks old C57BL10 male mice and transplanted in syngeneic streptozotocin induced diabetic animals (1,000islets/recipient). Study groups included: A (n= 4) intra portal and, B (n=4) under the kidney capsule ITX. Both groups were compared to control animals (n=9). Blood glucose levels and body weight (BW) were monitored. Intra peritoneal glucose tolerance tests (IPGTT) were performed at 1, 3 and 6 months post ITX. Glucose response at different IPGTT time points where compared and p value was calculated to determine significant differences.
Results: All animals included reversed diabetes within 1 week post ITX and no significant differences were observed in glycemic control and BW during the study period. However, when IPGTT were compared, the islets transplanted in the liver had a significant worse performance for all post ITX follow-up periods. One and 6 months post ITX mean IPGTT curves are shown in the figure (* $p<0.05$).
Conclusions: These data demonstrate that pancreatic islets transplanted in the liver have an early and late post ITX impaired response to glucose challenges suggesting that the liver might not be the ideal site for ITX.



P104

Continuous exenatide infusion markedly improves insulin sensitivity and disposition index in partially pancreatectomised baboons. Is Exenatide the long sought-after "Elixir of Life" for the Islet of Langerhans?

Franco Folli[1], Francesca Casiraghi[1], Alberto Davalli[1,2], Gregory Abrahamian[3], Amalia Gastaldelli[1,4], Raul Bastarrachea[5], Subhash Kamath[1], Roberta Petz[4], Pengou Zuo[1], Andrea Ricotti[1,4], Ana Paez[6], Paul Higgins[5], Anthony Comuzzie[5], Glenn Halff[3], Ralph DeFronzo[1], Alberto Chavez[1]

[1]Medicine-Diabetes, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [2]Medicine, HS Raffaele, Milan, Italy; [3]Transplantation Center, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; [4]Institute of Clinical Physiology, CNR, Pisa, Italy; [5]Genetics, Texas Biomedical Research Institute, San Antonio, TX, United States; [6]Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States

Exenatide (EXE) improves glycemic control in T2DM by enhancing insulin secretion. However, the effect of continuous chronic EXE infusion on α and β-cell function is not well understood. We studied the non-diabetic baboon (NDB) model to assess islets compensatory mechanisms and chronic effects of EXE.Baboons received a two-step hyperglycemic clamp followed by IV Arginine (0.15 g/kg) (HC+A) at baseline and underwent a partial pancreatectomy (PP) (~30%). For 13 weeks they received a constant IV infusion of: i) EXE (0.014 ug/kg h, n=12) or ii) saline (SAL, n=12) in NDB. Upon completion of treatment HC+A was repeated and remnant pancreas collected. β-cell function (Disposition Index=DI) was calculated as ISR*M/I, α-cell function as % suppression of glucagon secretion from baseline. A significantly decrease in body weight (EXE 18.24±0.81 to 17.23±0.65 p=0.034, SAL 18.49±0.62 to 16.67±0.7 kg p=0.006), fat mass (EXE 1.54±0.5 to 0.93±0.2 p=0.044, SAL 1.29±0.2 to 0.86±0.2 kg p=0.035), and lean mass (EXE 15.65± 0.4 to 15.22± 0.5 p=ns, SAL 16±0.5 to 15± 0.6 kg p=0.033) were observed. M/I increased ~68% after EXE and decreased by 19% in SAL. ISR increased significantly in SAL group (Basal-ISR 2.0±0.3 [Pre] to 4.3±1.0 [Post] pmol/min/g pancreas, p<0.04, Total-ISR 5.6±0.4 to 7.7±1.1 nmol/g pancreas, p=0.08) but not in EXE (Basal-ISR 3.0±0.7 to 4.5±1.1 pmol/min/g pancreas; Total-ISR 7.2±1.0 to 8.0±1.6 nmol/g pancreas, p=ns). DI increased after EXE (1.0±0.1 to 2.2±0.3, p<0.01), but not after SAL (1.5±0.2 to 1.7±0.3, p=ns). Glucagon suppression or insulin/glucagon ratio between groups did not change.EXE increased IS and DI, whereas SAL treated NDB had compensatory increase in ISR, following PP. Our study offers mechanistic insights on the functional pancreatic endocrine secretory capacity of NDB after PP and marked improvement in insulin sensitivity after EXE treatment through β-cell "rest", and suggesting a role in preserving long-term pancreas and islet transplants in humans.

P105

Schwann cell implantation for spinal cord injury repair: Maximum tolerated dose in a clinically-relevant contusive SCI model

Jeffrey Datto[1], Johana Bastidas[1], Miguel Martinez[1], Dimitri Tahal[1], Athauda Gagani[1], Alexander Marcillo[1], Damien Pearse[1,2,3]

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Schwann cells (SCs), the glia of the peripheral nervous system (PNS) are critical to PNS regeneration and when implanted into the injured spinal cord, improve both anatomical and functional recovery in experimental models. Pre-clinical studies to date using clinically-relevant contusion spinal cord injury (SCI) paradigms have largely only employed a single (efficacious) dose of SCs for spinal cord implantation of 2 million cells; however, for safety and toxicity purposes, it is unclear whether this dose is the maximum tolerated dose (MTD). The current pre-clinical study sought to determine the SC MTD in adult female Fischer rats receiving a moderate contusive SCI at the thoracic level using the MASCIS impactor. At 1 wk post-SCI a single stereotactic injection of either medium or SCs (in medium) at volumes of 3, 6, 9, 12, 15 or 18 µl (300,000 SCs per µl) was made into the injury site. Assessment for MTD was based upon both anatomical and functional outcome measures including; (1) leakage of the injectate from the site of medium/cell deposition after spinal cord injection, (2) a worsening of gross locomotor function as measured in the open-field using the BBB score, and/or (3) an increase in pain sensitivity thresholds on the paws as measured by cutaneous allodynia or thermal hyperalgesia responses. Examination of injection volumes revealed that while a 15 µl volume was tolerated by the injured spinal cord (medium or cells), a volume of 18 µl or greater resulted in significant extrudate, technically limiting the SC MTD to 15 µl. Subsequent behavioral analysis (sensory/locomotor) showed no worsening of behavior compared to injured, non-injected controls with all injected

volumes (medium or SCs), confirming 15 µl as the SC MTD for this SCI paradigm. This study provides important safety and toxicity data for translating SCs as a therapy for human SCI clinical trials.
Support: Dept. of Defense Congressionally Directed Medical Research Programs, The Miami Project to Cure Paralysis.

