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

Journal of Biomechanics 36 (2003) 1409-1424

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

Lab on a Chip (2004) 4 91-97

Bioreactor Design

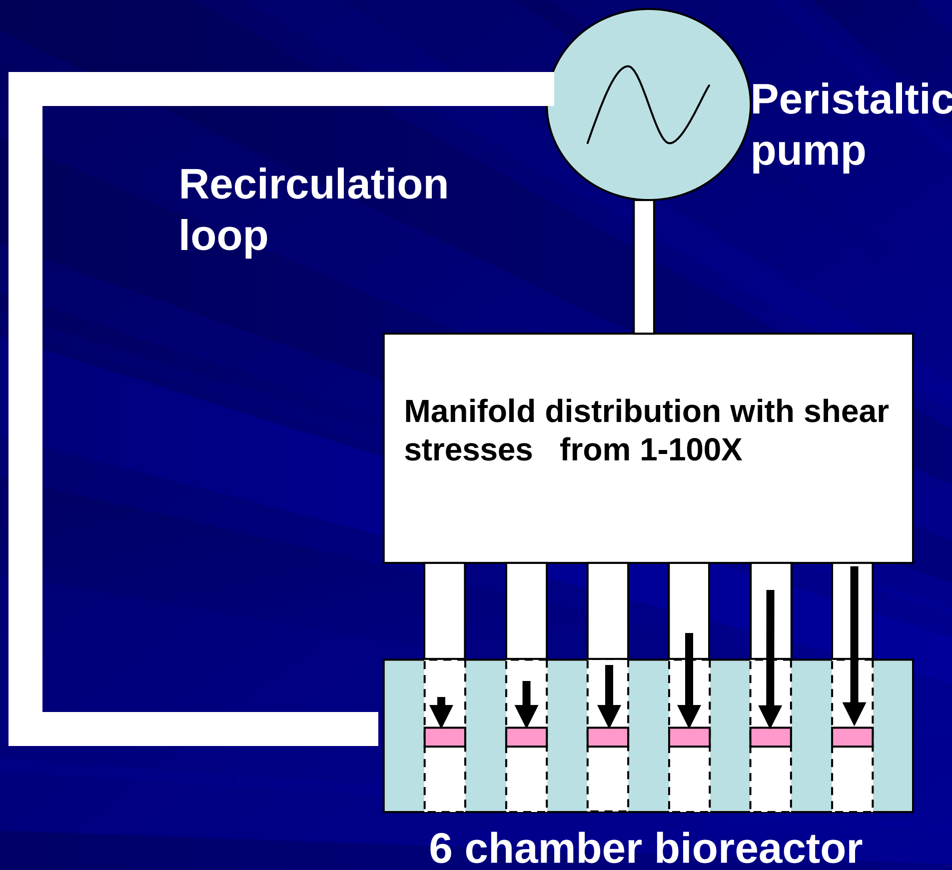


Figure 1: Diagram of bioreactor.

Bioreactor Design

- Poiseuille's law for laminar fluid flow

$$Q = \Delta P/R = \Delta P \pi r^4/8\eta l$$

$$\tau = (\eta Q)/(d_s \varepsilon D^2)$$

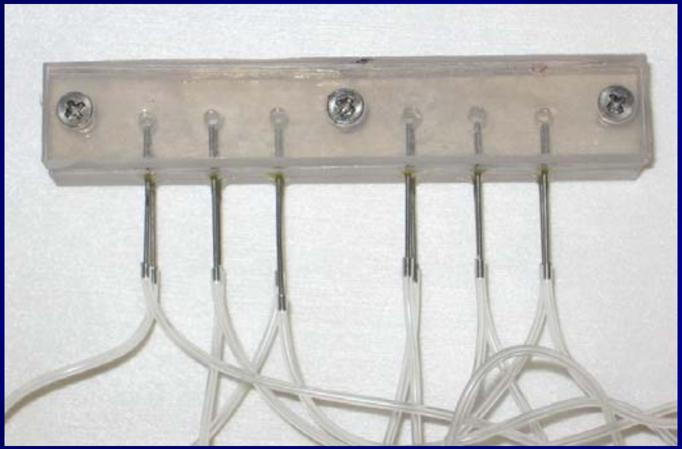


Figure 2: Image of bioreactor chamber.

