JOHNS HOPKINS
WHITING SCHOOL of ENGINEERING

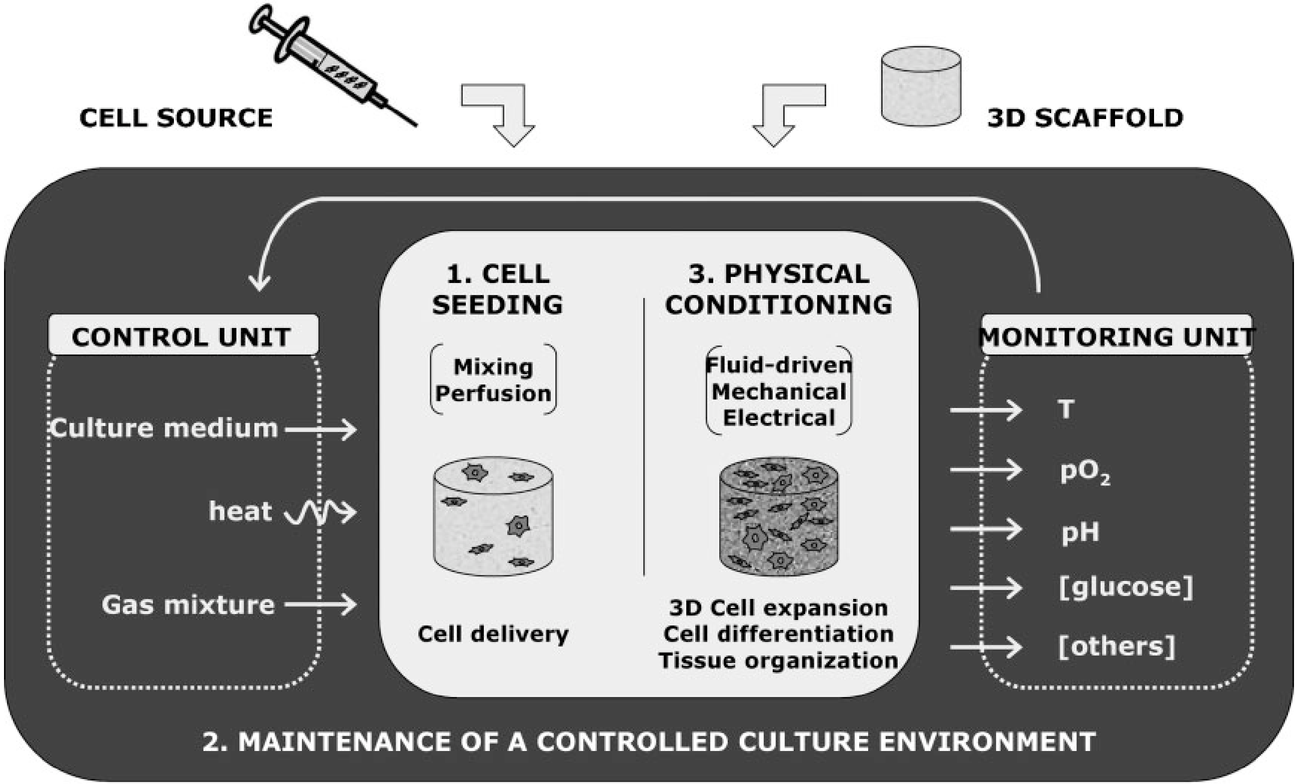
Cell and Tissue Engineering

Bioreactors – Warren Grayson, Ph.D.

Bioreactors and Beer

- Term ‘bioreactors’ originally used to describe systems regulating fermentation processes i.e. unicellular microbial organisms.
  - Application of chemical engineering approaches to biological processes
- Bioreactors in tissue engineering
  - Overcoming inhomogeneities
- Complexity in multi-cellular tissues makes it challenging to correlate effects of input parameters with specific outcomes
- Various uses of bioreactors
  - Cell expansion
  - Creation of functional tissues
  - Micro-systems – modeling cellular responses (drug testing)

What are Tissue Engineering Bioreactors?



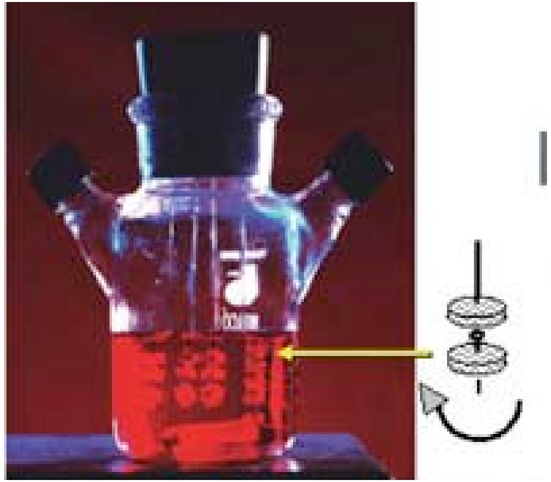
Wendt et al, 2009

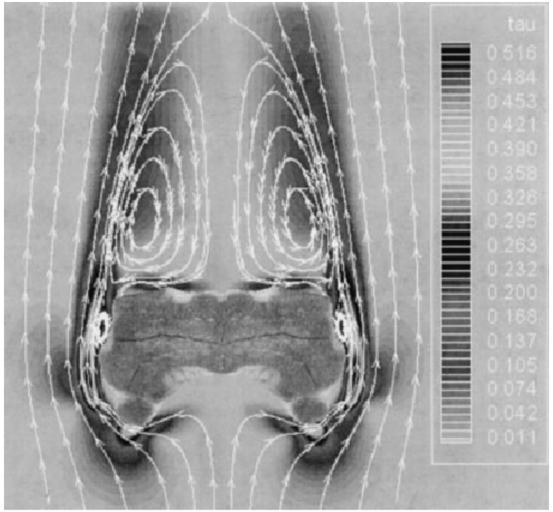
Principles of bioreactor design

- Maintenance of sterility
- **Improved mass transfer of nutrients to cells**
  - for growing large constructs
- Adequate gas exchange to bulk culture medium
- Temperature & pH control
  - Critical for ‘stand-alone’ systems
- Replenishment of spent medium
- **Biophysiological stimulation**
- Imaging compatibility