P106
A porcine model of intraportal islet allotransplantation: An in vivo model for the study of the instant blood-mediated inflammatory reaction (IBMIR)

Peta Phillips[1,2], Helena Hurst[1,2], Joanne Hawkes[1], Heather Burns[1], Sussan Davies[1], Shihani Stoner[1], Philip O'Connell[1,2], Wayne Hawthorne[1,2]

[1]Centre for Transplant and Renal Research, Westmead Millennium Institute; [2]Western Clinical School, The University of Sydney; Westmead, Australia

In the immediate post-transplant period as many as 50-70% of islets are destroyed primarily due to an inflammatory and thrombotic process termed IBMIR. IBMIR is characterised by rapid activation and binding of platelets to the transplanted islets, along with activation of the complement and coagulation systems. The aim of this study was to develop a relevant animal model of IBMIR and identify the early innate immune response to islet transplantation. Porcine neonatal islet cell clusters (NICCs) were transplanted intraportally into allogeneic recipients as a model of IBMIR. Four pigs (20-25kg), were transplanted with 10,000-14,000 IEQ/kg of NICCs. Blood samples were collected at multiple time points post-infusion and used to assess parameters of complement activation and thrombosis. Liver biopsies were performed 2hrs, 24hrs, and D3 post-islet transplantation.By 6 hours post transplant there was a prompt and significant increase in fibrinogen (1.8±0.2g/L to 3.7±0.3g/L p=0.03), thrombin antithrombin (TAT) levels (6.4±1.3µg/L, to 53.5±11.7µg/L, p=0.03) and a decrease in antithrombin III (ATIII) levels (108±4.4% to 86±7.8%, p=0.06) which all returned to baseline by 48-72hrs. By contrast lymphocyte numbers steadily decreased in the first 12hrs (p=0.03), followed by a significant increase (p=0.04) by 48hrs. Corresponding with changes in fibrinogen and TAT levels, neutrophil numbers increased (p=0.03) at 6hr post-transplant, returning to baseline by 24-72hrs. Liver biopsies showed NICCs were heavily infiltrated by T cells at 24hrs with an increase in the T cell infiltrate out to D3. Following NICC allotransplantation there was an immediate activation of thrombosis and neutrophils. This was followed by a rapid recruitment of T cells into the graft, suggesting that the early innate immune response is an important initiator of a strong T cell immune response in islet transplantation. The data suggests this is a good pre-clinical model of IBMIR similar to that seen clinically.

P107
Bio-response of a rodent hemi-nephrectomy model to implantation of Neo-Kidney Augment prototypes composed of selected renal cells and biomaterials

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Continual loss of renal function over a time span of months or years is the operational definition of chronic kidney disease. Current renal function replacement therapy includes dialysis and eventual kidney transplant. An unmet need exists for new treatments to restore renal function thereby delaying or eliminating dialysis and transplant. Towards addressing this need, Tengion has developed a unique integrated regenerative medicine technology platform capable of catalyzing regeneration of tissues and organs. In the current study, we report on the development of a Neo-Kidney Augment (NKA) product prototype, comprised of biomaterials and selected regenerative renal cells (SRC), which facilitate regeneration of kidney tissue. SRC are obtained from enzymatic digestion of a kidney biopsy and density gradient separation of cells. Gelatin based hydrogels were used as biomaterial.
Bio-response of mammalian kidney towards implantation of NKA prototypes has previously been evaluated in healthy adult rodents (Basu et al., 2011, *Cell Transplantation*). However, removal of single kidney from rodents (hemi-nephrectomy) increases sensitivity of the model, permitting detection of systemically acting toxicological effects. In this study, 20 hemi-nephrectomized rodents were injected with NKA prototypes within the renal parenchyma of the remnant kidney. Physiological indices derived from whole blood, serum and urine chemistries were evaluated at 2 and 4 week time points post-implantation. Animals were sacrificed at 4 weeks post-injection and remnant kidney examined histologically for evidence of inflammatory or fibrotic bio-response. Implantation of NKA prototypes did not significantly affect key renal physiological indices, and presented minimal evidence of

inflammatory, necrotic or fibrotic bio-response. Therefore, NKA prototypes based on SRC in gelatin based hydrogels are well tolerated by remnant kidney in the rodent hemi-nephrectomy model.

P108

Immunomodulatory properties of porcine, bone marrow-derived mesenchymal stromal cells and comparison with their human counterpart

