BEST ABSTRACTS SESSION II

Abstract 1: Ultrafast Volumetric Bioprinting of an Endocrine Pancreas Using Functional Human iPSC-Derived Islets

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Introduction

Generating functional islets from human induced pluripotent stem cells (iPSC) could unlock an unlimited cell source to tissue engineer an endocrine pancreas. Despite significant progresses in the differentiation protocols, current cell cultures cannot replicate intricate islet-environment interactions. This absence of reliable, native tissue-like environments limits the progress on pancreatic regenerative medicine, and in the development of new in vitro models to understand islet biology. Bioprinting technologies, biomaterials, and (stem) cell engineering provide a novel technological toolbox to pave the way towards the design of bioinspired systems that recapitulate salient organ functions. This study combines iPSC-derived islets with ultrafast volumetric light-based bioprinting to engineer an advanced pancreatic endocrine construct for modelling and regenerative medicine.

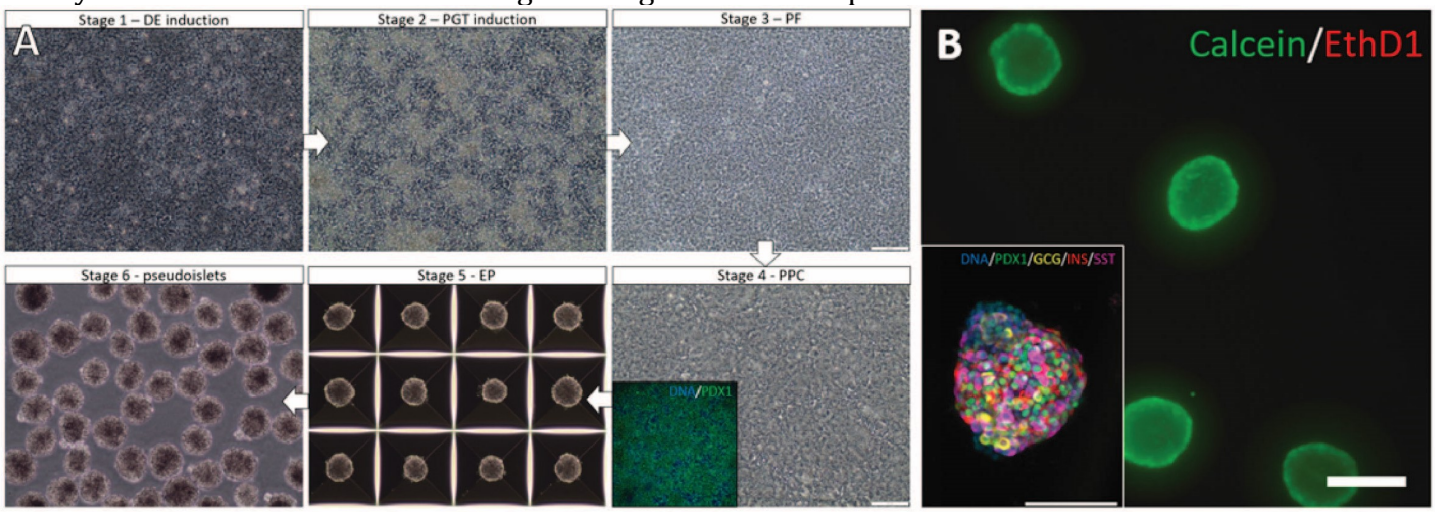
Method

Human pancreatic islets were generated from iPSC following a seven-stage protocol (1). When reaching stage 7, iPSC-derived islets were collected and pancreatic constructs were generated via layerless and shear stress-free, light-based Volumetric Bioprinting (VBP) (2). Concentrations ranging from 4%-7% (w/v%) of optically tuned gelatin methacrylate (GelMa) were used for bioprinting. iPSC-derived islets were characterized via staining and single cell transcriptomics. Insulin release on bioprinted constructs upon glucose exposure was assessed with static and dynamic glucose stimulated insulin secretion (GSIS). Bioprinted constructs were implanted in immunodeficient Balb/c NSG RIP-DTR mice and circulating human insulin levels measured over 3 months.

