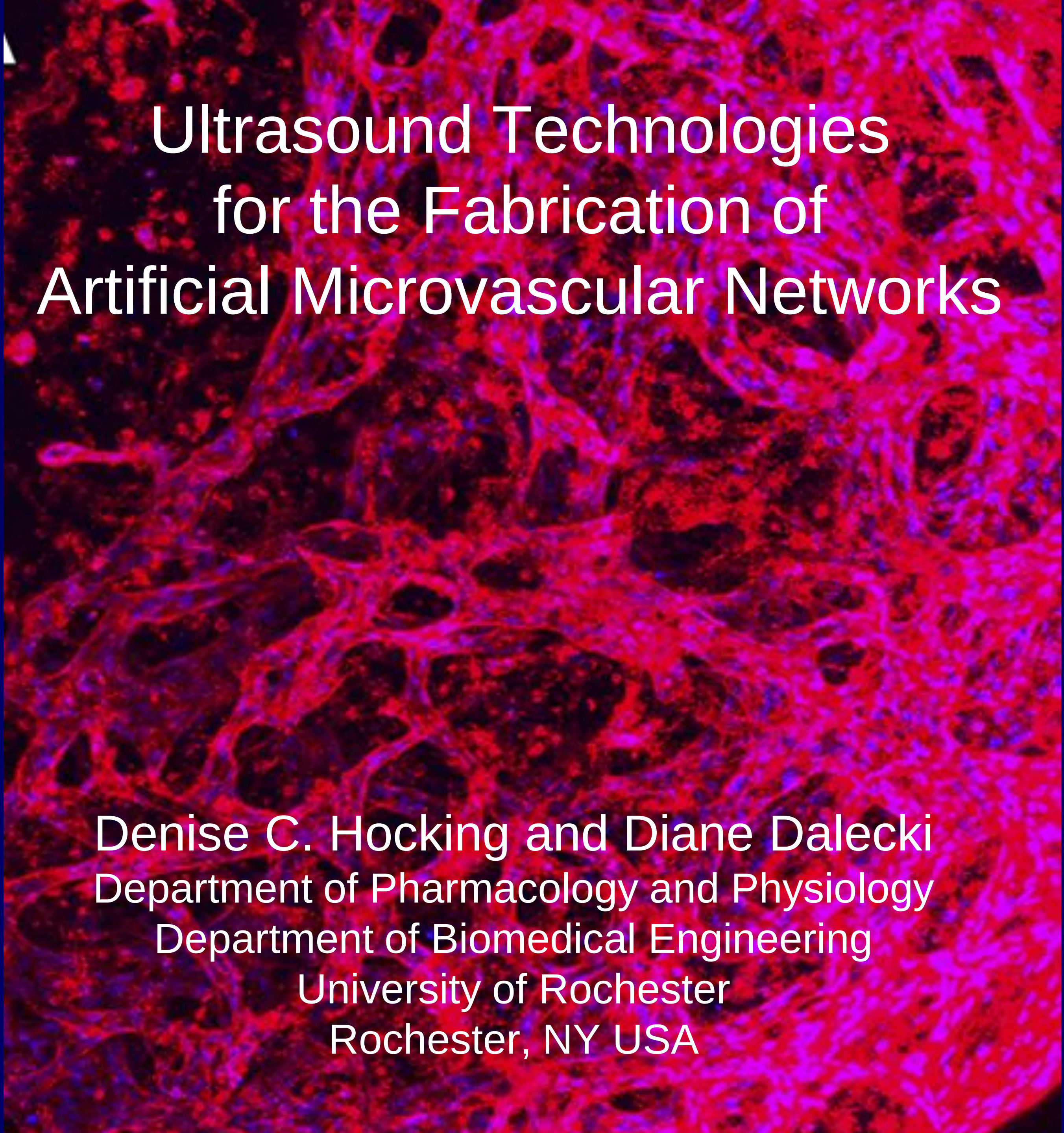

Ultrasound Technologies for the Fabrication of Artificial Microvascular Networks

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Department of Biomedical Engineering
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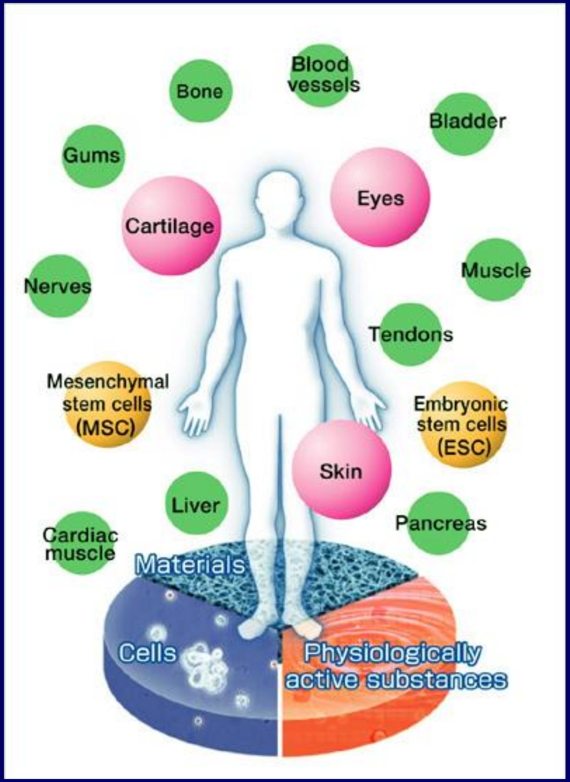
Tissue Engineering and Regenerative Medicine

Goal: To recreate tissues and organs in vitro

Engineered tissues would enable:

•Repair or replacement of diseased or damaged tissues

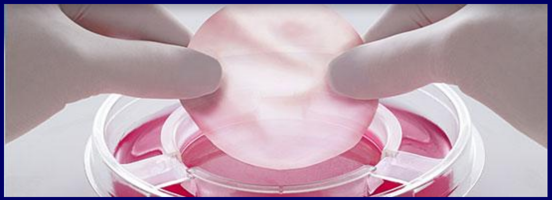
•Provide preclinical in vitro models
  – Replace traditional animal models
  – Drug discovery and product testing



www.jpte.co.jp/english/technologies/index.html

Current Progress in Tissue Engineering

- Commercially-available skin substitutes and cartilage replacement therapies



www.apligraf.com

- Successful implantation of bladder analogs and urethral segments

Atala et al., 2006