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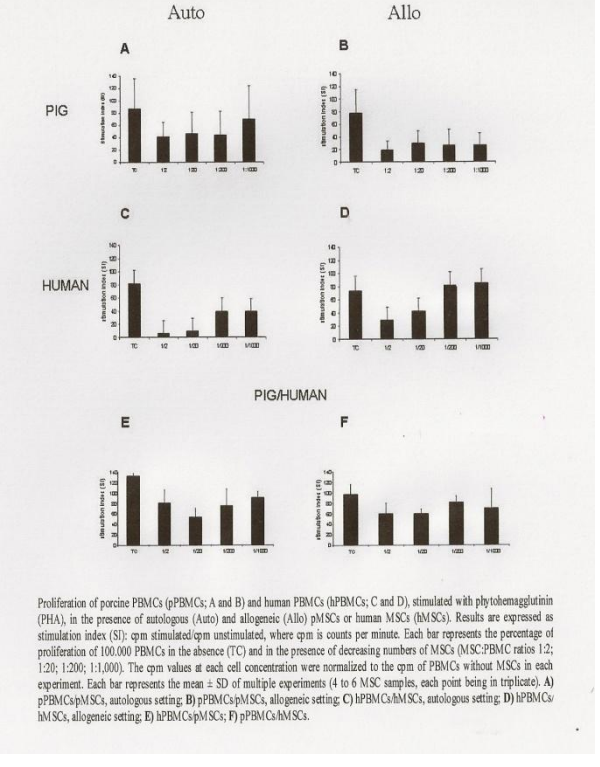
Thanks to their immunonodulatory properties, mesenchymal stromal cells (MSCs) are a promising strategy for preventing/reducing the risk of graft rejection after hematopoietic cell and solid organ transplantation. We have previously demonstrated that porcine MSCs (pMSCs) can be isolated from bone marrow and display similar morphology and differentiative capacity as compared to human MSC (hMSCs). In this study, we investigated the *in vitro* immunomodulatory properties (namely the ability to suppress lymphocyte proliferation in response to phytohemagglutinin and the cytokine production in the culture supernatants) of pMSCs from six Large White 6-month old piglets. Similarly to hMSCs, pMSCs reduced the phytohemagglutinin-induced lymphocyte proliferation. High levels of IL-6 were found in culture supernatants, whereas IL-10 and TGF-β were not detectable. In conclusion,*ex vivo* expanded pMSCs share selected biological/functional properties with hMSCs. pMSCs may be used in *in vivo* models to investigate novel approaches of prevention of graft rejection in solid organ transplantation.



Proliferation of porcine PBMCs (pPBMCs; A and B) and human PBMCs (hPBMCs; C and D), stimulated with phytohemagglutinin (PHA), in the presence of autologous (Auto) and allogeneic (Allo) pMSCs or human MSCs (hMSCs). Results are expressed as stimulation index (SI): cpm stimulated/cpm unstimulated, where cpm is counts per minute. Each bar represents the percentage of proliferation of 100.000 PBMCs in the absence (TC) and in the presence of decreasing numbers of MSCs (MSC:PBMC ratios 1:2; 1:20; 1:200; 1:1,000). The cpm values at each cell concentration were normalized to the cpm of PBMCs without MSCs in each experiment. Each bar represents the mean ± SD of multiple experiments (4 to 6 MSC samples, each point being in triplicate). **A)** pPBMCs/pMSCs, autologous setting; **B)** pPBMCs/pMSCs, allogeneic setting; **C)** hPBMCs/hMSCs, autologous setting; **D)** hPBMCs/hMSCs, allogeneic setting; **E)** hPBMCs/pMSCs; **F)** pPBMCs/hMSCs.

P109

Cross-validated plasma level quantification of anti-CD154 antibody 5c8 in nonhuman primates

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Inhibition of the CD40-CD154 costimulatory interaction is one of the most promising therapeutic strategies in pancreatic islet cell transplant models, and the 5c8 monoclonal antibody that binds CD154 (CD40Ligand) is particularly effective in nonhuman primates. Because of its extensive use in both allo- and xenotransplant models, accurate quantification of its circulating levels is of considerable importance to establish pharmacokinetic parameters as well as therapeutically effective levels both in baboons as well as cynomolgus monkeys. We have developed two different enzyme-linked immunosorbent assay-based methods to measure serum 5c8 concentrations with high sensitivity and, therefore, we were able cross-validate them. One method is based on the extension of our previous method (*J.Mol.Med.* **2009**, *87*, 1133) and measures the amount of antibody present indirectly by quantifying the blocking of the binding of Flag-tagged soluble CD154 to plate-bound CD40 (CD40L_ELISA), whereas, the other method measures the amount of mAb binding to plate-coated CD154 via an anti-Ig Fc antibody (mAbIg_ELISA). Plasma samples from five cynomolgus monkeys and two baboons receiving multiple doses of 20mg/kg 5c8 (NIH Nonhuman Primate Reagent Resource) i.v. (typically on POD -1,0, 3,10,18,28 for induction and every 14-28 days thereafter for maintenance) were used to cross-validate the methods, and the plasma levels obtained with the two different methods are in good agreement. The method measuring the amount of mAb binding to plate-coated CD154 has a larger calibration range and is somewhat more reliable. In two parallel experiments, animals with higher 5c8 levels had better function and maintained graft function longer. The ability of being able to accurately quantify the level of circulating 5c8 levels is an important development since it will allow the assessment of the pharmacokinetic and pharmacodynamics aspects of this antibody (see accompanying paper) and, hence, the fine-tuning of this important immunosuppressive regimen in different species of nonhuman primates.

P110
Exploratory pharmacokinetic-pharmacodynamic wtudies of the qnti-CD154 qntibody 5c8 in cynomolgus monkeys and baboons

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We have recently developed a cross-validated method for the quantification of the plasma levels of the anti-CD154 (CD40L) monoclonal antibody (mAb) 5c8 in nonhuman primates (see accompanying paper). These data made possible exploratory pharmacokinetic-pharmacodynamics (PK-PD) analyses in a few cynomolgus monkey and baboon islet allotransplant recipients treated with 5c8 (NIH Nonhuman Primate Reagent Resource) for which collected plasma samples were retrospectively available. For PK modeling, a one compartment first-order elimination PK model was assumed to calculate plasma levels, and good fit could be obtained with experimental data. In one cynomolgus monkey with sufficiently long follow-up, terminal elimination half-life was calculated as 18 day, which is in excellent agreement with results published previously from detailed PK analysis in cynomolgus monkeys suggesting terminal elimination half-lives of 13-21 days. For baboons, there is not yet sufficient follow-up data to clearly determine PK parameters, but PK models for two animals suggest elimination half-lives in the 10-15 day range – similar, maybe slightly shorter than in cynos. In a series of experiments exploring the potential of macroporous organosilicone scaffolds as support environment for cell transplantation, two baboons received similar islet allotransplants of ~25,000IEQ/kg in scaffolds implanted at the omental pouch site and were treated with 20mg/kg 5c8 i.v. alone. One of the recipients (islets alone) became insulin independent on POD130 and remained so at POD410 with excellent c-peptide levels, whereas the other recipient (islets+MSC) experienced significant reduction of exogenous insulin requirement, but rejection episodes precluded insulin independence. Retrospective assessment of 5c8 levels showed that while in the first recipient, plasma levels never dropped below 150-200µg/mL, in the second recipient, they dropped below 100µg/mL, and rejection treatments consisting of more frequent administration had to be initiated. These results prove the utility of such analyses even if they are only exploratory and on a limited number of animals.

