Developing biomaterials for 3D islet tissue engineering

Roberto Gaetani[1,4], **Kim-Vy Nguyen-Ngoc**[2,3,4], Heinz Strassle[1,4], Karen Christman[1,4], Sander, Maike[2,3,4]

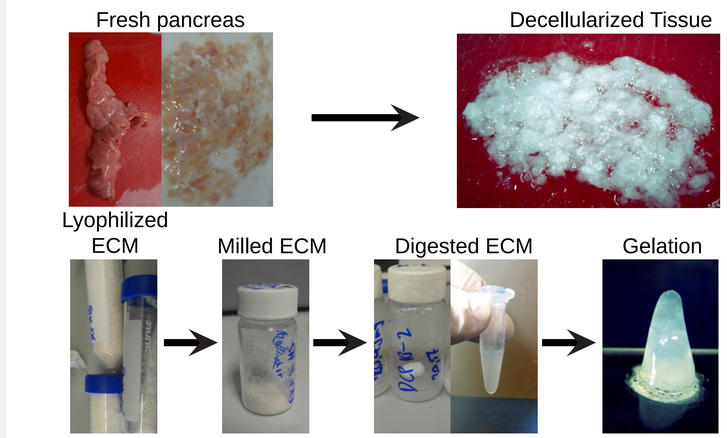
[1]Department of Bioengineering, [2]Department of Pediatrics, [3]Department of Cellular & Molecular Medicine,
[4]Sanford Consortium for Regenerative Medicine, University of California, San Diego, La Jolla, CA, USA

Abstract

Loss or dysfunction of pancreatic insulin-producing beta cells is the hallmark of diabetes; however, in vitro models to study disease mechanisms or to test novel therapeutics are lacking. Our goal is to create a 3D islet culture model that more accurately mimics the rich cell-matrix interactions that characterize the in vivo microenvironment of beta cells, as lack of these cues could explain why functionally mature beta cells are difficult to generate or to maintain in vitro. To this end, we developed a protocol for the generation of pancreas-specific extracellular matrix (pECM) hydrogel for 3D culture of human islets. By testing different detergents and varying their concentration and incubation time with pancreas tissue we have developed a decellularization protocol that preserved ECM components and the ability of the matrix to gel. Using quantitative mass spectrometry analysis of pECM components, we confirmed the presence of multiple types of ECMs. We were able to demonstrate that human islets remained viable for up to two weeks in culture in the pECM hydrogel. Furthermore, to develop a model of human islets derived from human pluripotent stem cells (hPSCs), we have established an “organoid” culture system, in which hPSCs are cultured in 3D-Matrigel to generate branched organoids consisting of pancreatic progenitor cells. Surprisingly, we found that Matrigel-only hydrogels were superior in promoting organoid branching morphogenesis when compared to hydrogels consisting of only pECM or a mix of pECM and Matrigel. Now, with the establishment of an optimized 3D culture system that recapitulates morphogenesis in vivo, we have created an artificial niche similar to the environment found in vivo.

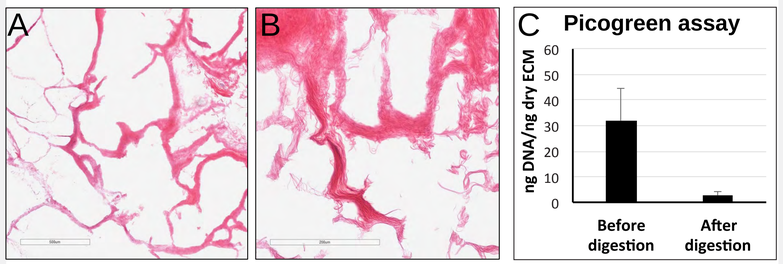
Methods

Figure 1. Generation of pancreas-specific ECM (pECM)



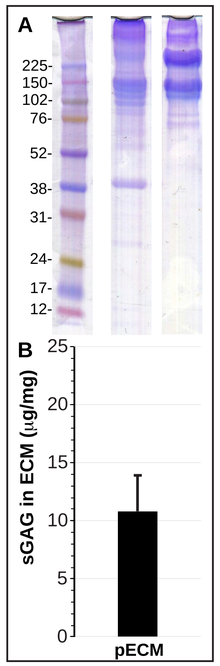
Results

Figure 2. Evaluation of decellularized pECM



(A,B) H&E staining, (C) PicoGreen assay.

Figure 3 and Table 1. Evaluation of pECM complexity

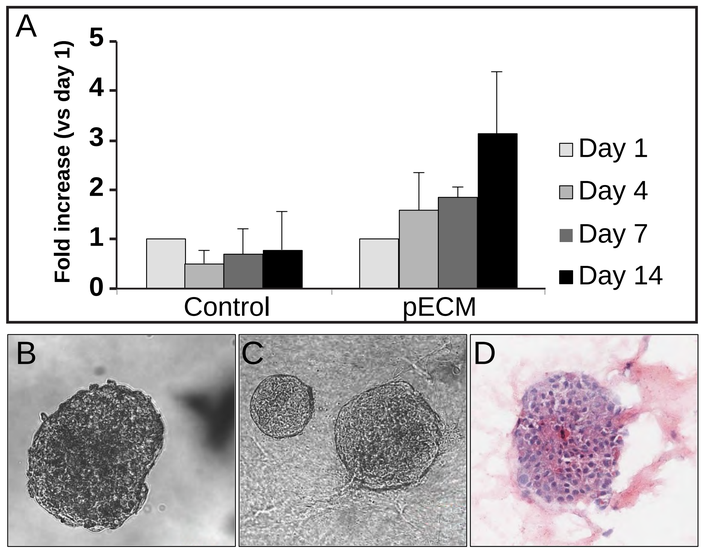


Evaluation of pECM content by (A) SDS-PAGE and (B) sGAG assay.

