A 3-Dimensional culture system for the cultivation and proliferation of Sca-1 positive pancreatic progenitor cells

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BACKGROUND

- When grown in 2 dimensional culture conditions primary cells isolated from all tissue types lose their original features and differentiate due to constraints of being grown in a monolayer.
- Culturing cells in a 3-dimensional environment creates a natural milieu hospitable to cell growth, proliferation, and differentiation that better mimics the environment seen during organogenesis.
- We have previously isolated a murine pancreatic progenitor cell (PPC), using Stem cell antigen-1 (Sca-1), a marker of hematopoietic stem cells. In two dimensional culture these cells differentiate into the multiple cell types that compose the pancreas.
- Using a novel 3D culture device we have been able to propagate and expand this PPC population . The cells maintain endocrine cell markers over extended culture, and secrete insulin in response to glucose stimulation .

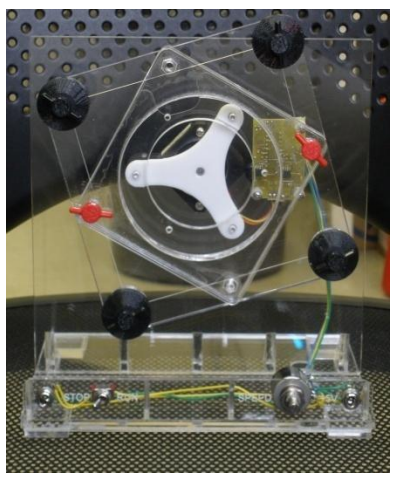
HYPOTHESIS

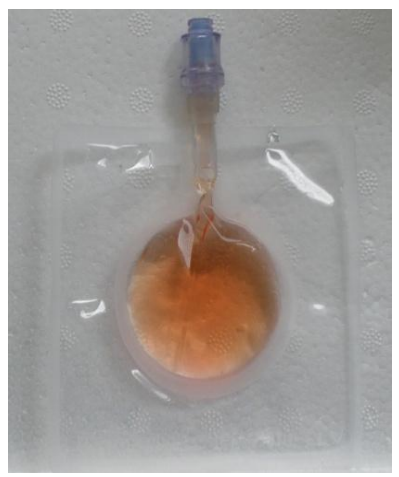
- We hypothesize that our rotating wall vessel bioreactor will improve pancreatic progenitor cell propagation thereby expanding a cell source with potential clinical applications.

MATERIALS AND METHODS

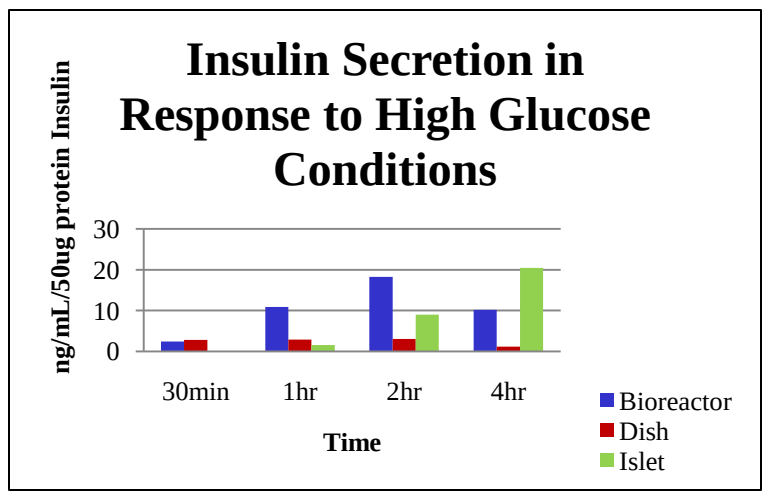
- PPC's were isolated from 2 week old GFP positive mice using enzymatic and mechanical digestion followed by purification for Sca-1+ cells using MACS®.
- Cells were allowed to attach to Cytodex 3 microcarrier beads and then cultured in VueLife™ fluoro ethylene propylene (FEP) bags attached to a rotating wall vessel (RWV) bioreactor.
- Cells were imaged over the course of two weeks in culture using GFP fluorescence.
- Insulin production in response to glucose stimulation was analyzed by ELISA.
- Cellular differentiation was assessed by western blot analysis.