P111
Assessment of the accuracy and reproducibility of a digital image analysis-based automatic islet cell counter (ICC) using microsphere mixtures as well as human and NHP islets

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There is an increasing need for good manufacturing practices (GMP) compatible standardized and reliable methods to quantitatively assess islet cell products, and fully computerized digital image analysis-based methods can provide a convenient approach to replace the current standard manual counting protocol. Here, we present results assessing the performance of an automatic islet cell counter (ICC; Biorep Technologies) that uses a digital imager and an image analysis segmentation method implemented in LabVIEW. Using a polymeric microsphere mixture that closely reproduces the distribution of isolated human islets (*Cell Transplant* **2009**, *18*, 1223), it has been shown that the digital image-based counter gave quick, reproducible microspheres counts. Counts obtained by the automatic ICC from different microsphere samples of known composition were in good agreement with both the fractional composition and the total islet equivalent (IEQ) content of the samples. They also agreed well with the average of all human operators (n=6) performing manual counts following the current standard operating procedure (SOP), but required much less time and effort. Recounting the same sample after remixing in the counting dish gave very similar results, which is unlikely to be the case with human operators where the intra-operator variability when recounting the same (photographic) sample seems to have a coefficient of variability (CV) of around 10%. Total IEQ counts obtained from the automatic ICC have been compared to those obtained by trained human operators using both human and nonhuman primate (NHP) islet samples, and good correlations (r^2>0.95) were obtained using either multiple operator (n=4) counts on different dilutions of the same sample or multiple samples counted by the same operator. In conclusion, if adequately standardized, the automatic ICC can provide a reliable and reproducible method to replace existing manual counting methods, which can also reduce inter-operator as well as inter-center variability in quantitating islet cell products.

P112
Gender is an important consideration for animal models, clinical trials and transplantation

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Research suggests that the sex of cells (recipient and donor in the case of cell therapies) is of paramount importance. Clear protection from stroke is observed in females for many years beyond menopause suggesting that although reproductive hormones and the presence of androgen or estrogen receptors on the cells may play a role, they do not explain all the differences. Male and female cells have been shown to respond differently to ischemia with the degree of damage being less sever in females. Males appear to be more susceptible to free radical mediated damage such as nitric oxide mediated-toxicity, whereas females are more likely to exhibit caspase-dependent apoptotic cell death. However not all cell types exhibit the sex differences since while female dopaminergic and hippocampal neurons are more resilient, female cerebellar Purkinje cells have no greater protection against experimental brain insults. Some forms of injury, such as H2O2 appear to be gender neutral.

Cellular therapy has considerable potential, but the role of gender remains controversial. Mesenchymal stem cells (MSCs) from males have been shown to release significantly greater quantities of proinflammatory factors and significantly less of proangiogenic or anti-inflammatory factors than female MSCs. This suggests that male MSC transplants maybe less beneficial than female cell transplants, such as in studies of endothelial progenitor cells and atherosclerosis. Female cells transplanted into females (or males) had a significantly greater benefit than male cells into males (or females).

We are therefore advocating that in determining the optimal source and type of transplantable cells for use in a cell therapy, much consideration should be given to the sex of both the donor and the recipient. While many studies are performed only in males, the results may not translate well to the female population. Treatments may need to be tailor-made to the sex of the patient.

P113
Warm ischemia during porcine pancreas procurement is significantly reduced by arterial and ductal flushing

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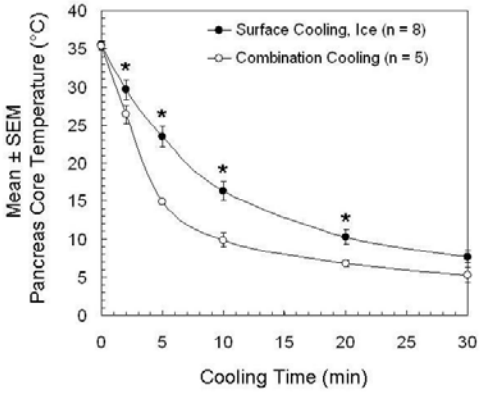
Introduction: Warm ischemia (WI) continues even after ice is applied to the surface of a large organ. We have shown that adding arterial and ductal flushing during porcine pancreas procurement can improve islet isolation yield

and post-culture islet viability. The purpose of this study is to characterize the cooling acceleration associated with the addition of arterial and ductal flushing.

Methods: 13 porcine pancreata were harvested following *en bloc* viscerectomy. 4 thermocouples were inserted at similar locations in the core of each pancreas and temperature was monitored for 30 min. Surface cooling was performed on all pancreata by covering the organ with crushed, frozen lactated ringer's solution (LRS) and providing surface irrigation with chilled LRS throughout dissection. In addition to surface cooling, 5 of the 13 pancreata were also cooled with a combination of intravascular and ductal flushing. The donors of these 5 pancreata were exsanguinated following systemic heparinization; the aorta was incised longitudinally to expose the celiac trunk and superior mesenteric artery, which were then cannulated and flushed with a total of 5 L chilled LRS; the pancreatic duct was identified, cannulated, and flushed with 60 mL chilled LRS.

Results: Average temperature drop was significantly higher ($P<0.05$) at 2, 5, 10, and 20 min of cooling with the combination cooling method as indicated in Figure 1 with asterisks (*). The difference exceeded 8 °C at 5 min. This cooling acceleration was achieved despite the average pancreas weight being 32% higher for the combination cooling group (261 ± 83 vs. 197 ± 20 g, mean ± SEM) as the pancreas size could not be known prior to allocation to each cooling method. Even for 2 very large organs (446-481 g) cooled with the combination method, average temperature drop was significantly higher ($P<0.05$) at 5, 10, and 20 min relative to the significantly smaller ($P<0.05$) surface-cooled organs.