- Artificial blood vessels, bronchial tubes, and cornea tissue substitutes in clinical trials

Macchiarini et al., 2008

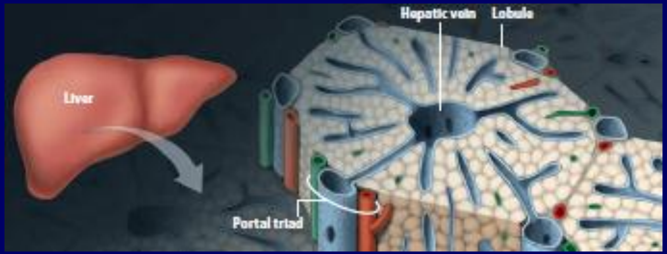
Fabrication of fully viable and functional tissues and organs that are of a larger size, have a more three-dimensional volumetric structure, and possess a more complex cell and extracellular matrix organization has been unsuccessful

Challenges in Tissue Engineering

- Reconstructing complex tissue organization

  Health and function of tissues depend closely on internal structure

  Patterning strategies to control spatial position of cells and/or extracellular matrix proteins

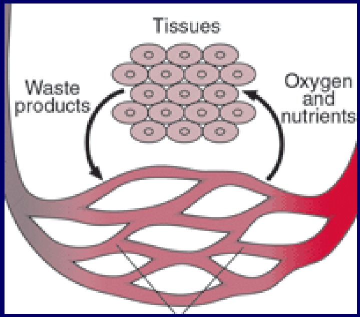


Khademhosseini et al., 2009

- Maintaining cell viability throughout the volume of engineering tissues

  Adequate supply of oxygen and nutrients

  Vascularization strategies



www.merckmanuals.com

Methods that address these challenges are needed to provide transformational advances in the field of tissue engineering