RESULTS

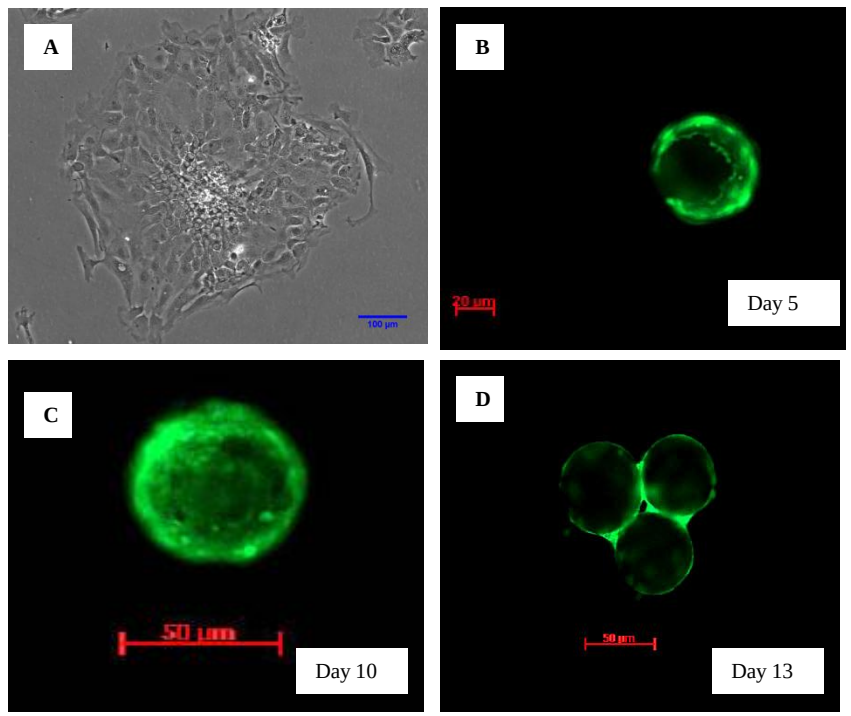




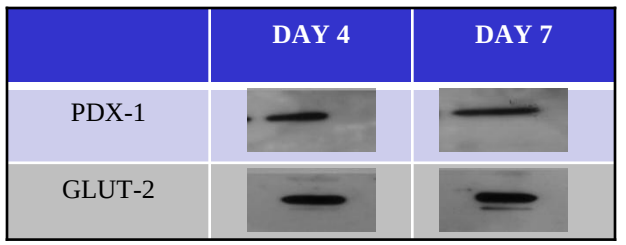
RWV bioreactor fabricated by Dr Bob Dennis and FEP cell culture bags used to keep cells in suspension culture.



Insulin production in response to glucose. Bioreactor cultures were compared to 2-D dish cultures and islets were used as a control.



A: PPC's at day 4 of culture when grown on 2D tissue culture dishes B,C,D: Growth and proliferation of PPC's on microcarrier beads in 3-D culture system at day indicated.



Western Blot analysis shows retention of pancreatic protein production up to 1 week in culture.

CONCLUSIONS

- Sca-1 positive PPC's remain viable and proliferate on microcarrier beads in a rotating wall bioreactor mechanism
- The unique 3-D environment supports cell adherence to microcarriers; allowing cell proliferation and aggregate formation with neo-tissue bridging between proximate beads.
- 3-D environment maintain s the cell's phenotype with expression of markers like Glut 2 and PDX-1.
- The bioreactor environment enhances rapid insulin secretion in response to a glucose challenge.