Understanding Cell Interaction Promoting Beta Cell-like Function and Maintenance

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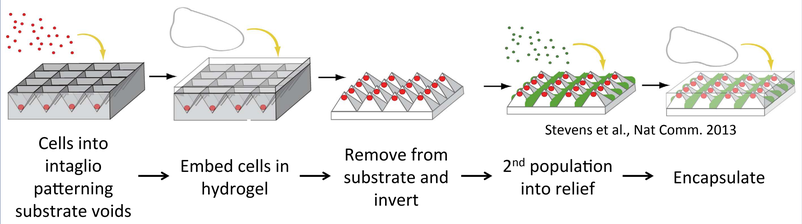
Abstract

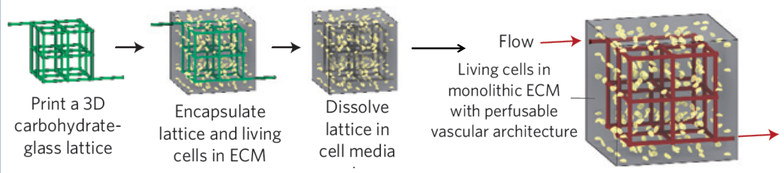
Cell-cell interactions play major roles to support organ function. The diverse endocrine cells within the pancreatic islets, together with micro-vascular endothelial cells and mesenchyme, regulate proper glucose homeostasis in the body. Interestingly, the inability to maintain islet function for extended period *ex vivo* underlines the importance of the pancreatic niche to sustain cell maturation and function. Merging classical stem cell biology techniques and novel bioengineers tools, we aim to generate biomimetic devices, which can be used to mimic the pancreatic microenvironment. Using the well established and validated "InVERT molding" method and co-culturing pancreatic precursor cells and human islets with different cell types, we will recreate the proper physiological conditions leading to long-term islets function, with the aim to understand the interactions between beta cells and other cells within the islets, supporting and maintaining function of the beta cell *ex vivo*. Here we present preliminary data of islets-like organoids generated co-culturing human ES cell-derived beta-like cells (Stage 7) with diverse cell types (HUVECs and hMSCs and adult islets) applying the initial steps of "InVERT molding" approach. Cellular interaction leads to the formation of 1000-cell 3D organoids one day after plating. Upon 6 days in culture pancreatic precursors retain clusters of C-peptide positive cells and appeared embedded in a network of endothelial cells. Proper amount of HUVECs and hMSCs, as well as optimal medium composition, are required to promote C-peptide release. These results indicate positive effects of hMSCs and HUVECs on Stage 7 cells *in vitro*.

1. Generating Integrated Biomimetics Devices Using "InVERT" Molding and 3D Printing Technologies

Intaglio-Void/Embed-Relief Topographic (**InVERT**)