Convective Mass Transfer

Spinner flasks provide convective mass transfer to cells

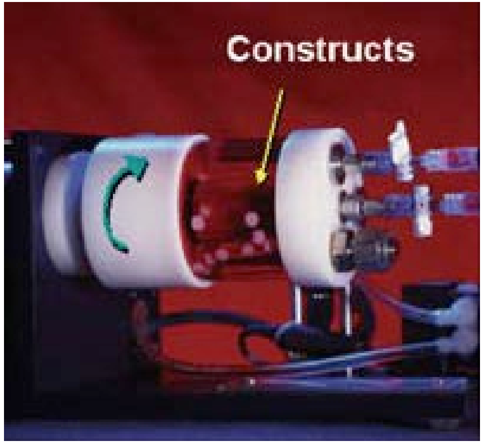


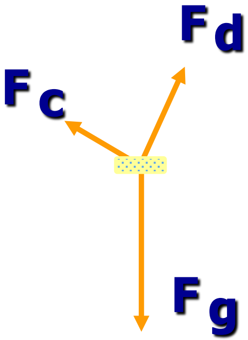


- Constructs in static cultures had huge necrotic cores
- Convection increased cell viability in central regions of the constructs
- Shear forces on surface may have detrimental effects on cells.
- Although convection increased nutrient transfer to the surface, nutrients still needed to diffuse from surface to center

Rotating Wall Vessels

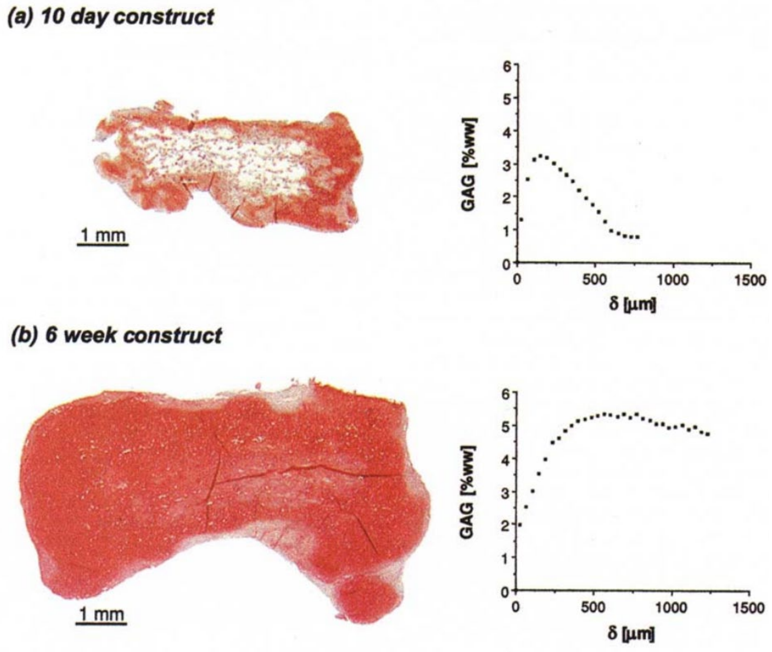
- Similar to stirred flasks in that convection increased nutrient transfer to the surface, nutrients still needed to diffuse from surface to center.
- Shear forces on surface are lower than those in stirred flasks
- Both systems resulted in more uniform tissue distributions however…





Mechanical Properties

- Improves tissue uniformity and protein production but poor mechanical properties
- Tissues develop appropriate structure in response to mechanical loads
- 2nd generation bioreactors investigated the application of physiological loads to developing tissue constructs



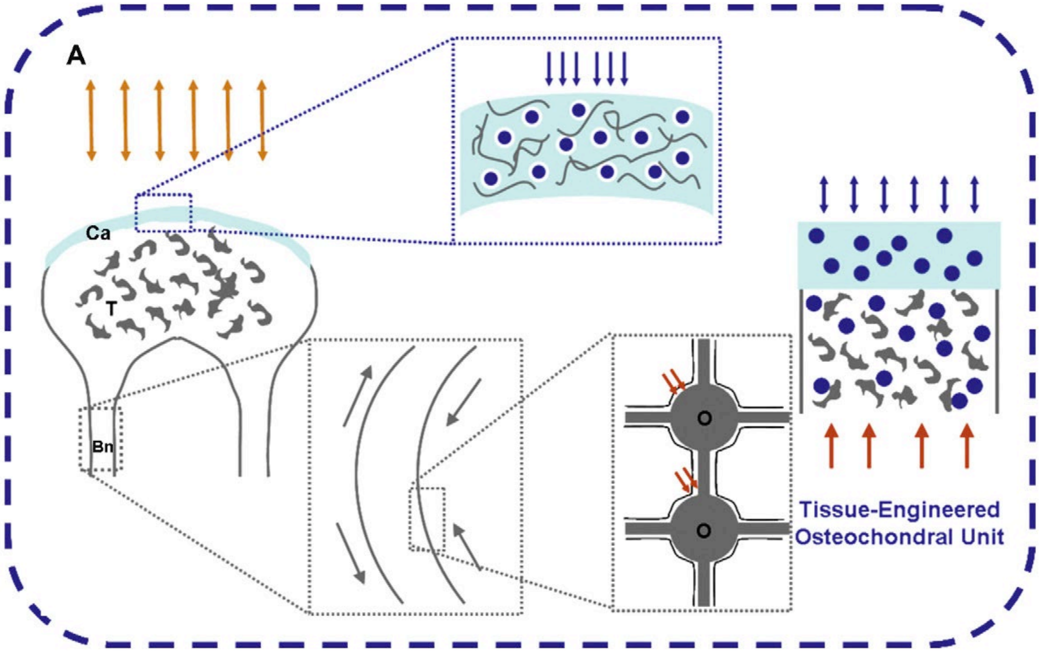
Chondrocytes cultured in PLGA scaffolds in rotating wall vessels for up to six weeks. The authors measured the distribution of GAGs in the matrix as a function of location within the scaffolds.