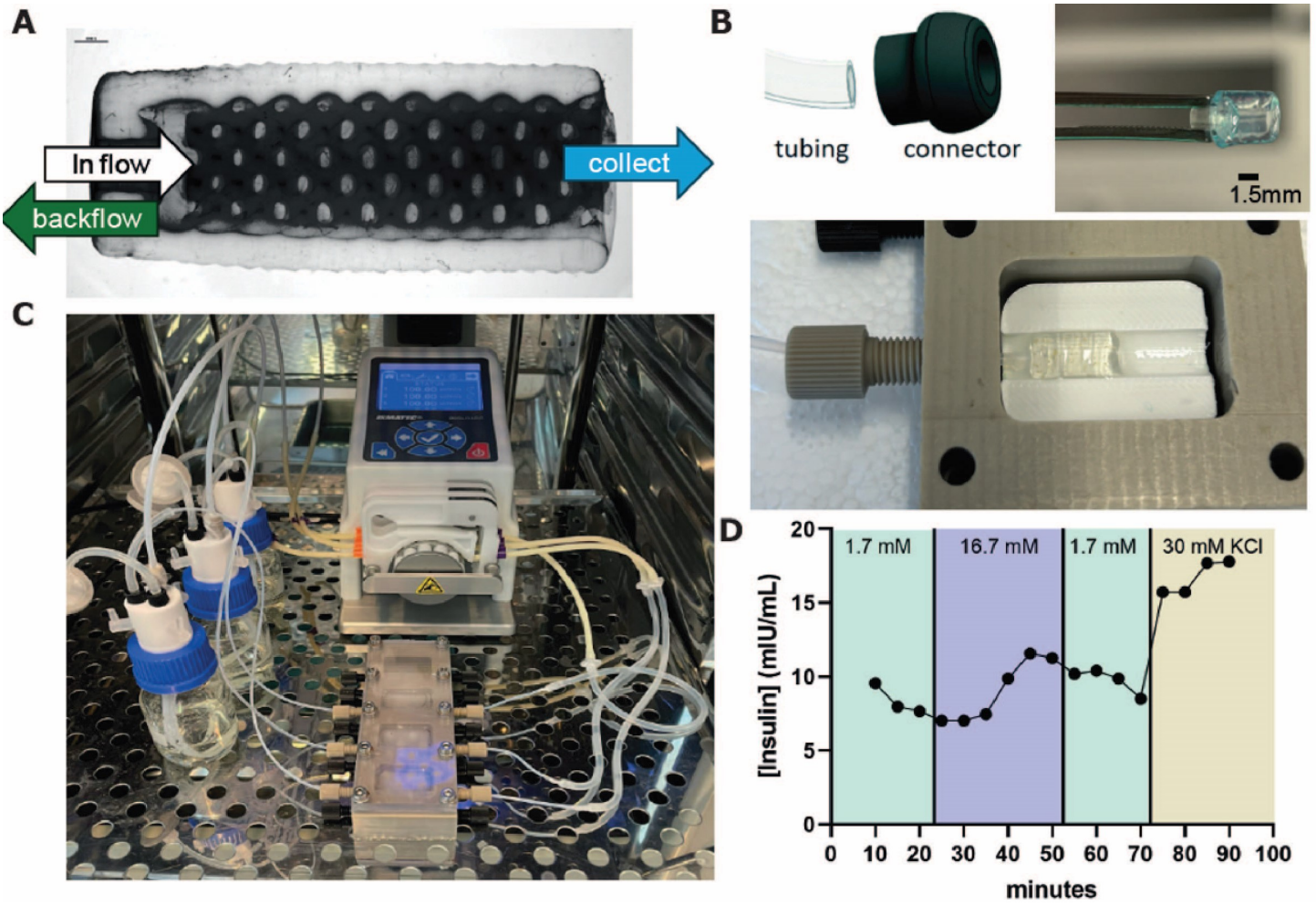
Results

VBP allowed to produce 50-60 mm3 pancreatic constructs in 30 s and posed no mechanical or chemical stress on iPSC-derived islets, which were highly viable for 21 days after printing (Figure 1). Islet metabolic activity remained constant over time during 21 days of culture. Volumetric bioprinted iPSC-

derived islets showed mature, insulin, glucagon and somatostatin, single-hormone producing cells after 21 days both characterized via staining and single cell transcriptomics.