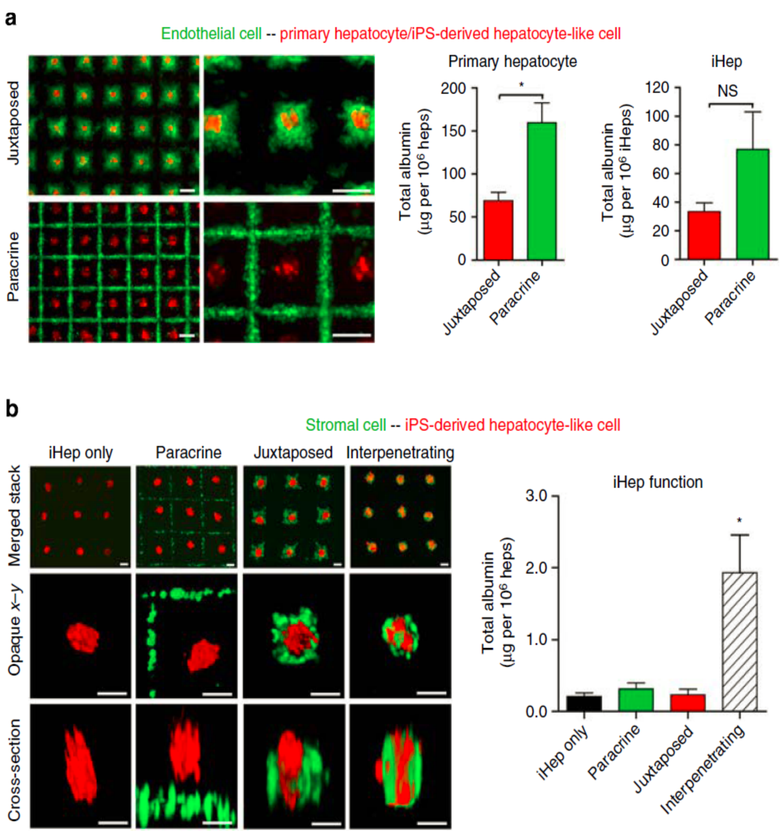


Generation of Fluidic Channels within Cellularized Tissue Constructs

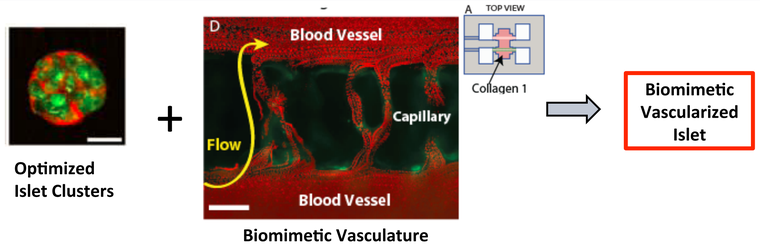


Miller et al., Nat Materials. 2012

2. Multi-compartmental placement dictates hepatic tissue function

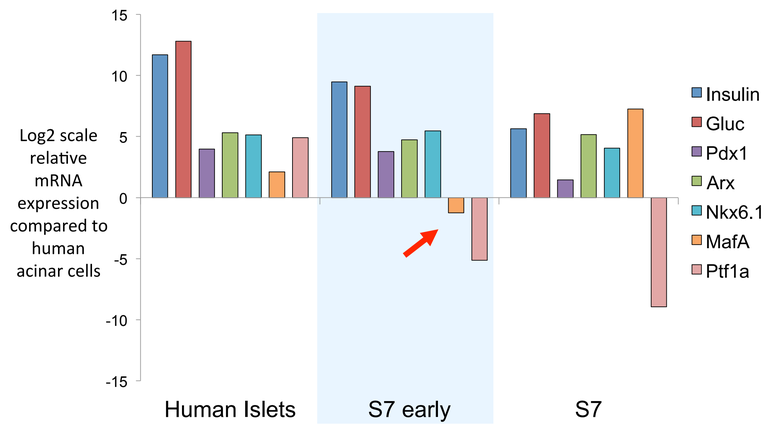


3. Integrating vascular network to maintain long term ex vivo culture of beta-like cells

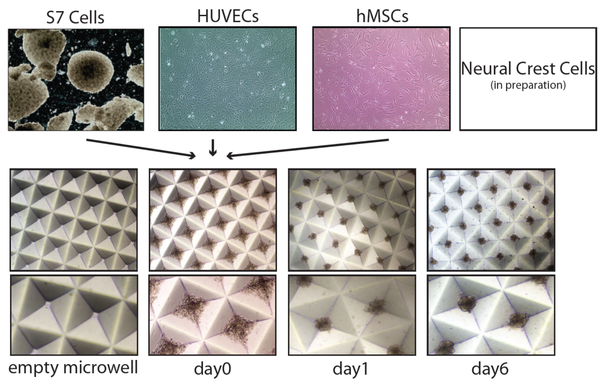


Interspersing optimized islet clusters into the interstitial compartment of the biomimetic vasculature, we will maintain beta-like cells' function and will improve maturity of beta-like cells *in vitro*.

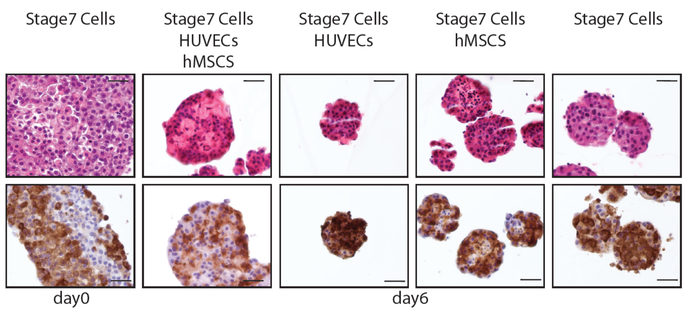
4. huES-derived Stage 7 Pancreatic Cells form 3D Organoids when Co-cultured with HUVECs and hMSCs



RT-qPCR shows expression of pancreatic endocrine markers in Stage7 Cells (early and more mature) comparable to human islets. Stage7 Cells express markers for both alpha and beta cells and are highly depleted in the expression of exocrine markers. Early Stage7 Cells show lower levels of MafA compared to human islets and more mature Stage7 Cells.