Obradovic et all, 2000

Biomimetic Principle

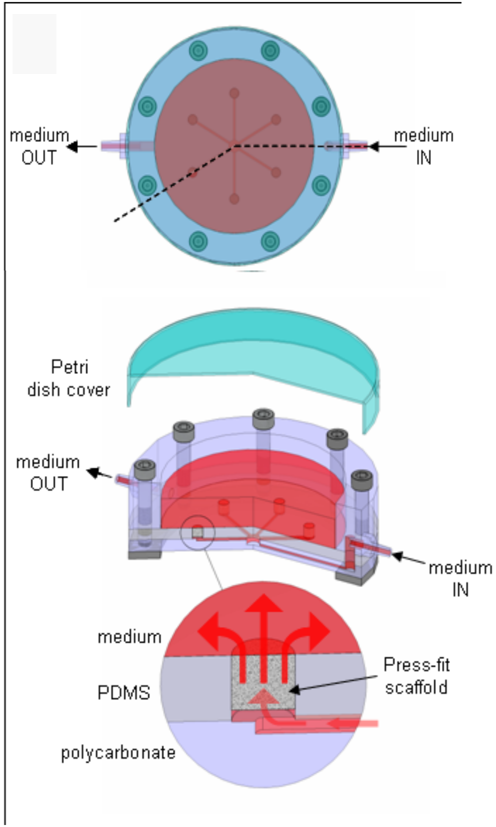
Providing tissue-specific biological cues

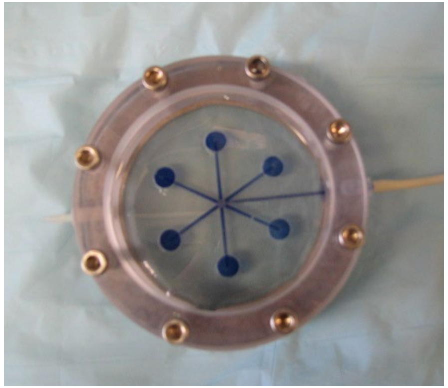
Example: What type of forces would we use to stimulate appropriate mechanical properties in engineered bone and cartilage tissues?