Conclusion: Combining surface cooling with arterial and ductal flushing significantly accelerates pancreas cooling and reduces WI, leading to improved islet isolation outcomes.



P114
Shipping of juvenile porcine islets in silicon rubber membrane (SRM) vessels

Bradley P. Weegman[1], Efstathios S. Avgoustiniatos[1], Michael J. Taylor[2], Simona C. Baicu[2], Jennifer P. Kitzmann[1], Kate R. Mueller[1], William E. Scott III[1], A.N. Balamurugan[1], John R. Wilson[3], Klearchos K. Papas[1]

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Introduction: Porcine islet transplantation (PITx) is emerging as a promising treatment option for patients with type 1 diabetes that can be potentially applied at the larger scale. The use of "medical grade pigs" is required for clinical application. Juvenile, as opposed to adult pigs are attractive as donors when considering the cost and logistics of raising them in specialized facilities. However isolation and culture of islets from juvenile pig pancreata is notoriously difficult. It is important to demonstrate that juvenile porcine islets can be successfully, isolated, cultured and shipped to distant facilities without substantial loss of tissue amount or viability. A method for shipping cultured juvenile porcine islets using gas-permeable silicon rubber membrane bottom (SRM) vessels in temperature controlled containers is investigated here.

Methods: Islets were obtained using standard protocol; briefly, pancreata were procured from living juvenile porcine donors and islets isolated using the Ricordi method. Islets were purified using Ficoll density gradients and cultured for 6-7 days in SRM vessels (Wilson Wolf Manufacturing Corp. New Brighton, MN). Islets were then packaged in phase change temperature controlled shipping boxes as described previously by our group and shipped overnight. Islets were quantified by DNA or assessed for viability by oxygen consumption rate normalized to DNA (OCR/DNA; values reported are Means ±SEM) both immediately prior to and post-shipment.

Results: OCR/DNA was on average slightly higher but not significantly different post-shipment (175±25 nmol/min/mg DNA; n=5) when compared to pre-shipment (159±16; n=5). DNA recovery post-shipment was 65±6% with n=4.
Conclusions: A slight increase in average viability and only a minor loss of viable tissue during shipment indicates that most of the viable tissue survived while primarily unhealthy tissue was lost. These results are promising considering the historic difficulty in the culture of juvenile islets. The temperature controlled shipment of juvenile porcine islets using SRM culture devices appears to be a preferred means of islet culture and distribution.

P115
Implications of thrombosis on the oxygenation of the intraportally transplanted islet

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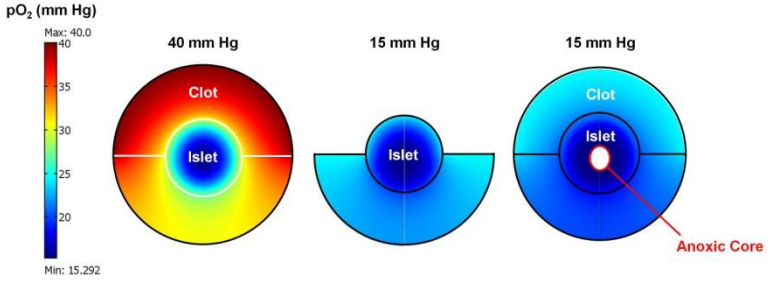
Introduction: Islet transplantation has yet to reach widespread acceptance, partly because the large number of islets required for long-term insulin independence. Many islets are believed to be lost in the early posttransplant period, suggesting that the liver may not be the optimal site for these grafts. Oxygenation at the individual islet level has not been studied extensively. We present a model that can predict the formation of an anoxic core within intraportally transplanted islets, with or without the presence of thrombosis.
Methods: Modeling of steady-state oxygen delivery into islets was performed using COMSOL Multiphysics (Burlington, MA). An islet was assumed to be a spherical body (with radius of 75-µm) of viable, oxygen-consuming tissue that has lodged in a terminal portal sinusoid and only has access to oxygen from a single side. Oxygen transport is by diffusion alone, either from the islet surface or the surface of a 100-µm thick clot which surrounds it. We obtained most model constants from literature, including oxygen diffusivities and solubilities (at 37°C) for both a clot and an islet. The oxygen consumption rate was representative of human islet preparations, as measured in our laboratory (~200 nmol/min·mg DNA). We explored reasonable values of surface pO_2 and their affect on the size of the anoxic core in an islet.
Results: Figure 1 illustrates representative results from the diffusion-reaction model, showing that the presence of a clot surrounding a lodged (intraportal) islet may result in the formation of an anoxic core.
The size of the anoxic core would increase for islets of a larger diameter, greater oxygen consumption rate (higher viability) or if surrounded by a thicker clot. It is also possible that islets could lodge together (not modeled here) which would result in additional oxygen limitations. Inflammation would further impair oxygen delivery – in that the infiltrate would compete for the same oxygen supply.
Conclusion: Oxygenation is important to consider, particularly since not much is known about the local oxygen supply at the level of the intraportally transplanted islet. It may be that the liver is not the best site for islet transplantation and that may be in part due to poor oxygenation.