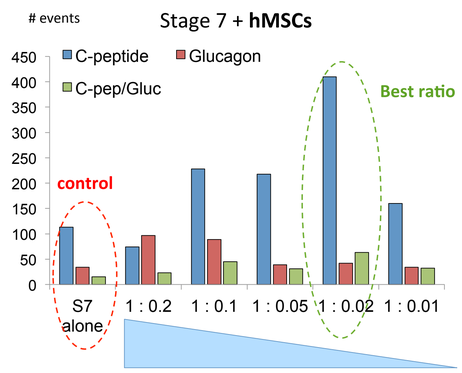


Stage7 Cells are mixed together with HUVECs and hMSCs and plated onto microwells in each single well of 96-well plate. Overnight, cells organize into a 3D structure which is maintained up to 6 days in culture, at which time analysis is performed

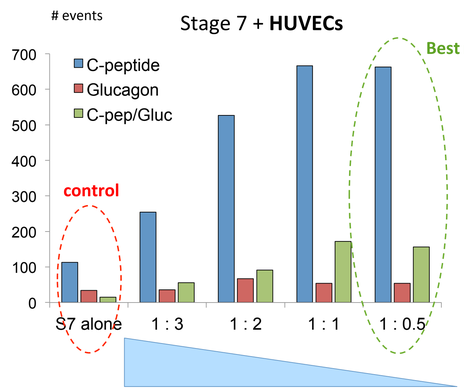


H&E staining reveals the presence of nuclei of similar size in all the co-cultured specimens analyzed. Organoids containing hMSCs show also nuclei free, protein rich (eosin positive) structures.

5. hMSCs and HUVECs Cells Affect Intracellular C-peptide (FACS) in Early Stage 7 Cells

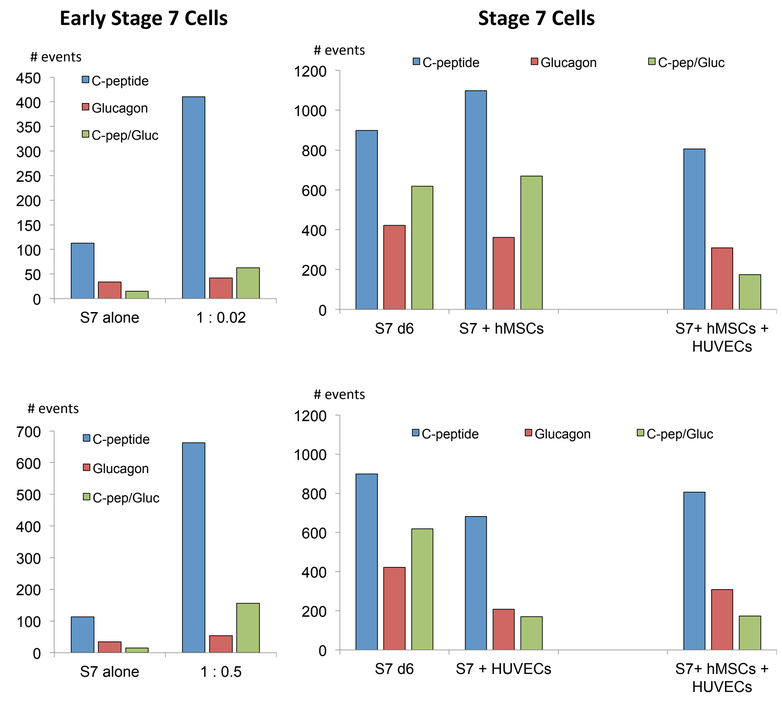


1 : 0.02 ratio represents the best mixture condition promoting the highest expression of C-peptide in hMSCs containing organoids.



1 : 0.5 ratio represents the optimal mixture condition promoting the highest expression of C-peptide in HUVECs containing organoids.

6. Early Stage 7 Cells Seem More Responsive to hMSCs and HUVEC than Stage 7 Cells



7. Future Directions

- **Assess early S7 vs. S7 cells response to combined MSC and HUVECs:**
  - gene expression
  - proliferation, apoptosis
  - GSIS by perifusion assay
  - extending culturing time
- **Add sympathetic neurons**
- **Assess scale: increased cell sizes**

8. Acknowledgments

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