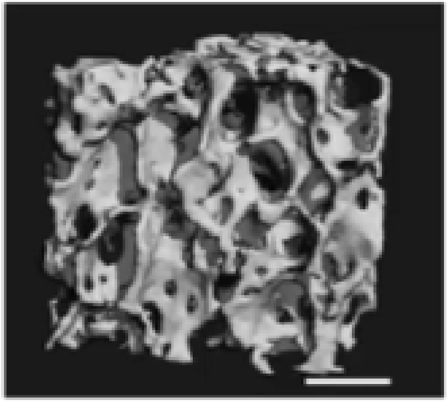


Perfusion Bioreactors

Improved mass transfer

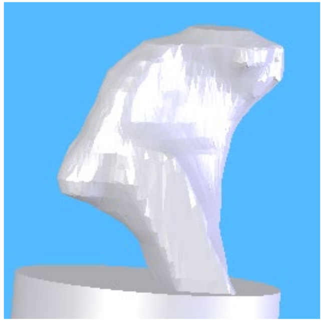


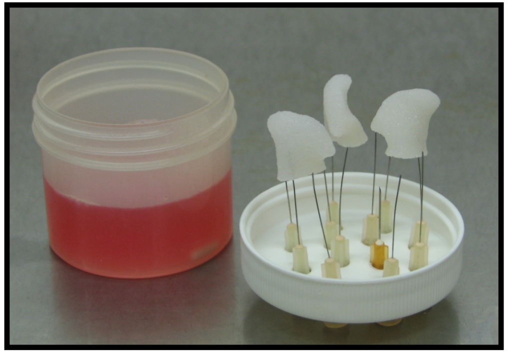


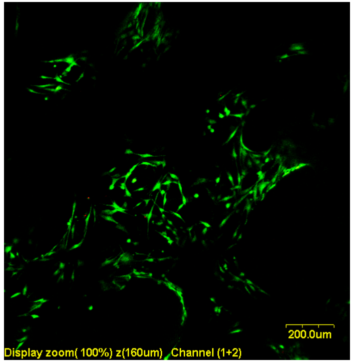


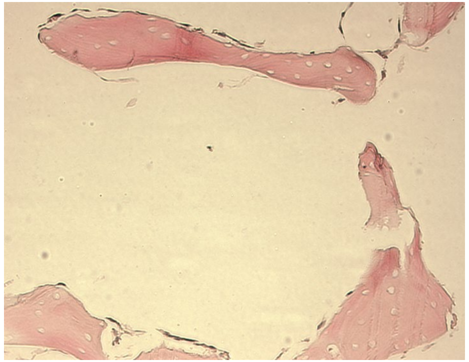
Grayson 2008

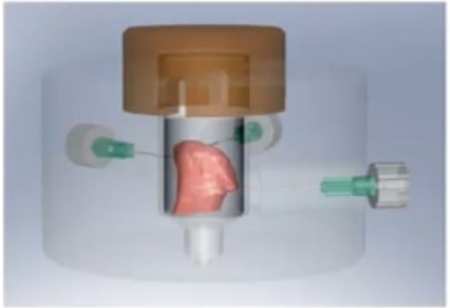
Obtaining Patient Specific Grafts

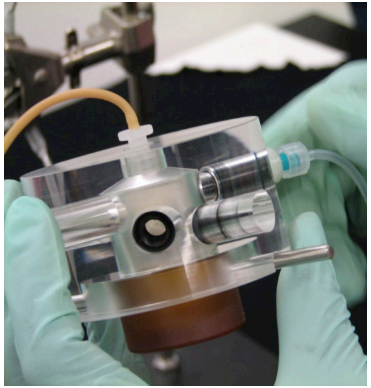