Table 1. Quantitative mass spectrometry analysis of pECM.

Protein Abundance in Porcine Pancreatic Matrix		
GENE	**Functional Classisfication**	**%**
COL4A1/5*	Basement Membrane	0.17
LAMC1	Basement Membrane	0.0020
COL4A1*	Basement Membrane	0.35
COL4A2*	Basement Membrane	0.16
LAMC1	Basement Membrane	0.0004
AGRN*	Basement Membrane	0.0002
COL12A1	FACIT Collagen	0.012
COL1A1	Fibrillar Collagen	65.51
COL1A2	Fibrillar Collagen	30.23
COL5A1	Fibrillar Collagen	0.26
COL5A2	Fibrillar Collagen	0.20
PRELP	Matricellular	0.07
FBLN5	Matricellular	0.0005
FN1	Matricellular	0.0021
COL18A1	Matricellular	0.0013
EMILIN1	Matricellular	0.0004
DPT	Matricellular	0.12
LUM	Matricellular	0.03
COL18A1	Matricellular	0.0013
COL6A1	Matricellular	2.22
COL6A2	Matricellular	0.04
FBLN5	Matricellular	0.0003
DPT	Matricellular	0.28
COL6A1	Matricellular	0.0082
COL6A3	Matricellular	0.19
COL6A1	Matricellular	0.0050
TNXB	Matricellular	0.0020
ANXA2	Other ECM	0.0003
ASPN	Other ECM	0.03
OGN	Other ECM	0.08
FBN1	Structural ECM	0.0012
FBN2	Structural ECM	0.0004
BGN	Structural ECM	0.03

Figure 4. Culturing human islets in pECM hydrogel



(A) Alamar blue assay confirming islet viability up to 14 days when cultured in pECM. (B-C) Brightfield images of human islets cultured in suspension (B), in pECM (C). (D) H&E staining of human islets in pECM.

Figure 5. Overview of the differentiation protocol used to generate hPCS-derived organoids

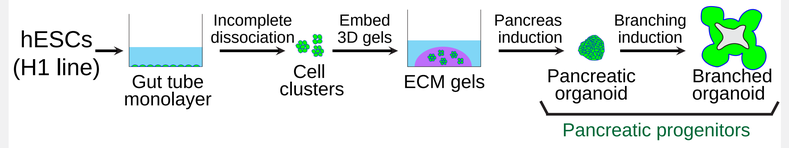
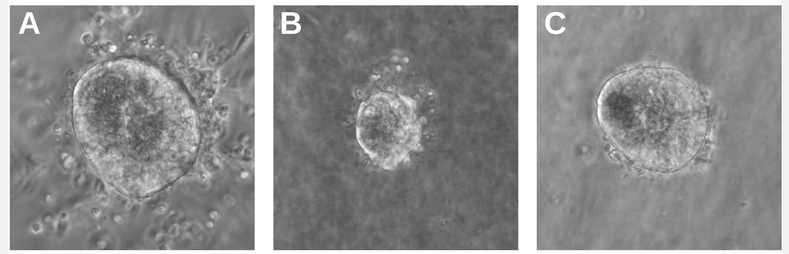
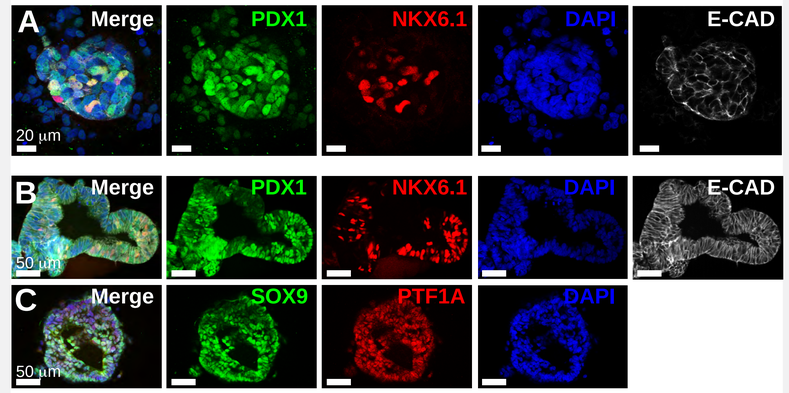


Figure 6. pECM has negative effects on organoid growth during pancreatic induction



(A-C) Cell clusters isolated at gut tube stage are induced toward the pancreatic endoderm stage in 3D gels with Matrigel:DMEM (1:1) (A), pECM (B), and Matrigel:pECM (1:1) (C).

Figure 7. Characterization of pancreatic organoids



Immunoflourescent staining showing (A) pancreatic organoids before branching, (B,C) after branching.

Future directions: Induce endocrine differentiation in organoids