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Can Cells Solve Mazes?- Understanding Cell Responses to Wound Healing

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MOTIVATION

- Wound healing is a process that occurs within the body to repair damaged tissue.
- This process is largely driven by cellular functions/interactions.
- Thus it is important to understand how cells migrate from all around the body to a site of injury.
- If we can understand how cells make decisions and respond to complex environments, then we can develop better technologies.
- The **current goals** of the project are:
    1. Develop a methodology for studying cell growth and migration in 2D confinement
    2. Understand the decisions of cells in response to confinement and chemical guidance

BACKGROUND

- One of the major cell types involved in wound healing is fibroblasts.
- Fibroblast movement requires the action of contractile forces within the cell and the formation of new attachment points to the surface on which the cell is moving.[1]
- It is driven by a response of the cell to growth factors (chemotaxis).[2]
- There is a need to more fully understand how fibroblasts respond to growth factor gradients along specific paths.
- To mimic this environment, maze-like structures can be used as a tool for creating these chemical gradients.
- Other cell types have been found to solve mazes for the shortest path to a food source.[3]
- This project aims to use similar techniques to understand fibroblast migration and growth specifically.

METHODS

Laser Cut Acrylic Mazes

- Mazes designed using Solidworks
- Design cut from plastic (acrylic) using a laser cutter
- Inverse channels created by pouring polydimethylsiloxane (PDMS) over acrylic maze cut-outs
- Gradient established by placing a hydrogel containing concentrated growth factor (40% FBS) at one end of the structure (Figure 1a)

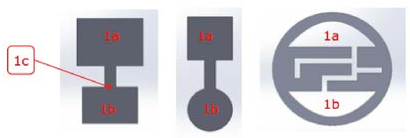

Figure 1. Select maze designs and inserts. 1a) End containing concentrated growth factor 1b) End at which cells are plated 1c) Channel entrance point

Modeling Cell Growth

- Once the cells reached a specific area (Figure 1c), pictures of the cell front were taken every 12 hours
- Pictures used to quantify cell growth rate
- Using a 1D diffusion model, data was inputted to calculate the cell growth rate

Cell Growth Assay

- A LDH assay was conducted to test the hypothesis of increase in growth factor (FBS) concentration lending to increase in cell growth
- The amount of LDH released is directly proportional to the number of cells present
- This test isolated the effect of growth factor concentration on cell growth

RESULTS

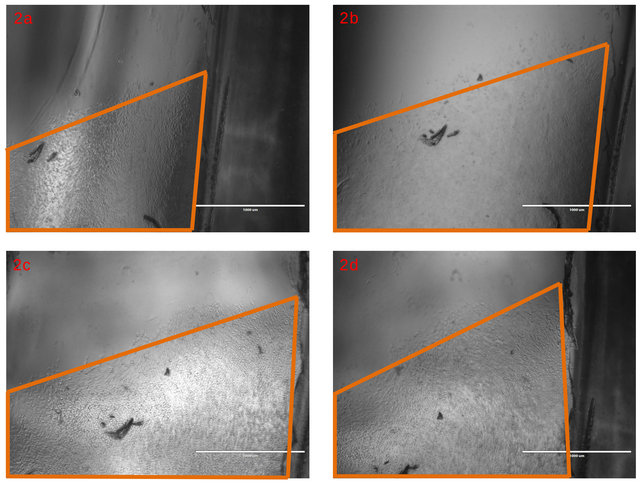

Figure 2.Time lapse images of cell front in maze moving through maze. Cell front marked in orange lines. 2a) 48 hours post plating 2b) 72 hours post plating 2c) 96 hours post plating 2d) 120 hours post plating

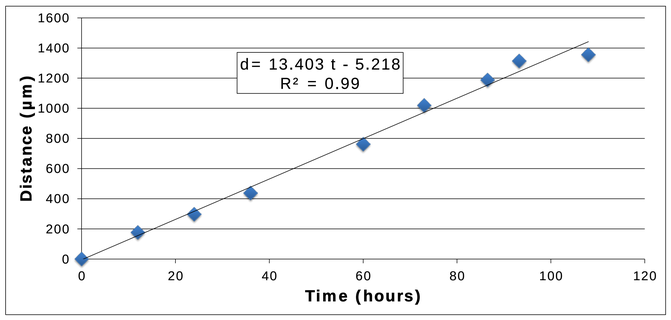

Figure 3. Cell front movement tracked by with time lapse pictures. Growth rate was calculated using the MATLAB polyfit function to yield average cell front velocity of 13.403 µm/hour.

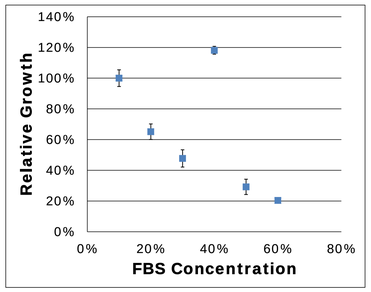

Figure 4. Cell growth in response to media supplemented with different concentrations of FBS. Cell growth measured with lactate dehydrogenase assay and standardized against recommended cell culture media (10% FBS).

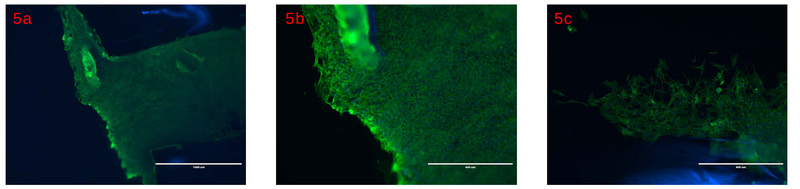

Figure 5. Eipfluorescent images of 3T3 Fibroblasts stained with Phalloidin 488 and DAPI. 5a) Cell front having reached end of channel at 4X magnification 5b) Cell front at 10X magnification 5c) Cell front extending towards end well at 20X magnification.

CONCLUSIONS & FUTURE WORK

Conclusion:

- Successful construction of an apparatus to measure cell migration and concentration gradients
- High FBS concentrations potentially work as an inhibitory growth signal

Future Work:

- Validate MATLAB model of growth factor diffusion
- Grow cells in more complex maze structures to continue the goal of understanding decisions made by cells
- Develop a model to aid in tissue engineering as a tool for regenerating damaged or diseased tissue

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