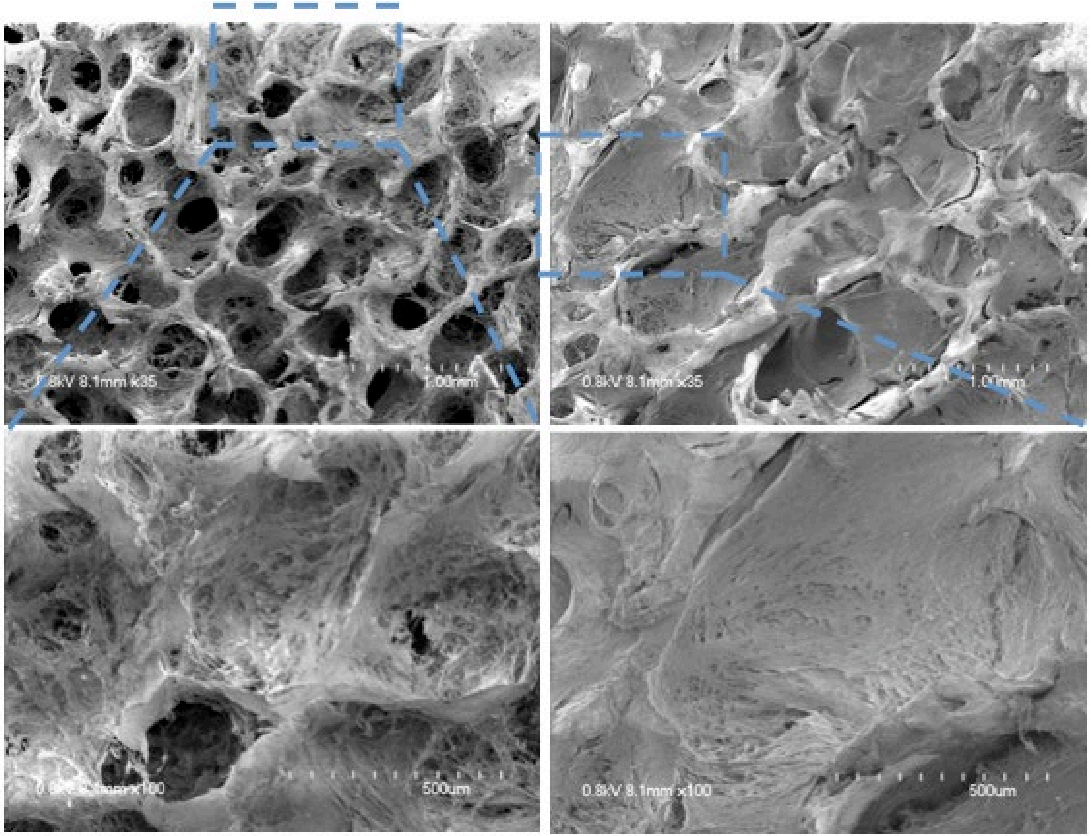




Grayson 2010

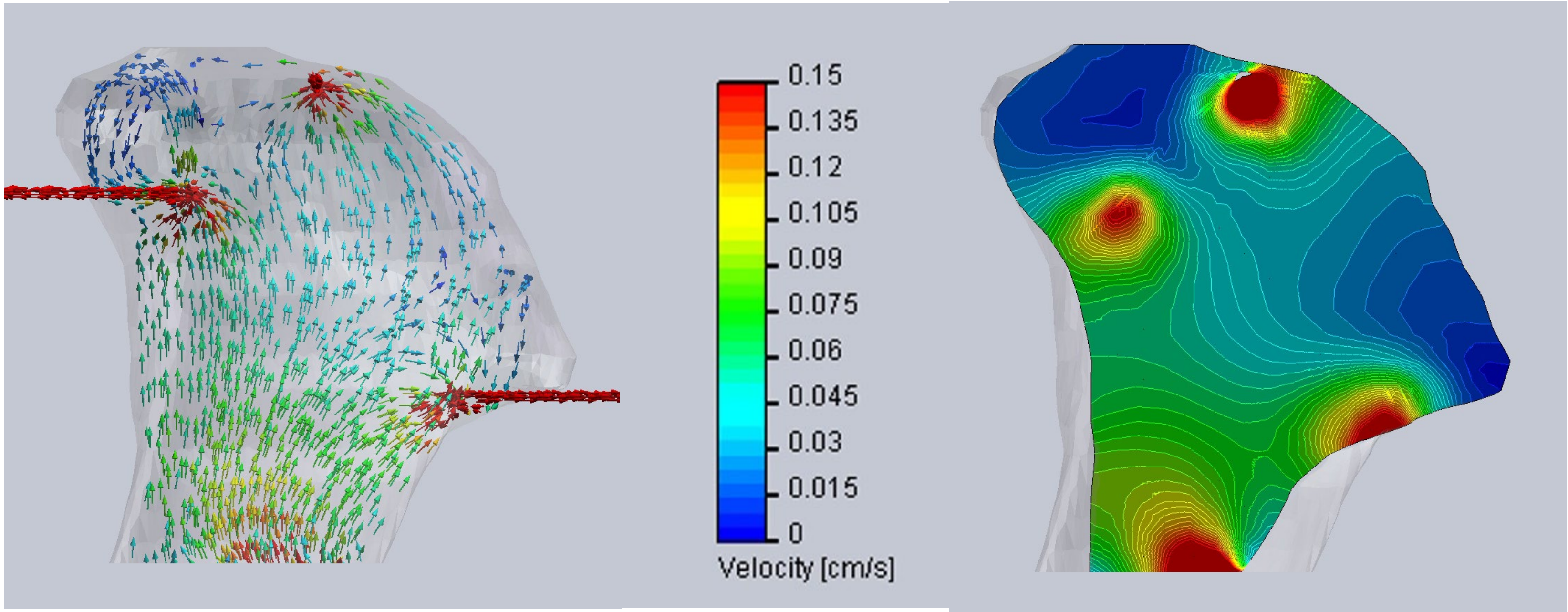
Difference of Perfusion on Cells

static



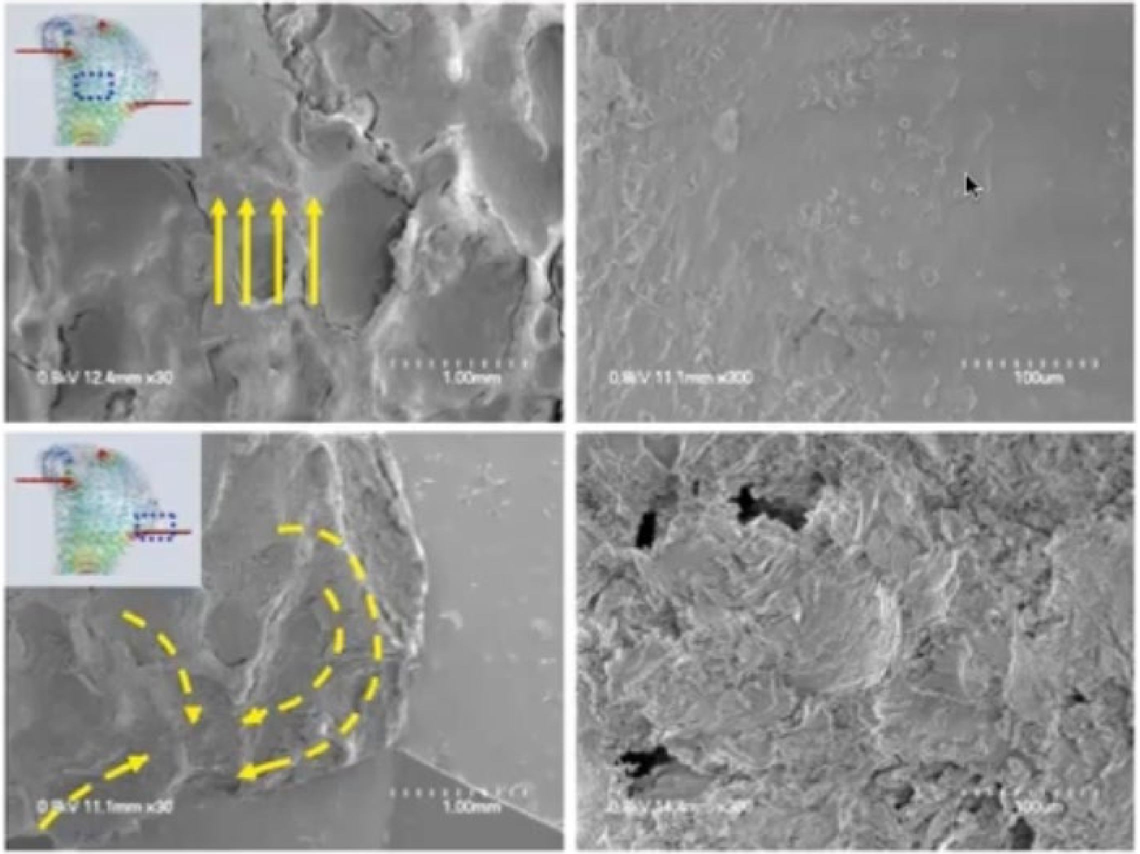
perfusion

Modeling flow through perfusion systems

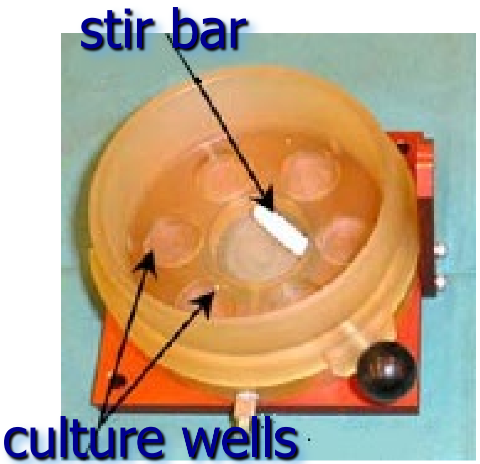


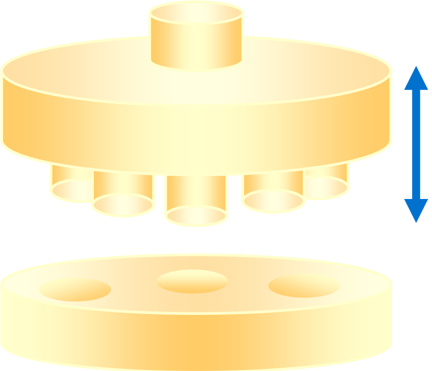
- Potential for optimizing flow conditions if correlation between flow properties and cell growth/tissue structure known