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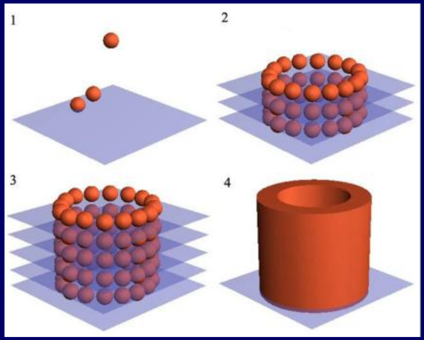
Limitations of Current Strategies

Patterning:

- Micropatterning of cell-adhesive substrates
- Force-mediated cell movement
  - Optical, magnetic, electrokinetic, fluidic
- Inkjet "organ printing" technologies

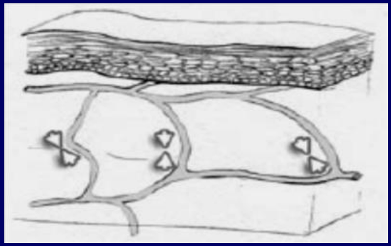


Mironov et al., 2009

Not adaptable to three-dimensions, slow processes, lack of large area patterning, loss of cell viability, adverse changes in cell behaviors

Vascularization:

- In vivo and in vitro strategies



Tremblay et al., 2005

Slow vascular growth, endothelial cell apoptosis, long processing time-scales

Development of novel patterning and vascularization technologies are essential for the advancement of tissue engineering

Acoustic Patterning using Ultrasound

Our overall goal is to develop ultrasound technologies for 3D patterning and vascularization of engineered tissues

Presentation Overview

- Ultrasound standing wave fields (USWF)
  - In a tissue engineering environment
- USWF-patterning in 3D engineered constructs
  - Using cells, cell-bound proteins, microparticles
- Fabricating microvascular networks with USWF
  - Control of microvessel morphology
  - Composite microvessel constructs
  - In situ acoustic patterning for tissue engineering in vivo

