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Tissue Engineered Representation of Human Blood Vessel Using Hagfish Protein

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Tissue Engineered Representation of Human Blood Vessel Using Hagfish Protein

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Utah State University
BIOLOGICAL ENGINEERING

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Utah State University

Background

- In tissue engineering, scaffolds help guide cells to grow into desired form.
- Scaffolds made of biocompatible materials increase the likelihood of acceptance when implanted into the body. For example, an engineered blood vessel grown on a biocompatible scaffold would be more likely to be taken in with the surrounding tissue.
- Previous work has shown cells can grow on fibers made of hagfish protein (1)
- For this reason, we tested the growth of both C2C12 (mouse smooth muscle) and HUVEC (human umbilical cord) cells on two different biocompatible materials. These cell types were selected because they are similar to cells present in blood vessels.

Methods

Cell Culture

- HUVEC cells were cultured using endothelial cell media until confluent on two T25 flasks
- C2C12 cells were cultured using DMEM media (10% FBS) until confluent on two T75 flasks (3)

Well Plate Preparation

- Hagfish protein was dissolved in 97% formic acid (3% w/v). 1 mL solution was added to each well, allowed to evaporate for 24 hours. The first column of wells was washed with PBS and EtOH. The second column was crosslinked with saltwater for an hour, then washed.
- Collagen wells were prepared using suggested protocol (2)
- The final column of wells was left bare

Cell Seeding

- Each HUVEC well was seeded with 0.5 mL culture media ($6x10^4$ cells/mL)
- Each HUVEC well was seeded with 0.5 mL culture media (10^5 cells/mL)
- Media was changed every other day for 7 days

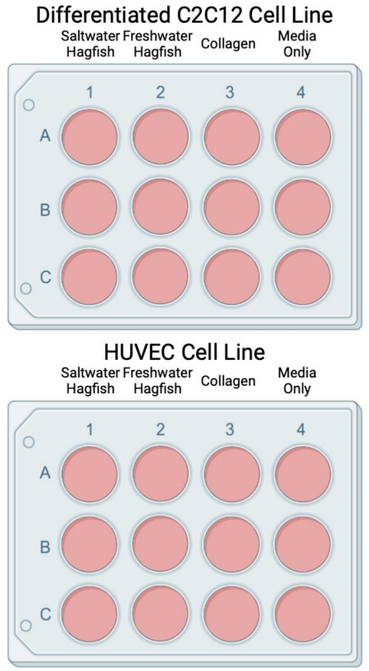

Fig.1: Experimental Setup for 12-well plate.

Results

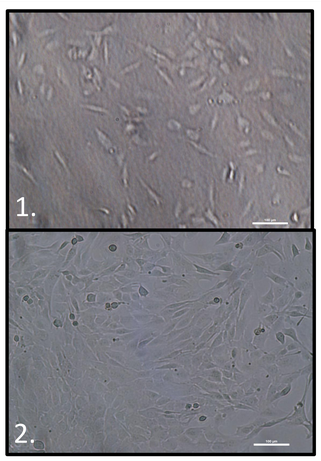

Figure 2: Cellular growth of HUVECs (1) and C2C12s (2) immediately before seeding 12-well plates.

Initially, the C2C12s grew well (Fig. 2.1), and required frequent passage to prevent differentiating before the treatment. HUVECs grew (Fig. 2.2) but became contaminated before seeding into 12-well plates. Growing HUVECs was attempted again but were again contaminated after seeding directly from cryo.

The media in hagfish protein wells was yellow and all cells seeded into them died within the first two days. No cells were observed in the performed DAPI stain (Fig. 3.a). Cells grew well in both the negative and positive controls, and visually it was confirmed that collagen, the positive control, was a superior substrate for cell growth of these cell lines than the negative control, the treated well-plate (Fig. 3.b-e).

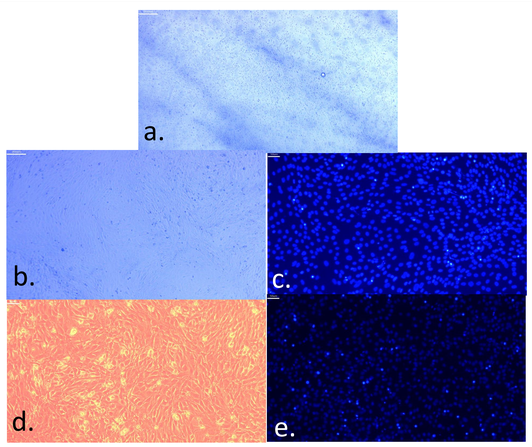

Figure 3: Imaging of cells. a) Hagfish DAPI stain 10x b) Brightfield collagen 10x. c) DAPI collagen 10x. d) Bright field media only 10x. e) DAPI media only 10x.

Conclusions/Future Work

The yellow shade of the media in hagfish membrane wells suggests the media was acidic, likely the cause of the complete cell death. Since this color change was not observed in the collagen or media-only wells, this low pH is likely due to residual formic acid in the membranes. To adjust for this, future replications of this protocol may use lower concentration formic acid or include more washes with a neutralizing solution.

HUVEC plates consistently developed contamination, potentially due to bacterial presence in the media or in the cryo tubes the HUVECs were stored in. Thus, we were unable to collect cell growth data for HUVECs.

Future work will include adjusting and repeating tests to determine if the hagfish membranes can sustain muscle and endothelial cell growth. If these are successful, tubular structures and bilayer co-cultures may be attempted.

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