Correlating flow patterns with tissue structure



Cartilage Tissue Engineering





Demerteau et al., 2003., Seidel, unpublished, Hung et al, 2004

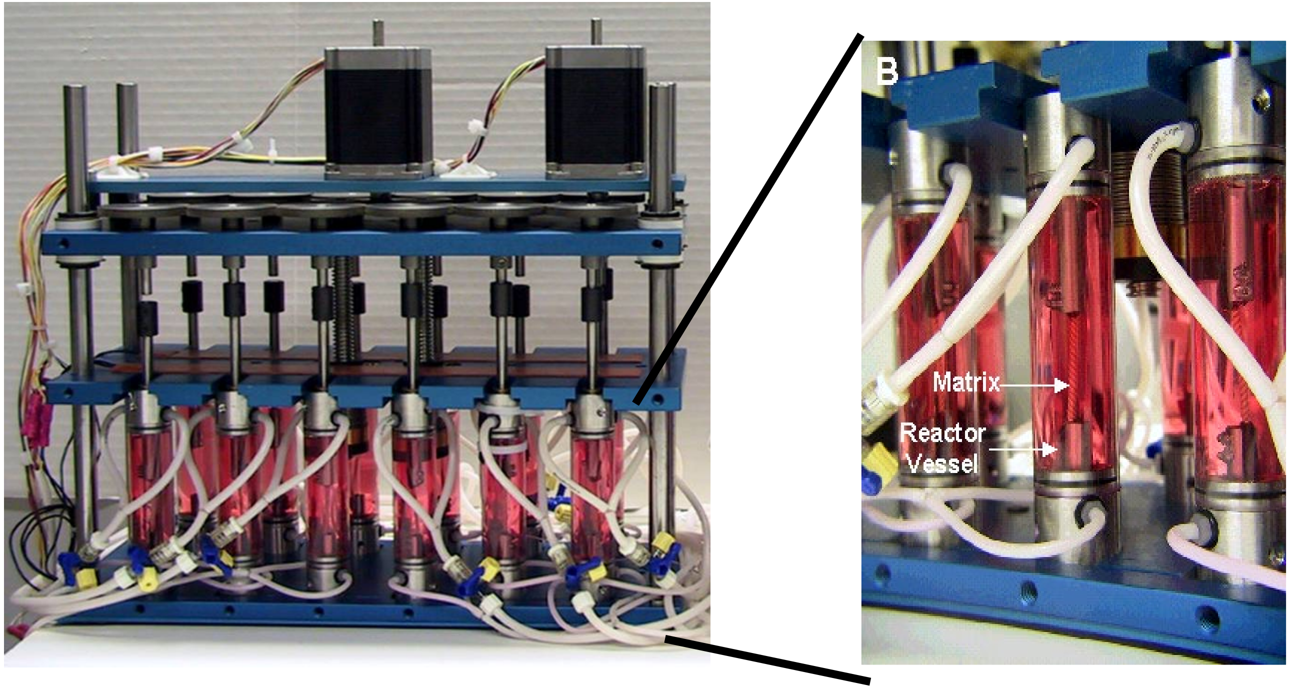
Engineering Ligaments

Dynamic torsion in combination with axial tension

- human and bovine bone marrow stromal cells

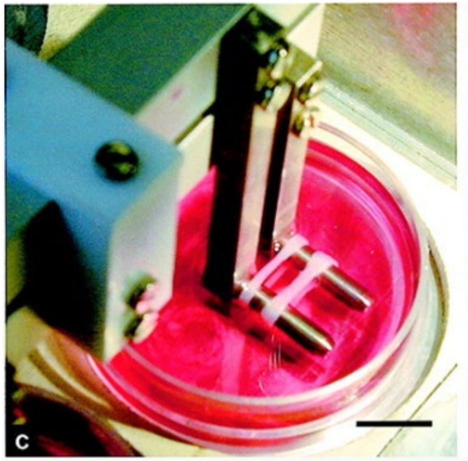
- scaffold: collagen gel, fibrous silk; 4 – 12 mm thick, 2.5 cm long

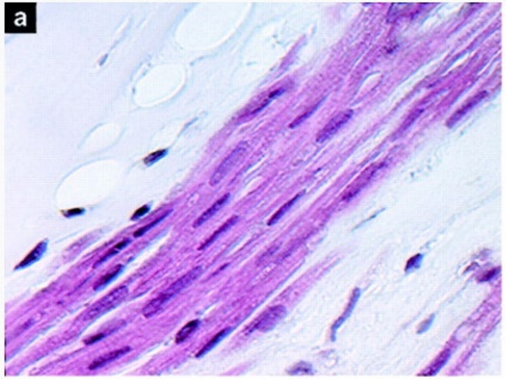
- 90 deg torsion, 10 % axial tension 0,0167 Hz; 20 min every 8 h

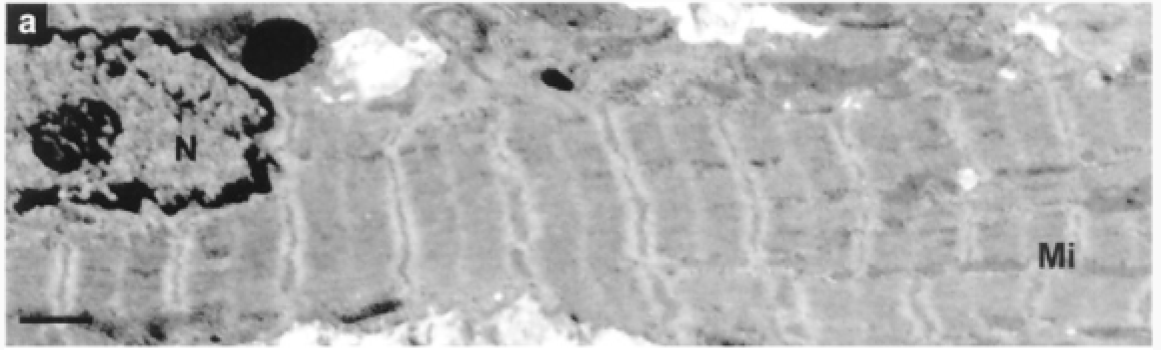


Altman et al. (2002)