P116
In vitro dedifferentiated Schwann cells to improve CNS repair after transplantation in the injured spinal cord

Paula Monje, Ryan Williams, Yee-Shuan Lee, Jennifer Soto, Ketty Bacallao, Randall Berg, Mary Bartlett Bunge

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Transplanted Schwann cells (SCs) mediate axon regeneration in the CNS and provide a unique advantage for autologous transplantation in the treatment of spinal cord injury. Whereas regeneration and remyelination of axons are increased after transplantation of adult nerve-derived SCs, functional outcomes remain modest. Recent evidence

has shown that SC precursors from embryonic nerves support better repair after spinal cord injury. However, using embryonic SCs may argue against potential clinical applications. Therefore, the objective of this study was to develop a clinically relevant method to convert adult SCs into highly immature precursor-like SCs to circumvent the use of embryos for transplantation strategies. We achieved this by treating SCs isolated from adult peripheral nerves with a defined cell culture medium that drives SC dedifferentiation in vitro prior to transplantation. The dedifferentiated SCs displayed both phenotypic and functional characteristics of SC precursors, as assessed by their expression of typical SC precursor markers (p75NGFR+/N-cadherin+/S100-/O4-/P0-) and their preference to interact with other SCs rather than axons, respectively. To determine the ability of dedifferentiated SCs to promote CNS regeneration, SCs were implanted as a bridge in the transected adult rat spinal cord. Dedifferentiated SCs not only integrated well with host tissue (i.e. association with astroglial processes) but also improved axonal growth into the bridge when compared to non-treated SCs. In conclusion, we have generated high numbers of SCs exhibiting characteristics of SC precursors suitable for transplantation aimed at CNS repair. This strategy is clinically relevant because SCs are induced to acquire embryonic characteristics without the need to use embryos, genetic intervention or viral delivery, while allowing for autologous transplantion.

P117

Immunosuppressive role of Fibrinogen-like-protein 2 (FGL2) in CD8+ regulatory T cells-mediated long-term graft survival

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Background: In a model of cardiac allograft in rat, blockade of CD40-CD40L interaction induces a long-term graft survival mediated by $CD8^{+}CD45RC^{low}$ regulatory T cells (Tregs) whose mechanisms of tolerance-induction remain unclear (1).
Materiel/methods: A lewis 1W rat heart is grafted in a heterotopic intra-abdominal position in a MHC mismatched Lewis 1A rat and infected with 2.10^{10}pi of adenovirus recombinant for CD40 molecule fused to the Fc part of an immunoglobulin (AdCD40Ig) the day of the graft. Tregs, effector $CD4^{+}CD25^{-}$ T lymphocytes (TL), and plasmacytoïde dendritic cells (pDC) from spleen are sorted by FACS Aria for *in vitro* tests. In *in vivo* studies, $4{,}5.10^{11}$vg of FGL2-recombinant adenovirus associated virus (AAVFGL2) are *i.m* injected in receivers 30 days before the graft. Splenocytes are transferred to irradiated rats by *i.v* injection the day before the graft.
Results: We have shown FGL2-overexpression in splenic Tregs and in the graft of AdCD40Ig-treated vs non-treated and naïve rats, by quantitative PCR and immunohistology. FGL2 involvement in Tregs immunosuppressive function has been proved by *in vitro* and *in vivo* experiments. Indeed, Tregs from AdCD40Ig-treated rats inhibit TL proliferation in response to allogeneic pDC. This inhibition is annihilated by FGL2-blocking antibodies (2) and can be mimicked by the adding of FGL2 protein. Moreover, AAV-mediated FGL2 overexpression in rat prolongs graft survival with a median of 18.5 days vs 11 days for controls. Furthermore, adoptive transfer of splenocytes from an AAVFGL2-treated rat, whose graft is not rejected after 120 days, to naïve rats, transmits long-term graft survival iteratively.
Discussion: FGL2 is a new immunosuppressive protein of major interest in immunotherapy. It could induce long-term graft survival, reducing or dispensing transplanted patients of broad immunosuppressors continuous taking.
(1) *Guillonneau, Transplantation, 2005*
(2) *Li, J Immunol, 2010*

P118

In vitro and ex vivo evaluation of Molday ION Rhodamine-B™ (MIRB) labeled cynomologous monkey Mesenchymal stem cells (MSC)

Benjamin Addicott[1,2], Melissa Willman[1], Jose Rodriguez[2], Kyle Padgett[3], Dora M. Berman[1], Joshua M. Hare[2], Norma S. Kenyon[1]

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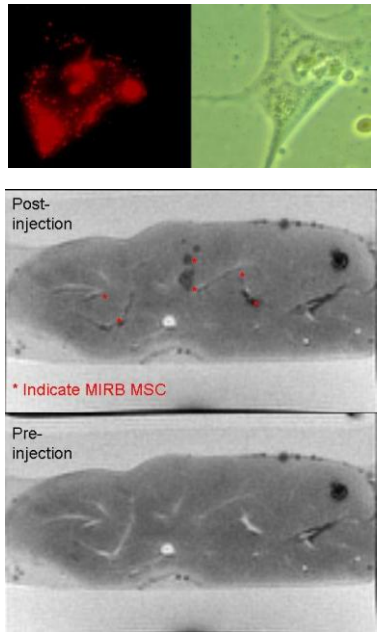
This study demonstrates the feasibility of using MIRB, a new SPIO contras agent to label MSC for *in vivo* tracking with MRI. MIRB is specifically designed for cellular internalization and is conjugated to Rh-B for fluorescent evaluation. *In vitro* experiments were conducted to study the effects of MIRB on MSC viability (7AAD) and

differentiation and to characterize cellular loading (Fe internalization) and MRI characteristics at 1.5T. *In vitro* scanning sequences were optimized based on contrast-to-noise ratio and contrast modulation of MIRB MSC pellets in agarose phantoms. Spin-spin relaxation rates (1/T2*) for gradient-echo (GE) sequences were approximated by fitting intensities at various echo times (TE) into a monoexponential decay as predicted by the Bloch Equation. A limit of detection was established by evaluating MIRB MSC pellets with various cell counts. Optimized *in vitro* parameters were applied to *ex vivo* monkey liver and porcine heart and modified to increase specificity for MIRB MSC. Images acquired before and after MSC injection were contrasted and MSC were detected as new areas of hypointensity. MSC presence was confirmed by histology. Results showed that MIRB did not affect MSC viability or the capacity to differentiate into bone or fat. Labeling efficiency was found to be approximately 100%. Optimal labeling concentration of 20 μg Fe/ml was determined based upon differential in contrast vs. labeling concentration and resulted in approximately 15 pg Fe/MSC. MIRB was observed to localize to perinuclear endosomes [**Figure 1**]. *In vitro* MRI analysis of MIRB MSC revealed that GE sequences provide the highest contrast for MIRB MSC vs. agar. Limit of detection for GE (TR/TE = 500/60 ms) was determined to be less than 1000 MSC. To increase ex*vivo* specificity, TE was decreased to 30 ms. At these parameters, MIRB MSC were detected after injection into *ex vivo* liver [**Figure 2**] and heart tissue. This data supports our ability to use MIRB for MSC labeling and *in vivo* MRI detection after infusion into monkey liver. The optimized parameters we have established will serve as a reference for designing future *in vivo* experiments.