Bioprinted islets required a four day adaptation time after printing to recover their glucose-responsive insulin secretion profile both in static and in perfusion systems. Mathematically defined gyroids (Figure 2) were bioprinted with a strut size of 500 µm and infill density of 33% considering the diffusion profile of human insulin to perform dynamic culture and GSIS. The system is compatible with supplementation with anti-diabetic drugs for drug screening. Implanted mice survived surgical implantation of bioprinted constructs and experiments are currently ongoing.



Conclusion

Overall, VBP allows to successfully and viably bioprint iPSC-derived islets into geometrically defined morphologies while retaining their functionality. This technology opens up to new possibilities on developing novel tissue engineered platforms for modelling and therapy.

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Abstract 2: Bio-fabrication of a Human iPSC-based vascularized endocrine pancreas for the treatment of Type 1 Diabetes

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Introduction: Intrahepatic islet transplantation in patients with type 1 diabetes is limited by donor availability and lack of engraftment. To address these issues, new sources of β cells as iPSCs and alternative transplantation sites are needed. Organ decellularization is an emerging strategy in organ regeneration. Based on our experience with decellularized rat lung as scaffold in generating a Vascularized Endocrine Pancreas (ECM scaffold repopulated by neonatal pig islet and BOEC cells), we bio-fabricated an iPSC-based version named iVEP (iPSC-derived Vascularized Endocrine Pancreas).

Methods: iPSC-derived β (iβ) and endothelial (iEC) cells were characterized by flow cytometry, then aggregated into functional vascularized iβ spheroids (SPH) for 7 days at a ratio of 90% iβ+10% iEC. Rat lung was decellularized by vascular perfusion with 1% SDS and 0.1% Triton and seeded with iECs and SPH from vascular and air accesses. The re-cellularized scaffold matured in vitro for 7 days in a customized perfusion bioreactor specifically designed to allow cell/compartment integration. iβ cell death was estimated during ex vivo organ maturation and compared to 7 days of iβ in vitro culture by evaluating miR-375 expression (droplet digital PCR). On day 7, fluoroangiography and dextran assays were performed to assess the vascular compartment structure and function while for the endocrine compartment, insulin production was measured by dynamic glucose perifusion and insulin quantification (ELISA/IF). Matured iVEPs were then transplanted subcutaneously into NSG diabetic mice followed for 30 days and compared to the deviceless (DL) implantation site. Explanted iVEPs were used for IF evaluation.

Results: iEC/iβ maintained for 7 days in vitro their phenotype expressing endothelial (>95% CD31+/CD105+/CD73+/CD90- cells) and β-cell (>60% PDX1+/insulin+ cells) markers respectively. Matured iVEPs showed regenerated vascular network (CD31+) able to sustain the direct distribution of a perfusate with SPH (Chromogranin A/Insulin+) fully integrated in the engineered vasculature. Also matured iVEPs reduce β cell death: the amount of lost iβ was ≤18% during organ maturation, while >70% during in vitro culture ($p<0.05$). In iVEPs, vascularized ECM was able to significantly sustain iβ engraftment, survival and promote phenotypical maturation of iβ with physiologic insulin secretion.

Preliminary preclinical results demonstrated the ability of iVEP to engraft and restore normoglycemia in diabetic recipient mice compared to SPH alone implanted in the DL site with a median time of 8 days. **Conclusion:** In vitro, iVEP platform enables iPSC based SPH engraftment and survival in a pre-vascularized ECM promoting iβ in vitro endocrine function. In vivo, iVEPs showed immediate function restoring normoglycemia in diabetic NSG mice. To our knowledge, we assembled the first iPSC-derived Vascularized Endocrine Pancreas able to provide both controlled insulin secretion in vitro and in vivo function.

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Abstract 3: A perfusable encapsulation device housing Stem cell derived pseudo-islets to treat type-1 diabetes