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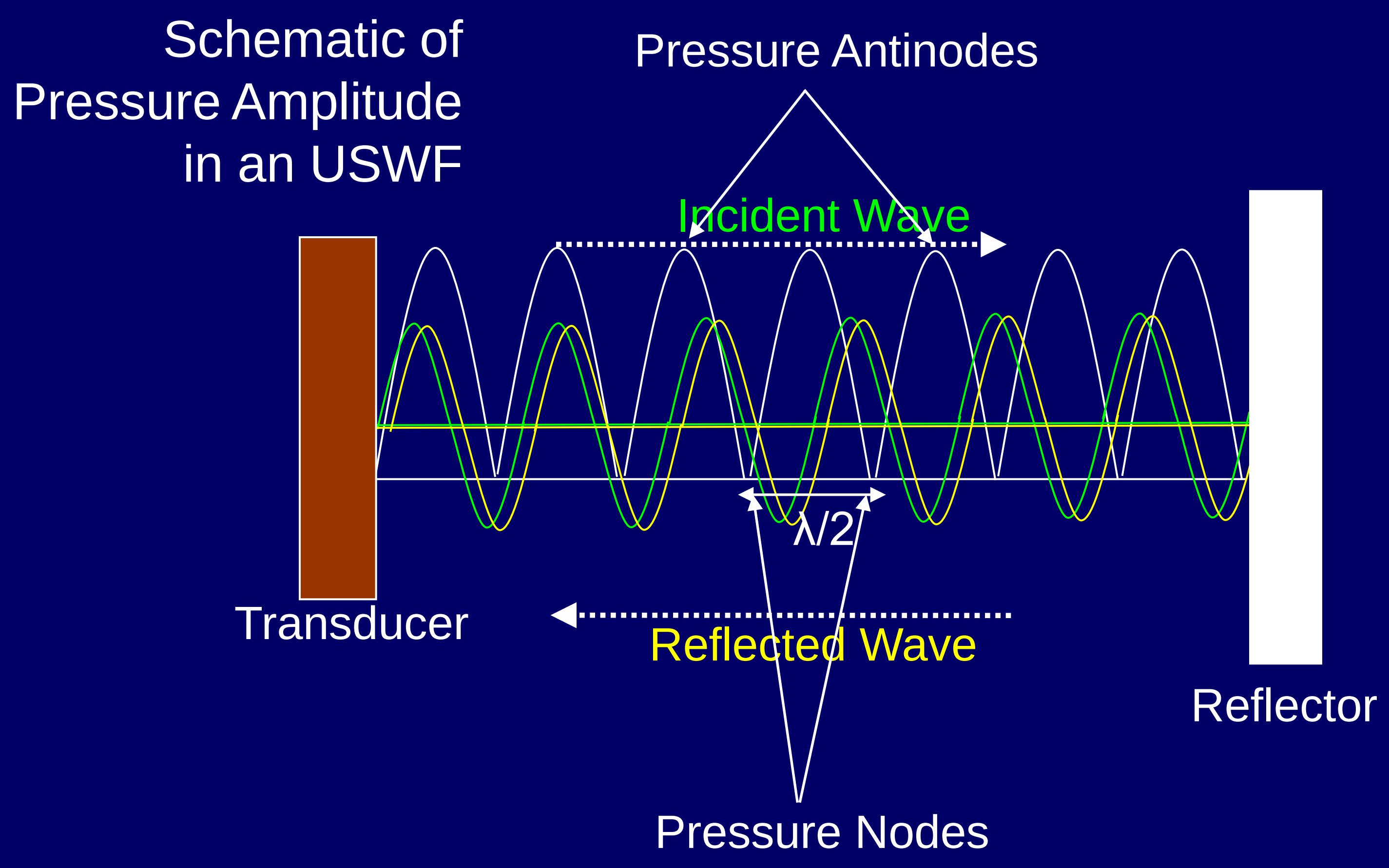
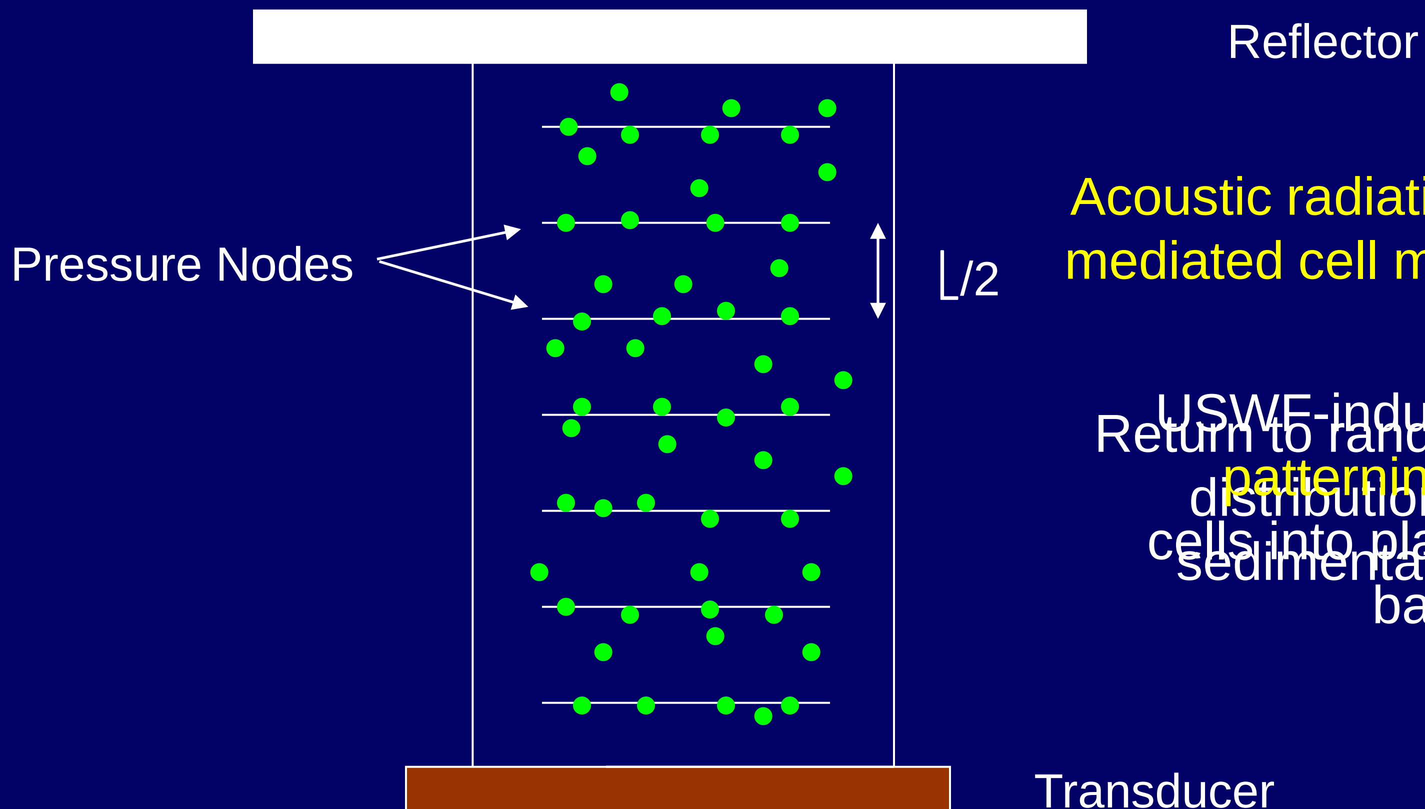
Ultrasound Standing Wave Fields (USWF)