P119
High field MRI in vivo tracking of therapeutic cardiac stem cells in infarcted porcine myocardium

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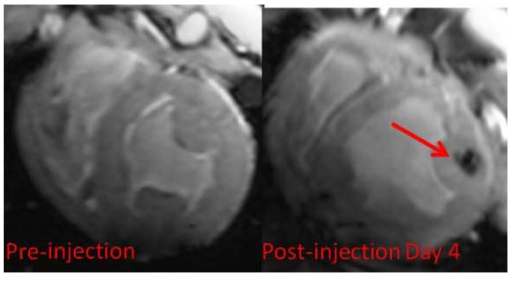
[1]Interdisciplinary Stem Cell Institute; [2]Diabetes Research Institute, University of Miami School of Medicine, Miami, FL, United States

Intramyocardial injection of cardiac derived stem cells is a highly promising therapy to restore cardiac function and reduce infarct size after myocardial infarction (MI). The mechanism through which these cells exert their ameliorative effects has not been fully characterized. In furthering our understanding of these mechanisms, it has become important to describe the migration patterns of stem cells after intramyocardial injection. This investigation was conducted to verify the feasibility of using high field strength MRI to track transplanted stem cells and correlate migration patterns with histological and functional parameters of myocardial viability. To accomplish this, we labeled cardiac derived stem cells with a superparamagnetic iron oxide (SPIO) MRI contrast agent (Biopal, Worcester, MA) and imaged the cells, *in vivo*, using a 3.0 T MRI. Cell labeling protocol was previously described in a series of *in-vitro* experiments done in collaboration with Diabetes Research Institute (Miami, FL). Four million abcg2+ human cardiac stem cells, grown to confluence, were labeled with SPIO at approximately 15 picograms Fe/ cell. The SPIO loading was verified under florescence microscopy. The labeled cells were then harvested and

prepared as four 0.35 ml saline suspensions. Myocardial infarction was created by balloon angioplasty of the left coronary artery for 60 minutes followed by full reperfusion. SPIO labeled cells were injected using thoracoscopic guidance to the infarct border zone at 14 days post MI. The animal was evaluated via sensitivity optimized cardiac and respiratory gated, susceptibility weighted gradient echo sequences immediately post injection and at post-injection days 2, 4, 7, and 14. MR images demonstrated labeled cells as new hypointensities, not seen in pre-injection imaging (**Figure1**). Cells were identified in myocardium at injection sites immediately post injection and remained detectable at day 14. Analysis of image data demonstrates evolving hypointense lesions originating at bolus injection sites with directed, asymmetric diffusion patterns developing over time post injection. These experiments demonstrate the feasibility of tracking therapeutic cardiac stem cells in vivo over time with MRI for correlating cell migration patterns with histological and functional measures of myocardial viability.



P120
Human pancreas-derived MSC-mediated apoptosis of pancreatic cancer cells in direct- and indirect co-cultures

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Background: Mesenchymal stem cells (MSCs) in cancer therapy have attracted extensive attention due to their accessibility, tumor-oriented homing capacity and the feasibility of autotransplantation. The inefficient treatment and poor prognosis of pancreatic cancer is mainly attributed to the lack of therapeutic specificity. Anticancer gene-engineered MSCs are capable of targeting tumor sites and producing specific anticancer agents locally and constantly. Focusing on personalized therapeutic strategies, this study was performed to detect the sensitivity of pancreatic cancer cells (PCCs) to MSC^{TRAIL} under different culture conditions.
Method: PCCs (Panc-1 and HP62) were analyzed by FACS for TRAIL receptors. Human pancreas-derived MSCs engineered with membrane-bound TRAIL ($mbMSC^{TRAIL}$) and secreting TRAIL ($stMSC^{TRAIL}$) were used for the direct co-cultures, while conditioning media from corresponded cultures were applied to Panc-1 and HP62 cells as indirect co-cultures. Wild-type MSCs and corresponding media were used as controls. The TRAIL expression was assessed by both ELISA and western blot analysis. Live/Dead assay was utilized to determine the cell apoptosis. Statistical significance was determined by Student t test.
Results: ELISA and western blot analysis results confirmed the TRAIL expression on both cell lysates ($mbMSC^{TRAIL}$ >$stMSC^{TRAIL}$) and culture media ($mbMSC^{TRAIL}$ < $stMSC^{TRAIL}$). Different patterns of TRAIL receptor expression were observed on Panc-1 ($DR4^-$, $DR5^-$, $DcR1^-$, $DcR2^-$) and HP62 ($DR4^-$, $DR5^+$, $DcR1^-$, $DcR2^+$). Both Panc-1 and HP62 cells responded to all types of MSCs (i.e. $mbMSC^{TRAIL}$, $stMSC^{TRAIL}$and wild-type MSCs), however, more apopototic cells were observed on the HP62/MSC^{TRAIL} and HP62/$stMSC^{TRAIL}$ co-culture compared with the same ratio of HP62/MSC co-culture. In the indirect co-culture study, a significant cell death was only observed on HP62 cells, which displayed a dose dependent apoptosis.
Conclusion: Pancreas-derived MSCs exhibit intrinsic inhibition on Panc-1 and HP62 cells. TRAIL-engineered MSCs exert additional inhibition on HP62 cells, depending on the expression of death receptor DR5 on these types of cells.

P121
Environmental monitoring (EM) program of the clean room facility

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Establishing an effective EM program in cGMP facility is an essential tool used to enable the detection of any change in the sterile environment required in a clean room. The EM program established at the Diabetes Research Institute effectively controls over the clean room environment and allows investigation of sterility issues. The EM program tests viable and non-viable air and surface particles in the clean rooms, which are regulated by HEPA filters, according to ISO guidelines.Methods: In this study we have described three ways of detecting particles in a clean room facility. Airborne particles are sampled as viable and non-viable particles, while for surface viable samples were collected.