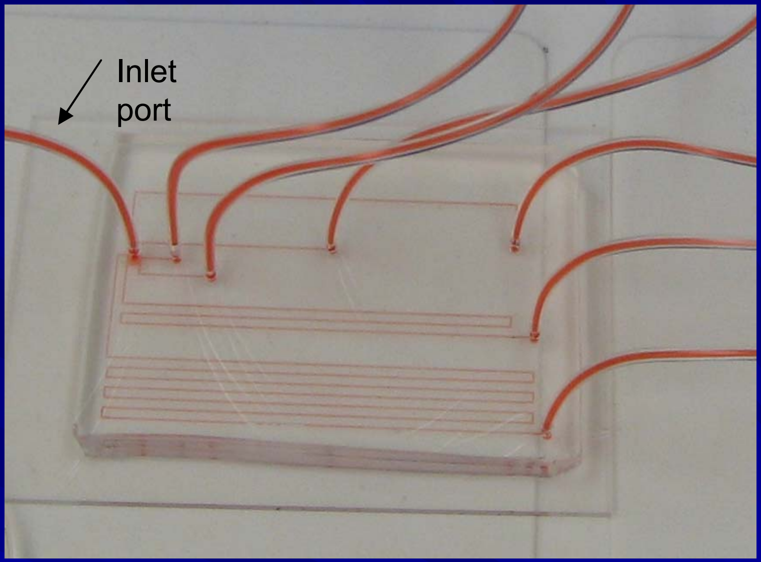


Figure 3: Image of manifold apparatus.

By varying the length of the channel vary the shear stress applied from 1, 2.51, 6.31, 15.85, 39.81, 100x

Biomaterials 22 (2001) 1279-1288

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% (ε)

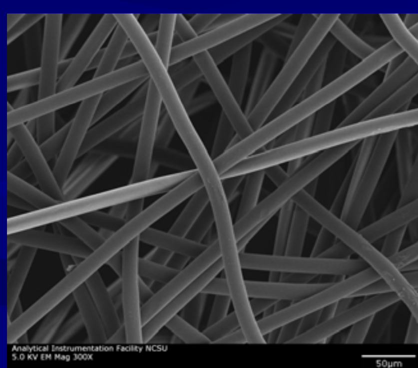


Figure 6: Images of spunbond PLA.