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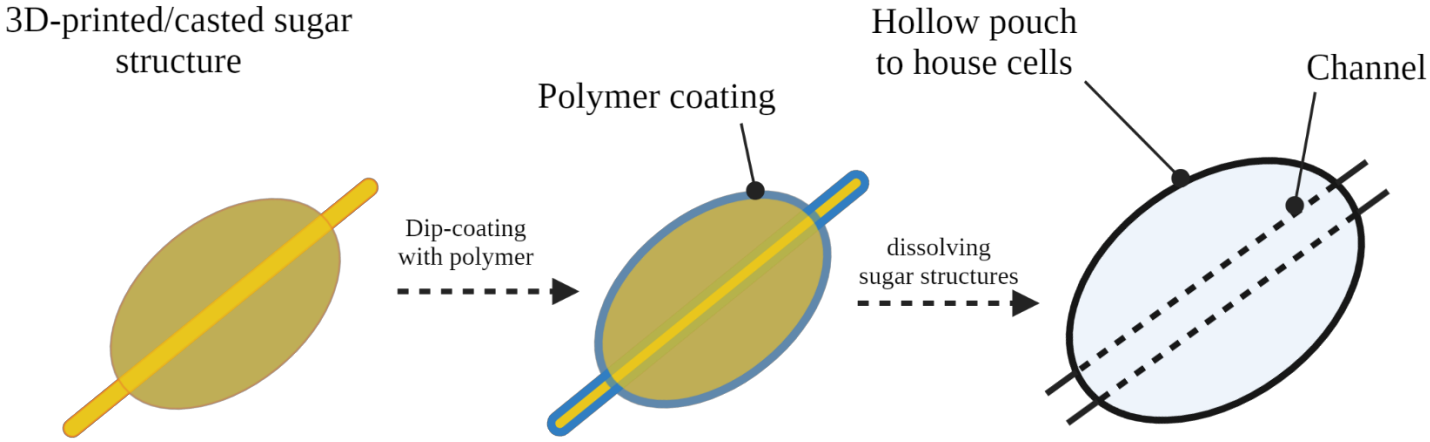
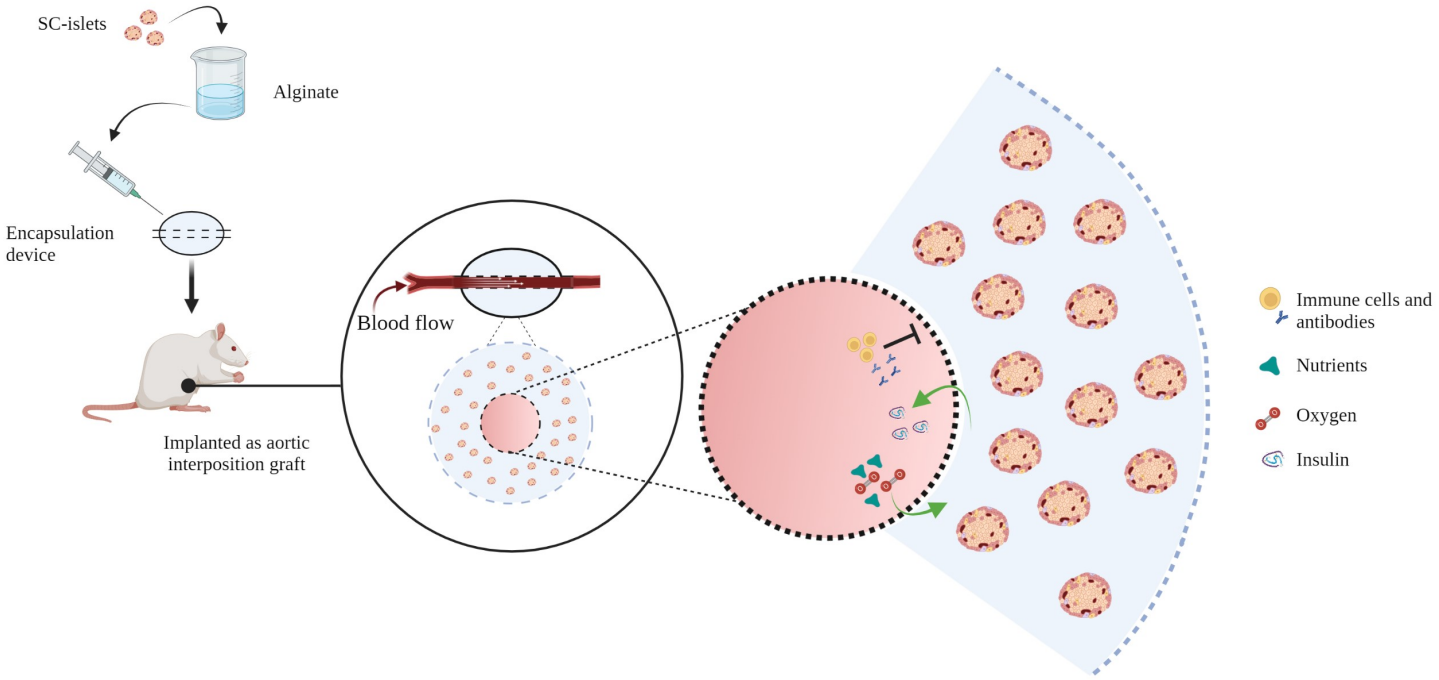
Type 1 diabetes is characterized by the autoimmune destruction of insulin-producing beta cells resulting in hyperglycemia due to insufficient insulin production. Islet transplantation is a promising long-term treatment option for type 1 diabetes which involves isolating the islets from the donor pancreas and implanting them as allogenic transplants. However, this strategy necessitates life-long immunosuppressants to avoid unwanted immune reactions against the grafted cells and the procedure is also severely limited by donor islet supply. To protect the transplanted islets from immune rejection, encapsulation strategies have been employed which involve immobilizing the islets in a permselective polymer matrix thereby creating a physical barrier from the immune system. However, nutrient supply to encapsulated cells occurs through diffusion which is often critically restricted post-encapsulation leading to graft cell loss. The objective of this project is to fabricate a cell encapsulation device with embedded vascular channels to facilitate perfusion with nutrient-rich media (in vitro) or blood (in vivo) to enhance mass transfer to the encapsulated cells. Further, stem cell derived islets-like clusters would be encapsulated in the device to eliminate the need of donor islets. Porcine scale devices were developed by dip-coating 3D-printed sacrificial sugar templates with polyurethane followed by the removal of the templates and encapsulating the cells of interest in alginate within the device.



