Introduction: The shortage of donor islets for treating diabetes highlights the urgent need for generating insulin-producing β-cell from human embryonic stem cells (hESCs). Despite the improvement in β-cell differentiation in in-vitro cultures, a major gap remains in the lack of cellular maturity. This maturation defect may be partly due to the lack of 3D structure and interactions with stromal cells, both of which influence embryonic pancreatic development in vivo. We attempt to re-engineer this organogenetic interaction by repopulating decellularized pancreas with hESC derived pancreatic progenitors and endothelial cells. We hypothesize that 3D micro-architecture from decellularized pancreas and co-culture interactions with endothelial cells would provide the optimal microstructure and microenvironment niche respectively to guide organogenesis and promote β-cell functionality.

Materials and Method: Cadaveric pancreata were isolated from adult mice (n=8) and decellularized via detergent (2% Triton-X, 1% DOC) perfusion. Resulting decellularized matrices were characterized for acellularity, preservation of ECM and microstructures. Pancreatic progenitor (PP) cells were generated from step-wise differentiation of hESC into definitive endoderm (Activin and Wnt for 4 days) followed by sonic hedgehog inhibition (cyclopamine) and retinoic acid induction for another 4 days. For recellularization, hESC-derived PP cells ($3x10^6$ cells) were combined with HUVEC ($3x10^6$ cells) and introduced into the acellular 3D pancreatic scaffold via portal vein in 3 steps, with 30 min interval between each step. The recellularized 3D construct was cultured on a perfusion bioreactor and evaluated via immunostaining, TUNEL, and qRT-PCR after 7 days.

Results and Discussion: Perfusion-decellularization of pancreas with detergent resulted in complete removal of cells. Characterization of the decellularized pancreas revealed preservation of ECM and 3D architecture. At day 7, qRT-PCR analysisrevealed that recellularized pancreatic construct(n=3) had significantly increased expression of early pancreatic marker genes (PDX1, NKX6.1, NGN3, ISL1) and maturation markers (MAFA, INSULIN) compared to cells seeded in matrigel (n=6) and control (n=6), $P<0.05$ (**Figure 1**). Consistent with this, immunostaining result showed significantly higher number of PDX1+ cells ($P<0.023$) were found in recellularized pancreas construct compared to matrigel. In addition, immunostaining result also showed that majority of PDX1+ cells (n=10, 82 ±11%) were detected in close proximity with endothelial cells (CD31+ cells), suggesting the involvement of stromal-cell-dependent factors in pancreatic maturation.

Conclusion: Perfusion-decellularization of pancreas efficiently removes cells while retaining ECM protein and microstructure. This perfusable 3D whole organ scaffold yields complex3D tissue construct that favored pancreatic differentiation when seeded with hESC-PP and HUVEC. This is a first step proof of concept to show that the 3D pancreatic maturation can be recapitulated in vitro by co-culturing pancreatic progenitors with endothelial cells in decellularized pancreas.

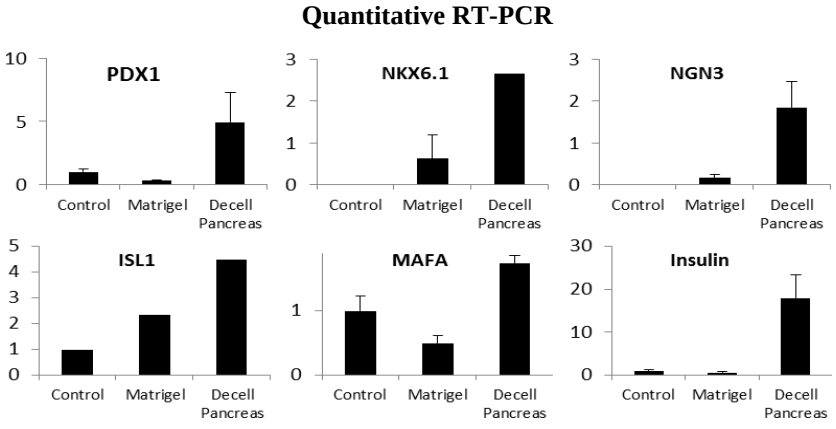


Figure 1.Quantitative PCR analysis of pancreatic marker gene expression in recellularized pancreas with hESC-PP and endothelial cells at day 7 of culture. Control samples were hESC:HUVEC that had been grown as separate cell types and mixed together at the same ratio of cell types as in recellularized pancreas construct.