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

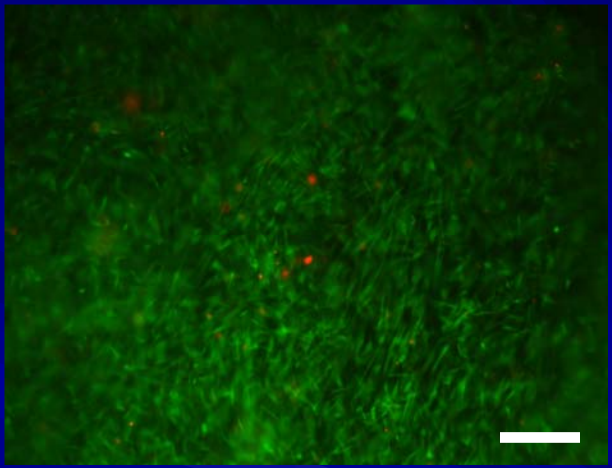
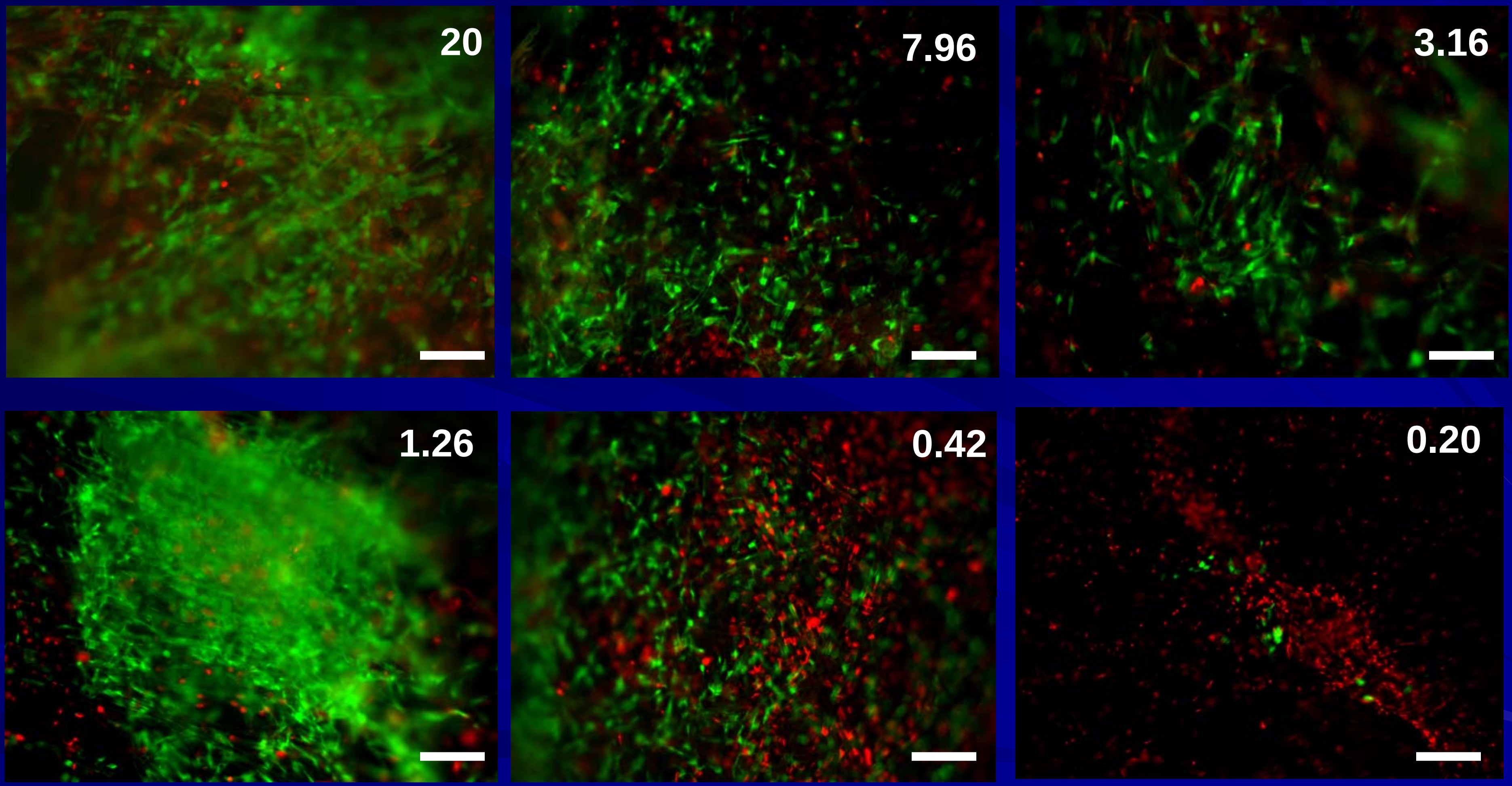


Figure 7: Image of hASCs before inoculation.