The perfusion devices exhibited high MIN6 viability, stem cell derived pseudoislets viability/maturation in vitro. The devices also remained mostly patent as arterio-venous shunt in porcine models in vivo.

Rodent scale devices were also developed for future studies on diabetic models and similar or better results are expected for these scaled-down devices as well. The rodent-scale devices will serve as a crucial model for demonstrating the preclinical efficacy of stem cell-derived islet grafts in diabetic rat models. The synergistic approach of using stem cell derived islets in a perfused immunoprotective implantation system could be significant in effectively treating type-1 diabetes.



Canadian Institutes of Health research. Diabetes Quebec. Natural Sciences and Engineering Research Council of Canada. Fonds de recherche du Québec.

Abstract 4: Identifying optimal engraftment sites for human stem cell-derived islets in a non-Human primate model

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Introduction

The recent development of robust protocols for generating stem cell-derived islets (SC-islets) from human origin has emerged as a promising alternative, offering an unlimited supply to overcome the scarcity of sourcing islets from deceased donors. This advancement could lead to a definitive cure for type 1 diabetes(T1D). In Vertex's VX-880 clinical trial, the transplantation of fully differentiated allogeneic SC-islets led to a remarkable milestone in 2021, as the first patient achieved independence from exogenous insulin for over two years by far. However, separate reports demonstrate that SC-islets also pose a potential risk of tumorigenicity, manifested as teratoma and other malignant tumors. This raises safety concerns, particularly with the currently favored intrahepatic approach due to the graft's irretrievability. A key objective in advancing stem cell-derived β cell technology to clinical application is to identify an optimal implantation site. We undertook an approach involving the transplantation of SC-islets into multiple locations in a nonhuman primate (NHP) to determine the optimal site for initial graft survival.

Methods

A single batch of 150,000 fully differentiated human stem cell-derived stage 6 islets, with an average size of 230 micrometers, was evenly divided into seven aliquots. One aliquot was utilized to measure the initial insulin content, while the remaining aliquots were simultaneously transplanted into six different sites of a nondiabetic, naive NHP. These transplantation sites included the omentum, liver, thigh muscle, pre-peritoneal space, liver subcapsular space, and subcutaneous tissue. The immunosuppressive regimen consisting of Thymo, Rituxan, and Enbrel for induction, with maintenance involving Belatacept, anti-CD40L, Rapamycin, and FK506. On day 8, the animal was electively sacrificed to assess early engraftment at each transplantation site.