Ultrasound Standing Wave Fields Manipulate Cell Location



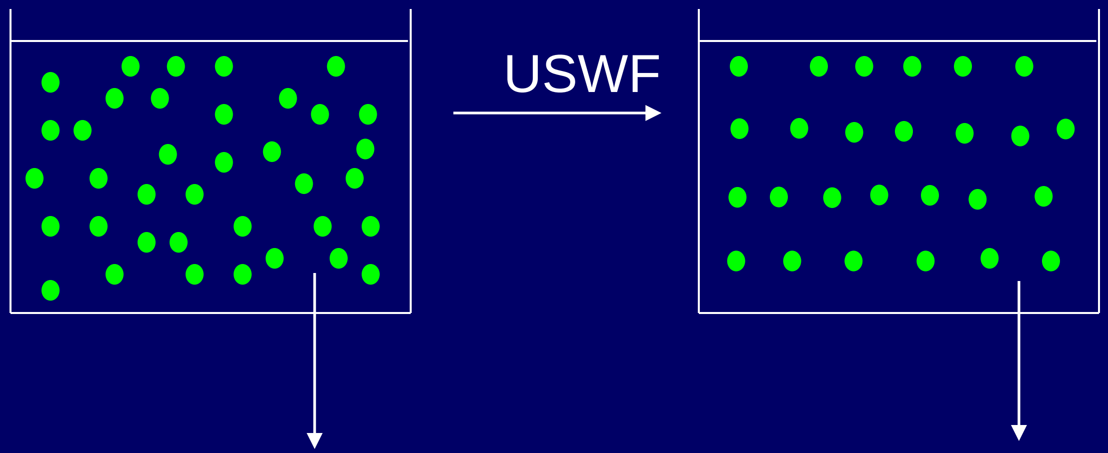
Acoustic radiation force-mediated cell movement