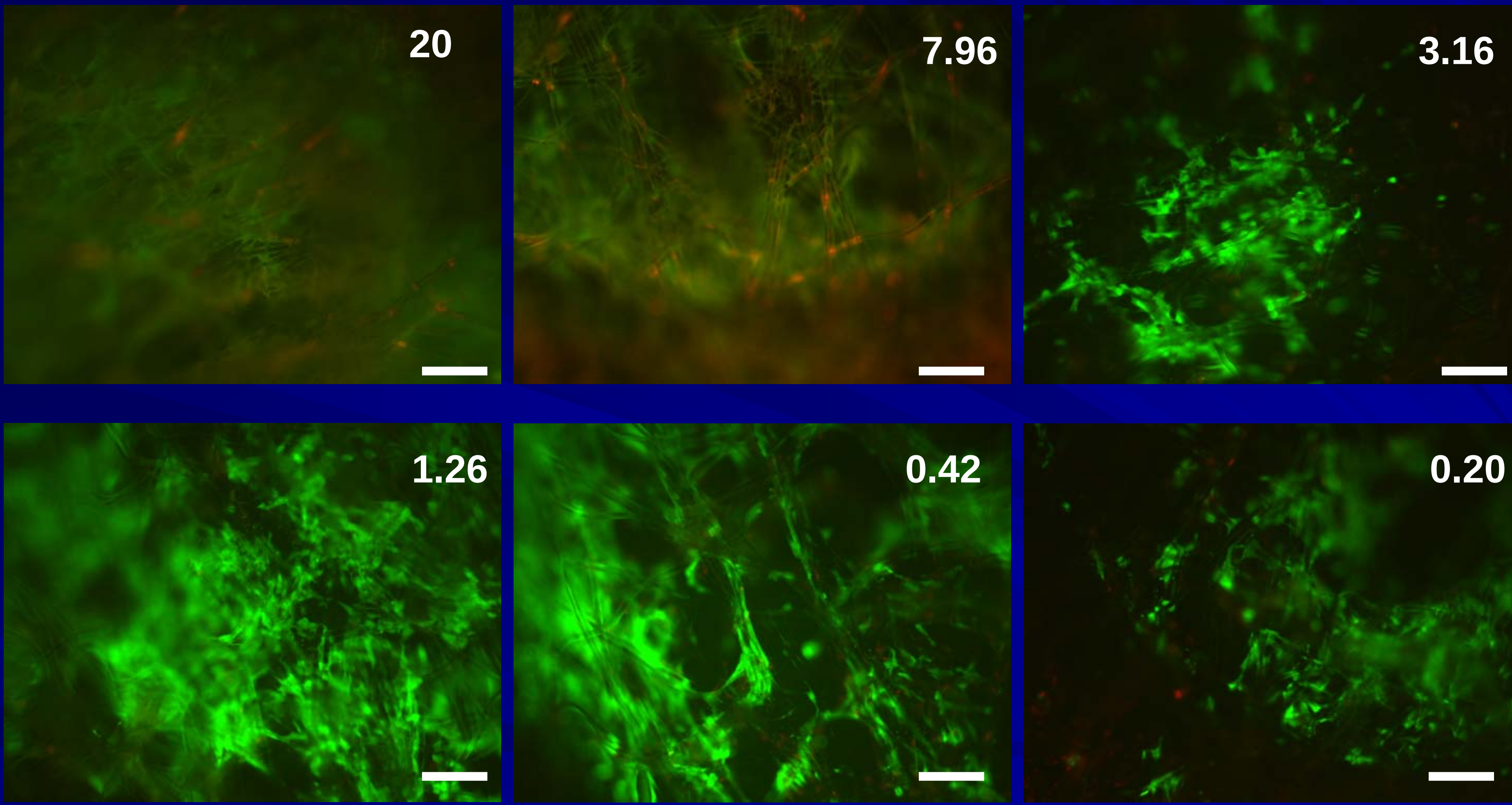
Scale bar = 200 μm

Cell Viability Day 1

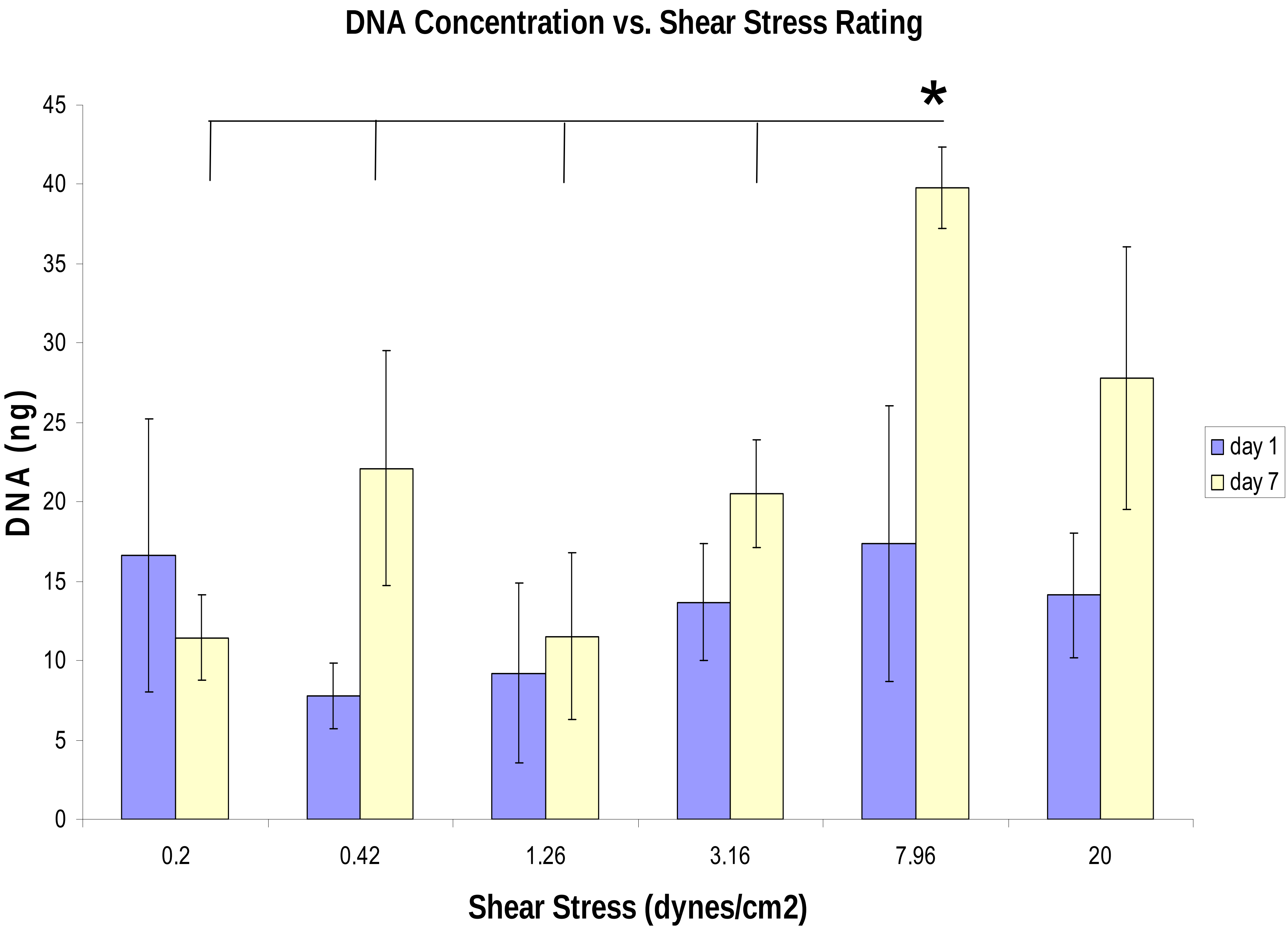


Scale bar = 200 µm

Cell Viability Day 7



Scale bar = 200 μm


DNA Concentration vs. Shear Stress Rating
*
45
40
35
30
25
20
15
10
5
0
DNA (ng)
0.2
0.42
1.26
3.16
7.96
20
Shear Stress (dynes/cm2)
day 1
day 7

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/cm^2
- Control of shear stress by simple design
- Future experiments will focus on effect of shear stress on osteodifferentiation

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