Results

The percentage of engraftment is calculated by dividing the recovered insulin from each transplant site by the starting insulin content. Data demonstrated that the omentum and liver sites exhibited the highest engraftment rates, with 41% and 36% recovery of the initial insulin content, respectively. These were followed by the thigh muscle (22%), pre-peritoneal (3%), liver subcapsular space (3%), and the subcutaneous site (2%). Examination of the graft at autopsy revealed preserved islet-like structures exhibiting robust insulin staining, with no evidence of lymphocyte infiltration.

Conclusions

In this SC-islet transplant model, a notable initial graft loss was observed, as anticipated for primary islet transplantation, ranging from 60% to 98% among different transplant sites. This study marks the first report of utilizing a single batch of SC-islets and simultaneously transplanting SC-islets into six sites of one recipient, aiming to eliminate batch and recipient variations. The omentum, a top-performing site, offers high initial graft survival and many advantages over the current favored intrahepatic approach, particularly graft retrievability in teratoma or tumor cases for SC-islet transplant. Conducted in a relevant NHP model, this study provides translational insights into stem cell-derived islet transplantation for T1D.

Abstract 5: Optimization of immunosuppression for beta cell replacement therapy: assessing the tolerability and efficacy of belatacept combined with reduced tacrolimus

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Background:
Significant progress has been made in beta cell replacement therapy with the introduction of stem cell-derived islet cell transplantation for clinical testing, showing promise as a functional cure for type 1 diabetes mellitus (T1DM). However, the toxicity of tacrolimus, a cornerstone of current immunosuppression, remains a major barrier to widespread clinical application. This study evaluates the strategy of minimizing tacrolimus using belatacept infusions to improve the tolerability and effectiveness of immunosuppression post-islet transplantation.

Materials and Methods:
Our study involved a cohort of 14 patients with T1DM who underwent cadaveric pancreatic islet transplantation. Induction therapy included Thymoglobulin for all patients. Maintenance immunosuppression comprised monthly administration of belatacept at 5 mg/kg alongside low-dose tacrolimus (target level 3-5 ng/ml). Ten patients (67%) received this combination as de novo therapy at transplantation, while the remaining four (33%) transitioned from tacrolimus/antimetabolite due to complications such as rising creatinine, chronic diarrhea, and increasing donor-specific antibodies.

Results:
The regimen of belatacept at 5 mg/kg monthly with low-dose tacrolimus effectively prevented de novo donor-specific antibodies (DSA) and antibody-mediated rejection (AMR) in all 14 (100%) patients during the 14-month follow-up, contrasting with only 33% (2/6) in a historical cohort treated with tacrolimus and antimetabolite. However, belatacept required modification in 7 (50%) patients, involving discontinuation in two cases and dose reduction to 2.5 mg/kg monthly in five (36%) patients due to adverse events such as chronic neutropenia, mouth ulcers with skin rash, reactivation of Epstein-Barr virus, recurrent norovirus, Clostridium difficile infection, and urinary tract or upper respiratory infections. To proactively prevent toxic or infectious complications, we also lowered belatacept to 2.5 mg/kg monthly in the remaining 7 patients. Following this adjustment, recurrent infectious complications and toxicity resolved, while islet graft function remained stable in all 14 patients.

Conclusion:
Belatacept in combination with low-dose tacrolimus effectively prevented de novo DSA, AMR, and islet

graft loss. However, reducing the belatacept dose to 2.5 mg/kg monthly was crucial to minimize the risk of toxicity and infectious complications, highlighting the delicate balance required for successful long-term immunosuppression in islet transplantation.

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Abstract 6: Islet vascularized ECM organoid (IVEO) constructs repair isolated islets native environment for successful engraftment and functionality post-transplantation