USWF-induced patterning of cells into planar bands

Return to random distribution or sedimentation

Acoustic Patterning of Cells in Hydrogels using USWFs

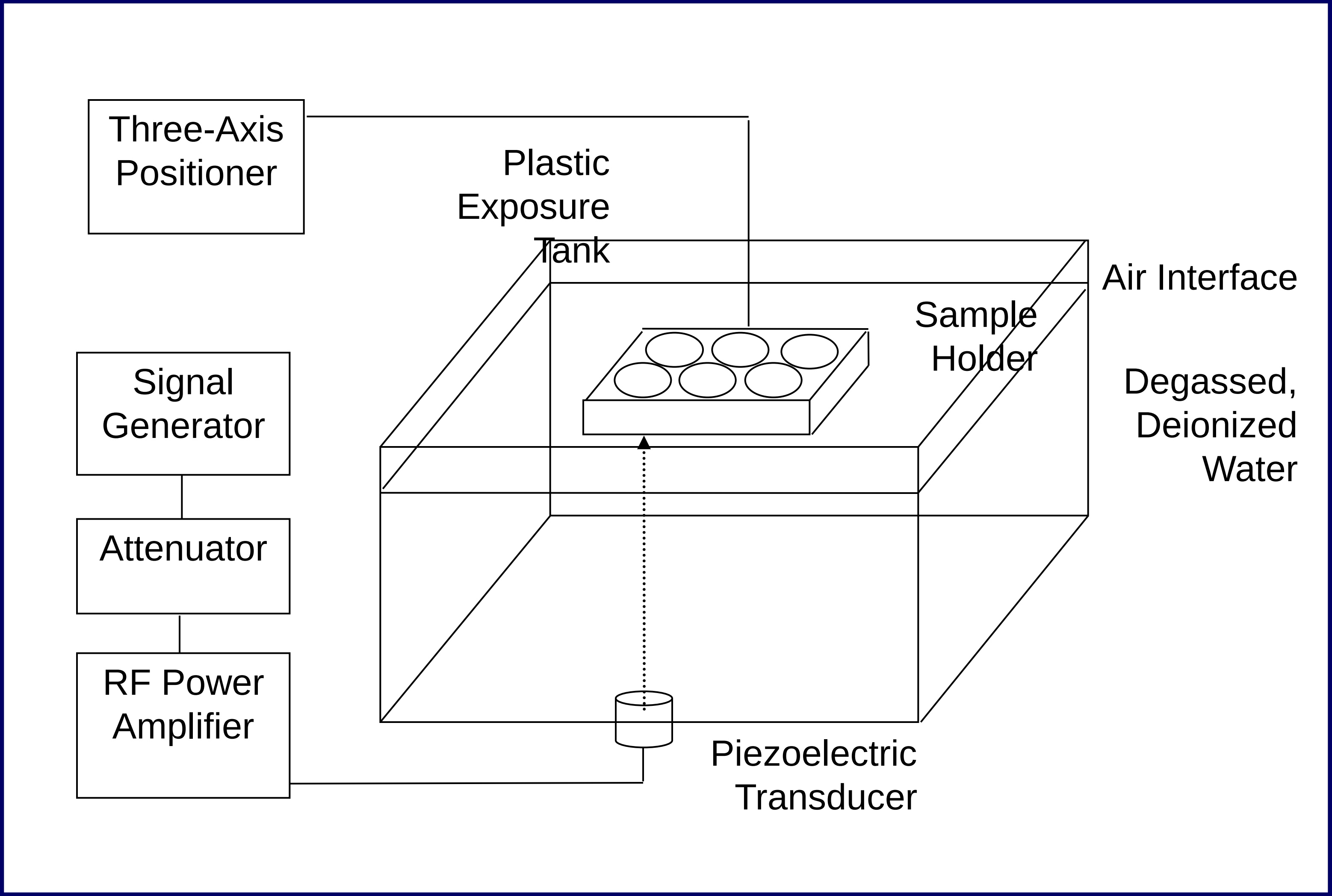
- Polymerization of collagen-I during US exposure locks in the acoustic pattern
  - -Natural component of the extracellular matrix
    - -Polymerization initiated through temperature rise



