A novel microfluidic platform for application of fluid shear stresses to cells in 3D culture

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Objectives

- Develop a microfluidic bioreactor to deliver a range of shear stresses (1-100x) to stem cells in a 3D microfluidic environment.

- Test cell viability and proliferation of human adipose-derived stem cells (hASCs) over a 7 day period under a 100-fold range of shear stresses.
Background

- Functional tissue engineering
  - Application of physical forces to manipulate stem cell behavior (differentiation)

- Bone provides more surface area for exchange and filtering of solutes than vascular or lymphatic system
  - Porous matrix
  - Fluid phase
  - Cells

- Transmission of mechanical signals via application of fluid shear forces to cells

Background

- **Fluid-induced shear stress**
  - Dynamic due to locomotion and normal homeostasis fluid movement in lacuna-canilucular network
  - Conversion into intracellular biochemical signals
  - Shear stress varies depending method of application
    - Viscosity
    - Flow rate (Q)
Microfluidic Bioreactor

Provide adequate platform
- Parallel analysis comparisons
- Minimum expenses
- 3D vs 2D (through the plane flow)

Laminar fluid flow

Simple manipulation based on Poiseuille’s law for fluid flow
Bioreactor Design

Figure 1: Diagram of bioreactor.

- Recirculation loop
- Peristaltic pump
- Manifold distribution with shear stresses from 1-100X
- 6 chamber bioreactor
Bioreactor Design

Poiseuille’s law for laminar fluid flow

\[ Q = \frac{\Delta P}{R} = \frac{\Delta P \pi r^4}{8\eta l} \]

\[ \tau = \frac{(\eta Q)}{(d_s \varepsilon D^2)} \]

By varying the length of the channel, the shear stress applied is varied from 1, 2.51, 6.31, 15.85, 39.81, 100x
Complete Bioreactor

Figure 4: Peristaltic pump and reservoir.

Figure 5: Manifold connected perfusion chamber.
Porous Matrix: Scaffold

- Commercially available spunbond PLA
- Fiber Diameter ~20 µm
- Pore Size: 40 µm ± 15 µm ($d_s$)
- Porosity: 89% ± 3% ($\varepsilon$)

Figure 6: Images of spunbond PLA.
Methods

- Seed 50k hASCs on spunbond PLA
- Culture for 2 days in growth medium
- Culture in bioreactors for 7 days in osteogenic medium
- Analysis on days 1 and 7
  - Cell viability and proliferation

Figure 7: Image of hASCs before inoculation.

Scale bar = 200 µm
Cell Viability Day 1

Scale bar = 200 µm
DNA Concentration vs. Shear Stress Rating

Shear Stress (dynes/cm²)

DNA (ng)

day 1

day 7

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Conclusions

- Limited cellularity
- Samples display varying viability based on shear stress
- Trend suggests strong proliferative response on day 7 for shear stresses greater than 0.2 dynes/cm²
- Control of shear stress by simple design
- Future experiments will focus on effect of shear stress on osteodifferentiation
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