Engineering Hydrogels for Encapsulating Islets within Fluidic Devices

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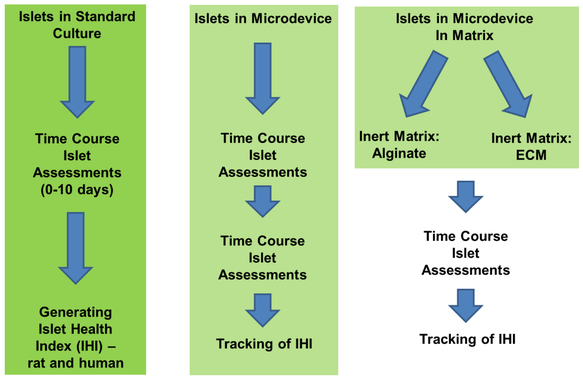
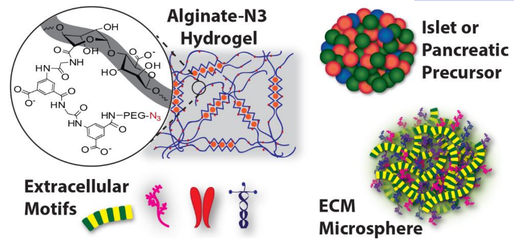
Objective

Specific Aim 2: Utilize a microfluidic platform to systematically delineate critical factors associated with the 3-D matrix and physiological microenvironment that are capable of supporting human islet maintenance

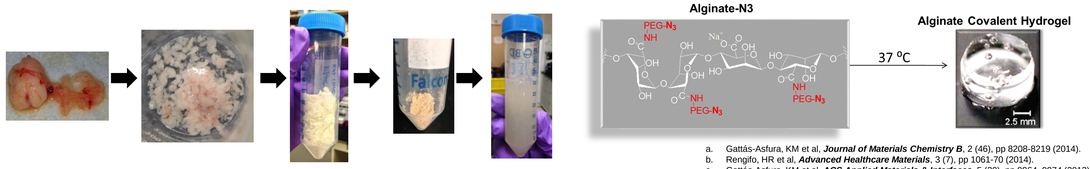
- **Identify critical islet assessments indicative of islet "health"**
- **Consolidate critical assessments into single islet heath index (IHI)**
- **Apply IHI to rodent and human cultures to evaluate impact of microfluidic platform**
- **Encapsulate islets within novel 3-D matrices and evaluate impact on IHI**



Engineering Hydrogels



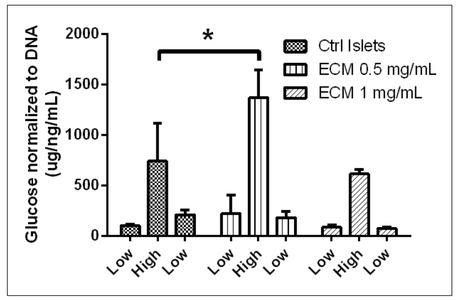
ECM Sources: Focus on ECM combinations similar to peri-islet BM (e.g. Collagen IV, laminin 511, entactin, and proteoglycans)
Alginate Sources: Focus on functionalized hydrogels for control of presentation of selected groups and/or material stability



a. Gattás-Asfura, KM et al, ***Journal of Materials Chemistry B***, 2 (46), pp 8208-8219 (2014).
b. Rengifo, HR et al, ***Advanced Healthcare Materials***, 3 (7), pp 1061-70 (2014).
c. Gattás-Asfura, KM et al, ***ACS Applied Materials & Interfaces***, 5 (20), pp 9964--9974 (2013)
d. Hall, KK ***Acta Biomaterialia***, 7, pp. 614-624 (2011).

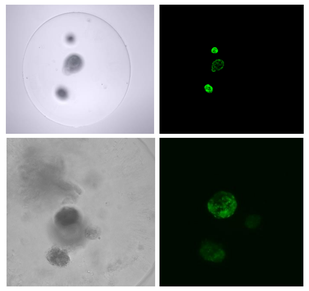
Screening of Impact of ECM

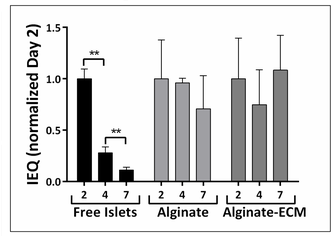
Methods: Islets were embedded within lung ECM 3-D gels

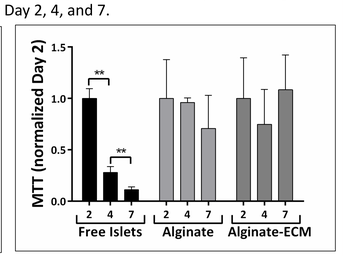


Screening of Inert (alginate) vs Bioactive (ECM+Alginate) hydrogels

Methods: Islets (~ 1 IEQ/bead) were mixed within Alginate (1.7% MVG) only or alginate + ECM (10 mg/mL decellularized porcine lung courtesy of K Christman) at 5:1 and dropped into bath of $BaCl_2$ for gelation. Assessments on Day 2, 4, and 7.