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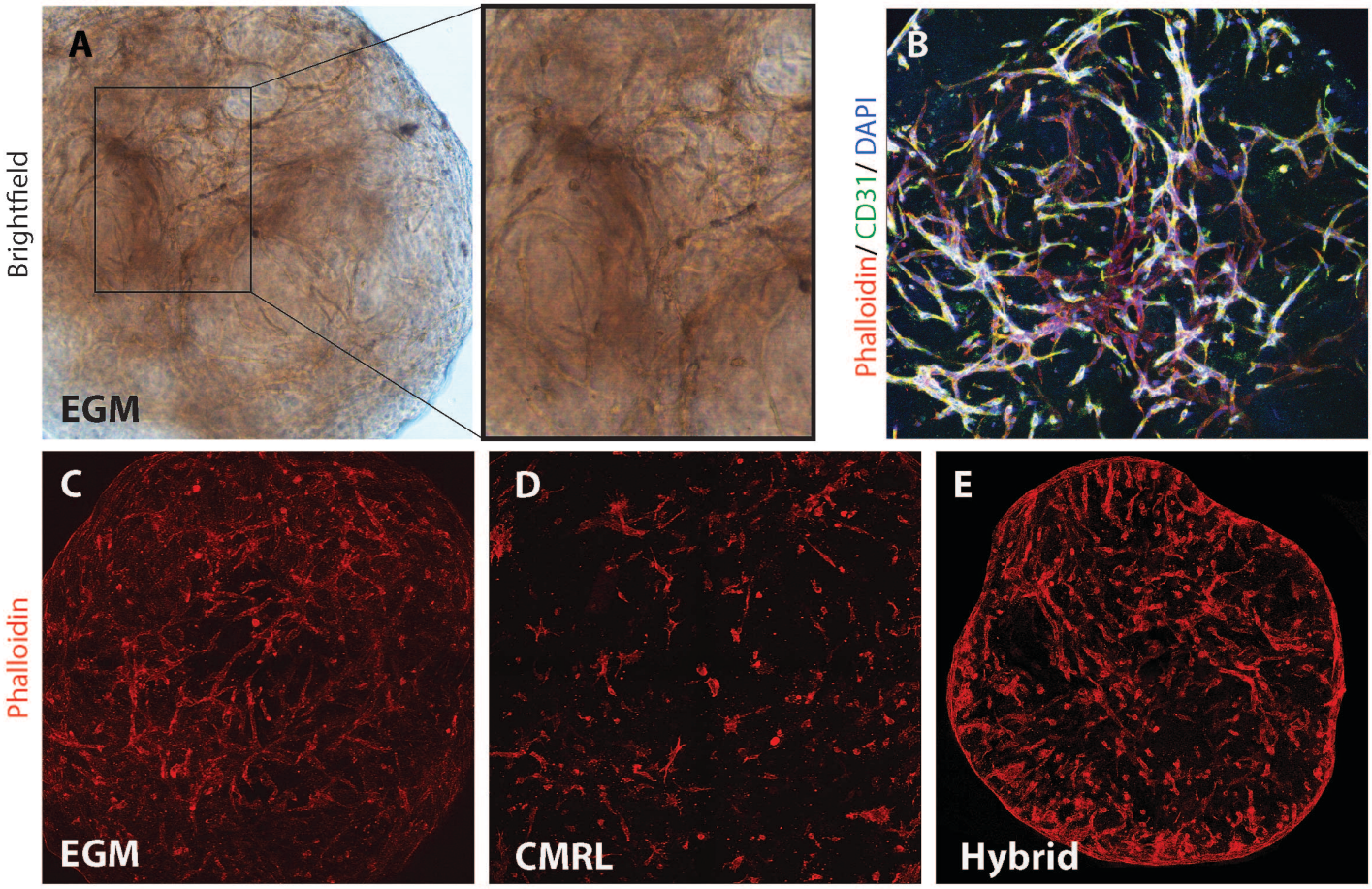
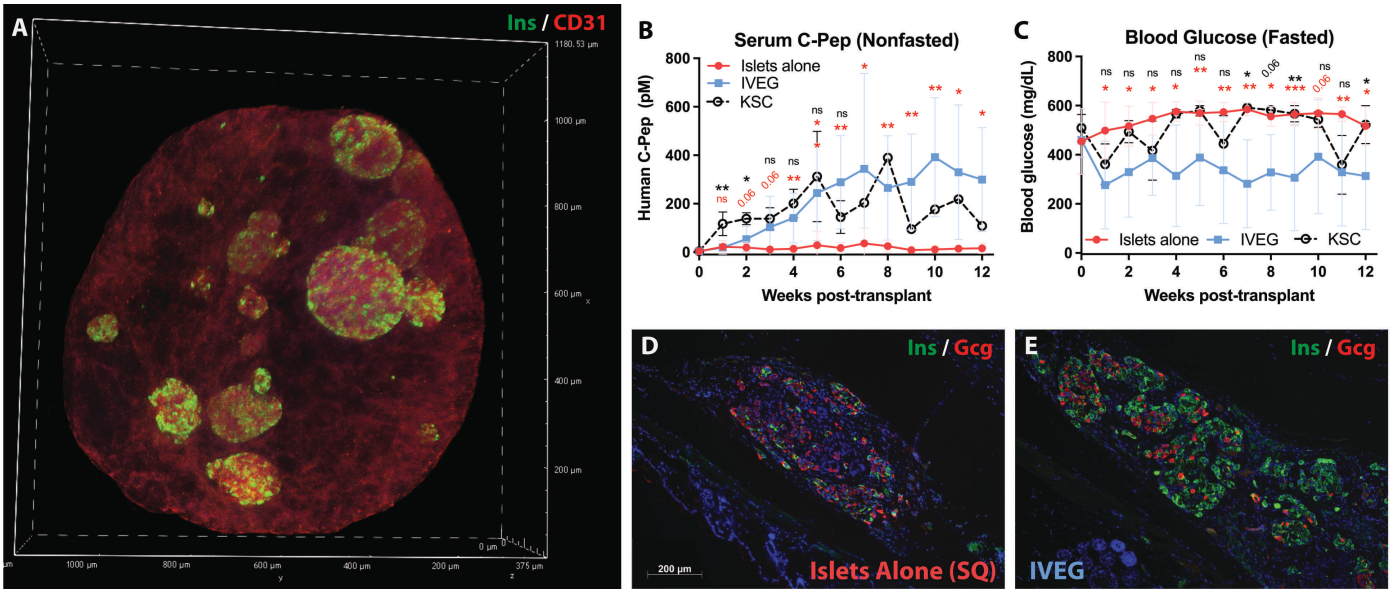
Introduction: Islet isolation results in extracellular matrix (ECM) damage, also severing connections with endothelial cells (ECs). Following transplantation, islets re-vascularize, enabling survival and function, but the time between isolation and revascularization results in significant islet loss. Further, much that is known about islet biology is based on using isolated islets, which are missing crucial connections and signals from the native environment. We developed a culture system that reconstructs the islet ECM and creates a network of capillary-like endothelial tubes in vitro, prior to transplantation. The islet vascularized ECM organoids (IVEOs) enable the study of crosstalk between islets and the islet microenvironment in vitro to reduce islet loss following transplantation.