• Viable air particles were detected using the "RCS High Flow." Air flows into this hand held machine towards the agar strips, leaving the air particles on the strips.

• Viable surface particles were sampled by gently touching agar contact plates on the area to be tested.

• Non-viable particles are detected in the air using an airborne particle counter. Air influx in this machine passes through a laser that detects and separates the size of the particles in the air. Samples collected on the agar strips and contact plates are sent to an outside reference laboratory for identification of viable colonies. Non-viable counts are obtained and compared with their specified classification limits.Results: Results obtained for viable CFUs in both the air and surface samples are well within the acceptable ISO limits at 90%. Air particle counts have a mean of 95%, also within ISO limits.Conclusion: An EM program in-house has specific benefits. Performing this monitoring in-house is extremely cost effective and corrective actions can be implemented immediately after the detection of any unexpected results.

P123

Phase I study; Adoptive transfer of IL-2 stimulated natural killer cells extracted from cadaveric donor liver graft effectively mounts an antitumor response in liver transplant recipients with hepatocellular carcinoma

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Tumor recurrence is the main limitation of liver transplantation (LT) in patients with hepatocellular carcinoma (HCC) and can be promoted by immunosuppressants. However, there is no prevention or treatment for HCC recurrence after LT. Here, we describe a clinical-scale method for an adoptive immunotherapy approach that uses natural killer (NK) cells derived from deceased donor liver graft perfusate to prevent tumor recurrence after LT. Liver mononuclear cells (LMNC) that were extracted from deceased donor liver graft perfusate contained a large percentage of NK cells (45.0% ± 4.0%) compared with peripheral blood mononuclear cells (PBMC) (21.8% ± 5.2%) from the same donor. Furthermore, interleukin (IL)-2-stimulated NK cells showed greater upregulation of activation markers and the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which is critical for NK cell-mediated anti-tumor cell death and increased production of interferon. Moreover, IL-2 stimulation induced LMNC to exhibit a stronger cytotoxicity against NK-susceptible K562 target cells compared with PBMC ($p < 0.01$). Finally, we also showed that the final product contained a very low T-cell contamination (0.02×10^6 cells×kg-1), which reduces the risk of severe graft versus host disease (GVHD). After obtaining approval from FDA and IRB of our institute, we successfully applied this approach to 7 liver cirrhotic patients (6M / 1F) with HCC, median age 61 years (range 55-66) (Clinicaltrial.gov #NCT01147380). The average number of NK cells that had been administered to recipients at 3-5 days after LT was $135.1 \pm 52.0 \times 10^6$ cells/body. Kinetic studies revealed that peripheral blood obtained from recipients that received this therapy exhibited a significant increase of NK cells compared with the recipients not receiving this therapy in early postoperative period (4W) (11.0 ± 5.7 %, 4.6 ± 2.7%, respectively, n=6, p=0.040). There are no study related adverse events. In conclusion, the administration of IL-2 stimulated cadaveric donor liver NK cells is well tolerated. Further investigations are proceeding in order to evaluate the long-term benefits of this approach.

P124

A novel strategy for intrastriatal dopaminergic cell transplantation: Sequential "Nest" grafting influences survival and behavioral recovery in a rat model of Parkinson's Disease

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Neural transplantation in experimental parkinsonism (PD) is limited by poor survival of grafted embryonic dopaminergic (DA) cells. In this proof-of-principle study we hypothesized that a first regular initial graft may create a "dopaminergic" environment similar to the perinatal substantia nigra and consequently stimulate a subsequent graft. Therefore, we grafted ventral mesencephalic neurons sequentially at different time intervals into the same target localization. 6-OHDA unilaterally lesioned rats received E14 ventral mesencephalon derived grafts into the DA-depleted striatum. In the control group we grafted all 6 deposits on the first day (d0). The other 4 groups received four graft deposits distributed over 2 implantation tracts followed by a second engraftment injected into the same site 3, 6, 14 and 21 days later. Quantitative assessment of the survival of tyrosine hydroxylase-immunoreactive neurons and graft volume revealed best results for those DA grafts implanted 6 days after the first one. In the present study, a model of short-interval sequential transplantation into the same target-site, so called "nest" grafts were established in the 6-OHDA rat model of PD which might become a useful tool to further elucidate the close neurotrophic and neurotopic interactions between the immediate graft vicinity and the cell suspension graft. In addition, we could show that the optimal milieu was established around the sixth day after the initial transplantation. This may also help to further optimize current transplantation strategies to restore the DA system in patients with PD.

P125
Highly efficient protocol to select null alpha 1,3-galactosyltransferase porcine cells

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Background: Cell selection to generate alpha 1,3-Galactosyltransferase (Gal) double knockout (DKO) pigs has been complex, inefficient and time-consuming. The objective of this study was to generate a highly efficient system to isolate Gal-DKO porcine liver derived cells (PLDC) using a combination of two sorting methods.
Materials and Methods: Gal single knockout (Gal-SKO) PLDC cells were cultured for seven to eleven passages. Thirty million Gal-SKO PLDC cells were stained with IB4-Alexafluor 488 and sorted for negative fluorescence. After sorting, cells were recovered in stem cell media (SCM) and cultured until confluent. A second selection was performed on the FACS-selected cells using biotin-conjugated IB4-lectin attached to streptavidin-coated magnetic beads. Gal-negative cells were collected in the supernatant and plated until colonies were formed.
Results: Forty-eight colonies developed; 44 (91.7%) were null colonies for the a-Gal epitope. Gal-DKO genotype was identified by PCR of genomic DNA of growing cells.
Conclusions: From cultured Gal-SKO cell lines previously developed in our lab, we isolated spontaneously mutated Gal-DKO cells. A combination of selection methods using flow cytometry and magnetic beads allowed recovery of a high percentage of Gal-DKO cells, for the production of Gal-DKO transgenic pigs.