Conclusions to date:

- Feasible to entrap islets in 3-D alginate-ECM hydrogels
- Preliminary studies indicate elevated stability and GSIR within gels

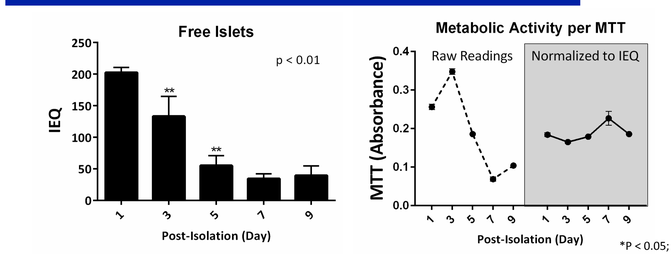
Islet Health Index

Screen a series of assessments to identify assays that are:

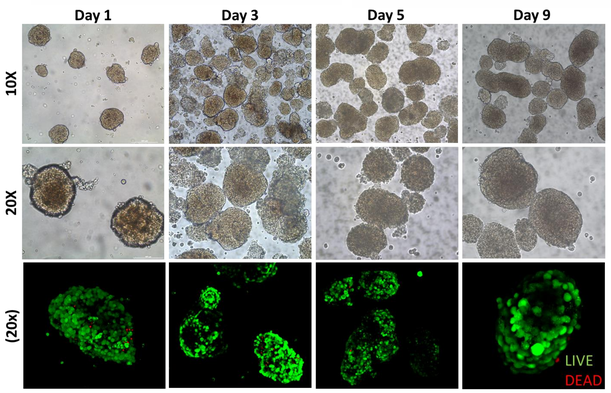
- Predictive of islet quality over culture period
- Capable of *in situ* analysis within microfluidic device

Initial Assay Candidates

Parameter	Assay	Collection	Temporal/ Terminal
Purity, Morphology, and IEQ	Visual Characterization	Imaging of Microwell	Temporal
Cell Loss	DNA	DNA assay of islets	Terminal
Metabolic Activity	MTT	Absorbance reading of islets	Terminal
Static or Perifusion Glucose Responsive Insulin Secretion	ELISA or Multiplex	Outlet Stream	Temporal
Static or Perifusion Glucose Responsive Glucagon Secretion	ELISA or Multiplex	Outlet Stream	Temporal
Static or Perifusion Glucose Responsive C-Peptide Secretion	ELISA or Multiplex	Outlet Stream	Temporal
Calcium or Zinc Imaging	Fluo doping and in situ imaging	Imaging of microwell	Temporal
Oxygen Consumption Rate	Oxygen sensors or hypoxia probes	In line or In situ	Temporal
Cytokine screening	ELISA or Multiplex	Outlet Stream	Temporal



*P < 0.05; **P < 0.01

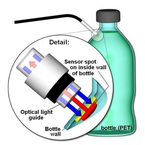


Conclusions to date:

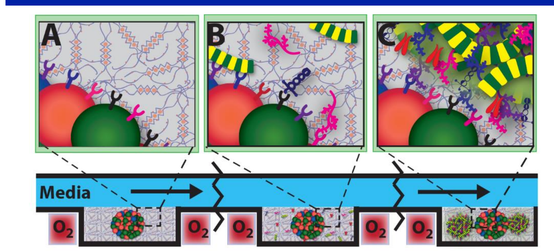
- Monitoring of IEQ over time correlates with invasive DNA or MTT assays
- When IEQ loss is accounted for, no decline in metabolic function or visual Live/Dead is observed
- GSIR declines over culture time

New Directions:

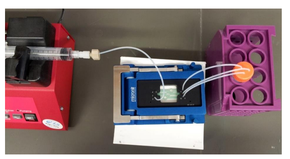
- OCR measurements promising assay
- Hypoxy probes alternative option
- Expand to multiplex to minimize volumes and permit more extensive measurements
- Visual probes to permit tracking *in situ*



3-D Matrix + Islets in Chip



Islets within Alginate Gel Slabs in Microwells



Islets within ECM Gel Slabs in Microwells

Conclusions to date:

Feasible to entrap and perfuse islets in 3-D matrices within chip design

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