Methods: Human pancreatic ECM hydrogel (hP-HG) was prepared by sequential decellularization, solubilization, and neutralization to form gel.[1] HUVECs were cultured in islet, EC, and hybrid media to optimize 3D tube formation (Fig 1A-B). Human islet function was assessed in IVEO constructs under ideal media conditions after 3 days of culture through static GSIS.
1000 IEQ were transplanted into diabetic immunodeficient mice (NSG RIP-DTR) alone subcutaneously (SQ), alone into the kidney subcapsule (KSC), or SQ within IVEG constructs.

Results: ECs cultured in hP-HG in EC medium (EGM) formed 3D tubes covering 29% of the area of a max-intensity image (Fig 1C). In islet medium (CMRL), the percentage was reduced (14.2%) (Fig 1D), but when cultured in a hybrid medium combining EGM with CMRL, the coverage was 31.7% (Fig 1E). This methodology enables the co-culture of endothelial tubes with islets in hP-HG for several days (Fig. 2A). Islets cultured as IVEOs had a significantly improved stimulation index (SI=10.0) compared to the same donor islets cultured in standard suspension culture (SI=2.1). IVEG constructs were transplanted following 3 days of culture and EC network formation.



Diabetic mice did not experience stable serum insulin levels following SQ transplantation with 1000 IEQ alone and did not have reductions in fasting blood glucose (FBG) levels (Fig. 2B-C). Mice transplanted SQ with the same donor islets after 3 days of IVEO culture saw reduced FBG and stable serum human insulin levels (N=3/grp). IVEO constructs transplanted SQ also outperformed islets from the same donors transplanted alone in the KSC. Grafts collected at 12 weeks post-transplantation showed visibly better retention of beta cell mass in the IVEO group compared to SQ islets alone (Fig. 2D-E).



Conclusions: ECs can form 3D tubes in hP-HG which maintain islet viability and function after 3 days of co-culture, enabling future study of islet-endothelial cell interactions in vitro. IVEO constructs more efficiently reduced hyperglycemia following SQ transplantation in diabetic mice than islets alone. Future studies will test additional doses in IVEO to achieve greater therapeutic effects in vivo.

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