Engineering Myocardium- Mechanical Stimulation

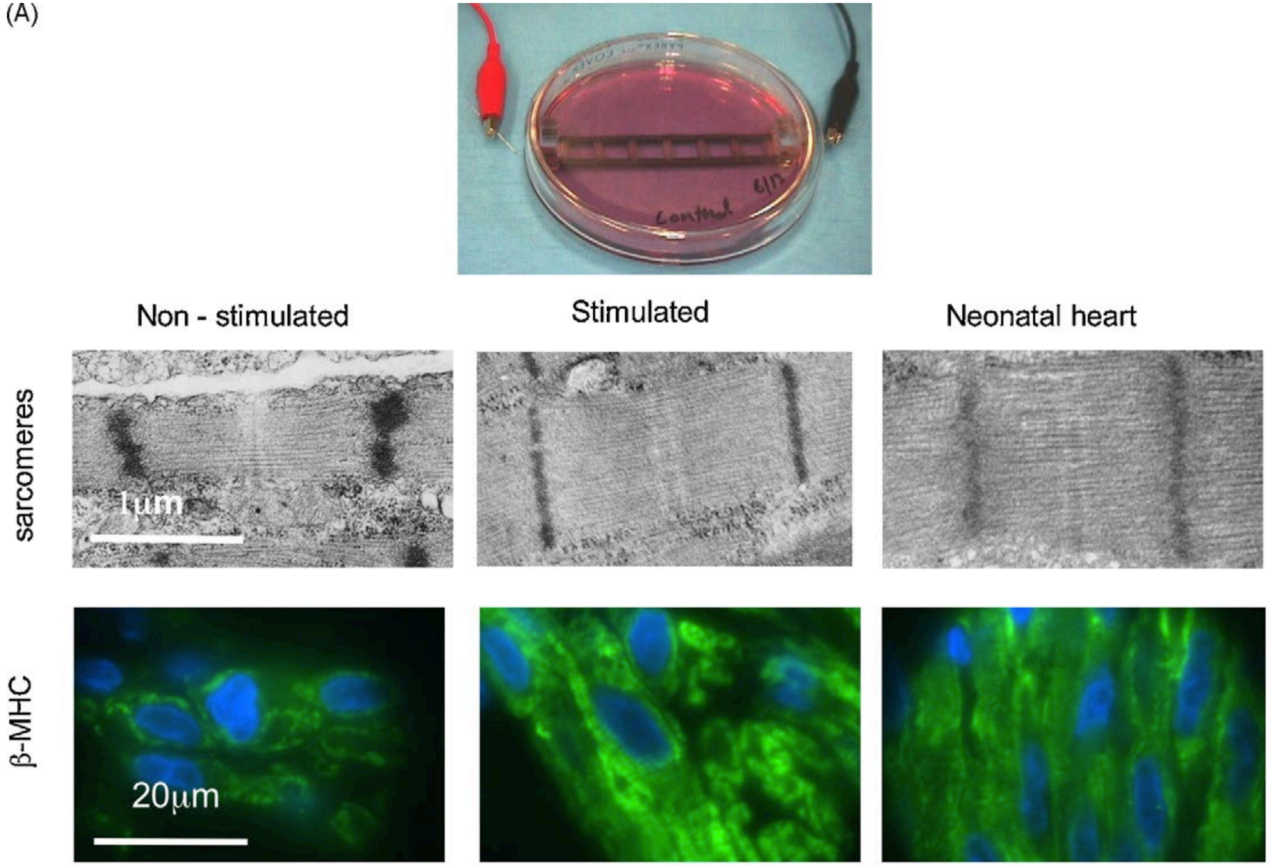






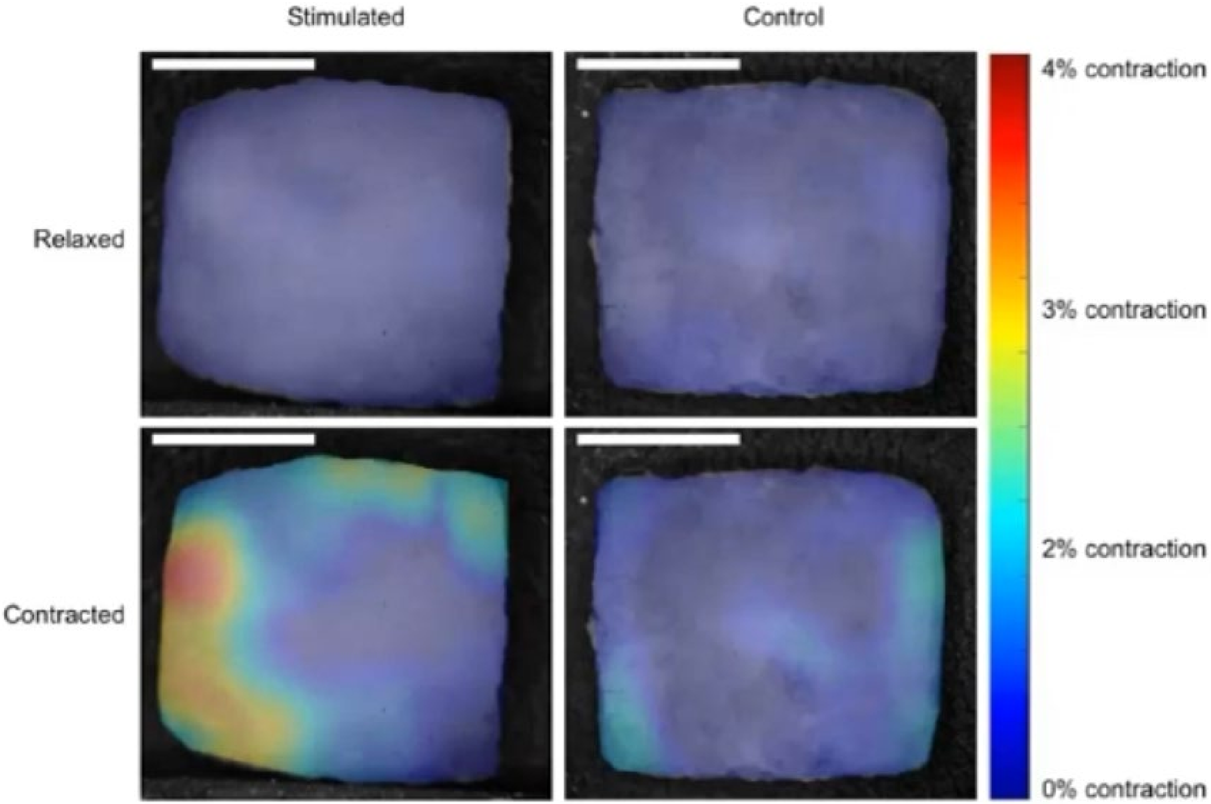
Zimmermann Circ Res 2002

Engineering Myocardium – Electrical Stimulation



Radisic PNAS 2004

Uniformity of Contraction

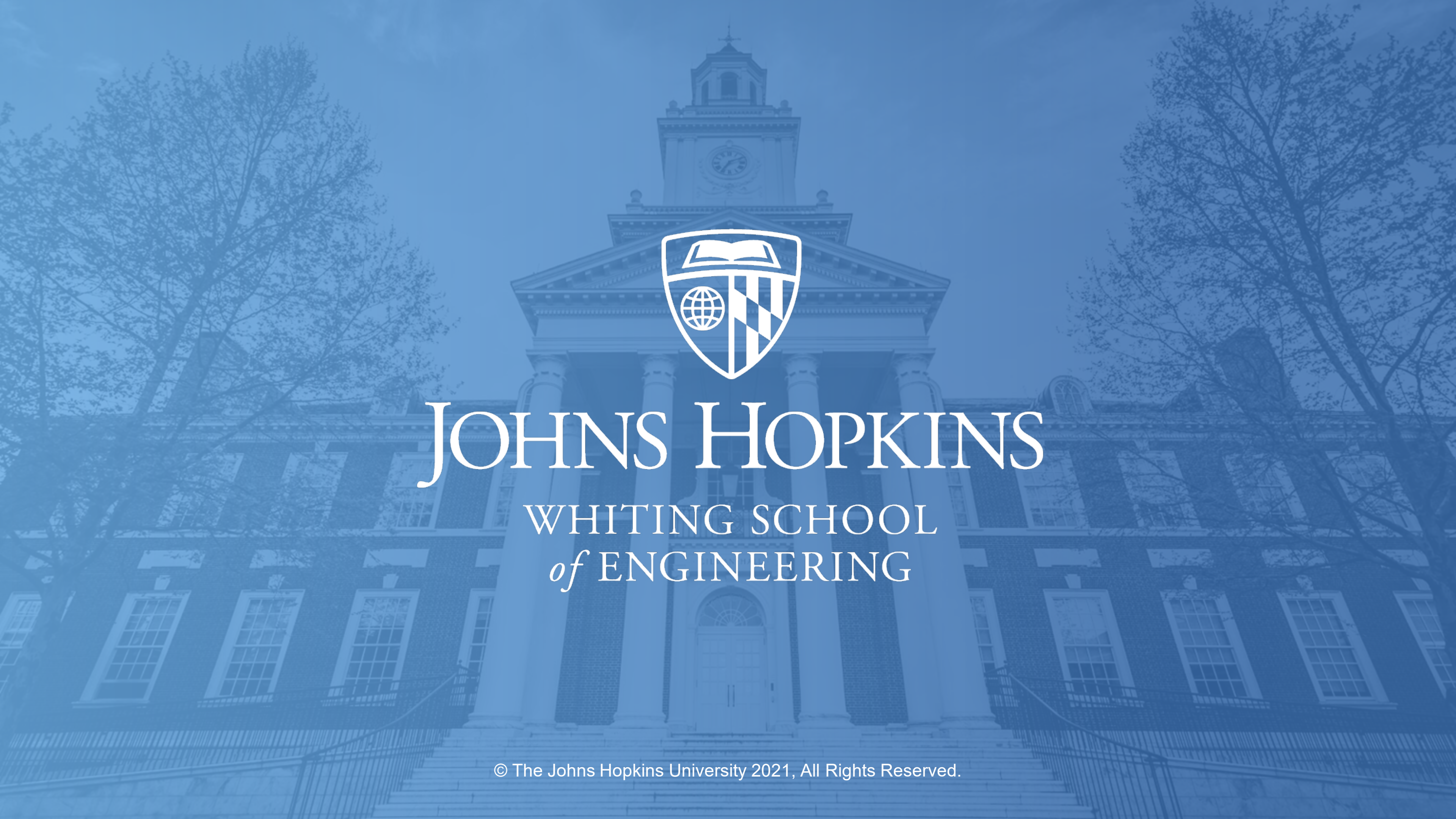


Summary

- Bioreactors provide a well-defined cultivation environment to enhance <u>uniform</u> tissue development.

- Certain key engineering principles must be considered in the general design of bioreactors

- Bioreactor design with respect to biophysical stimulation is tissue-specific
  - Bone – Shear Stress
  - Cartilage – Dynamic Compression
  - Ligaments – Torsion and Tension
  - Cardiac Muscle – Tension and Electrical Stimulation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