Collagen solution

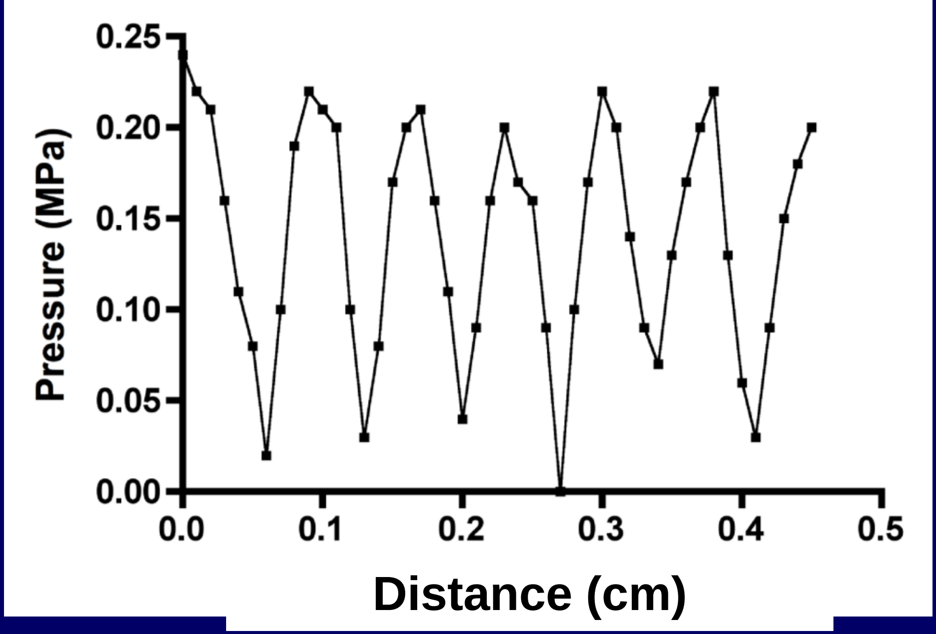
Polymerized Collagen Gel

Acoustic Exposure Set-up



Garvin et al., 2010

USWF Axial Beam Pattern within Sample Volume



1 MHz frequency

1” diameter unfocused transducer

Far-Field

0.1 MPa output pressure

Garvin et al., Ultrasound Med Biol. 36: 1919-1932, 2010

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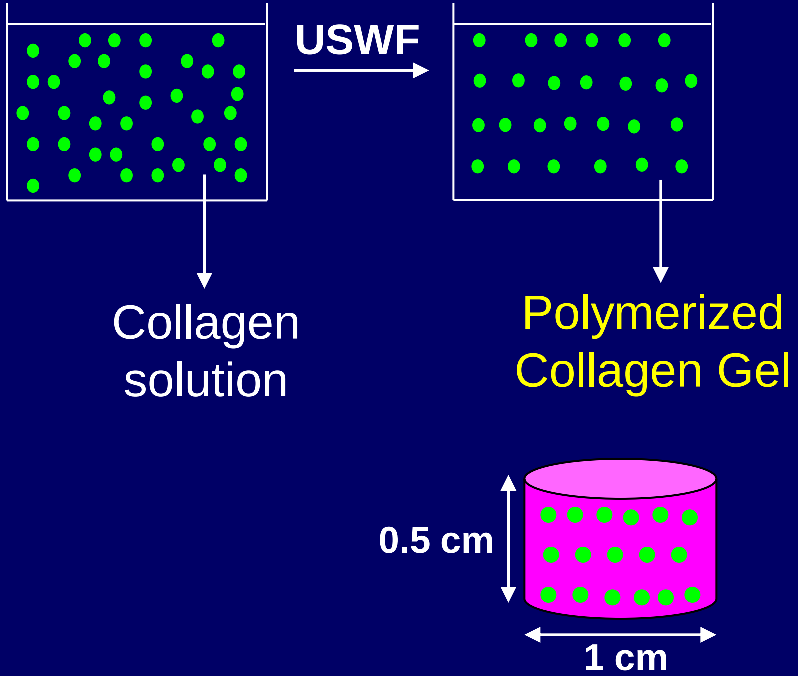
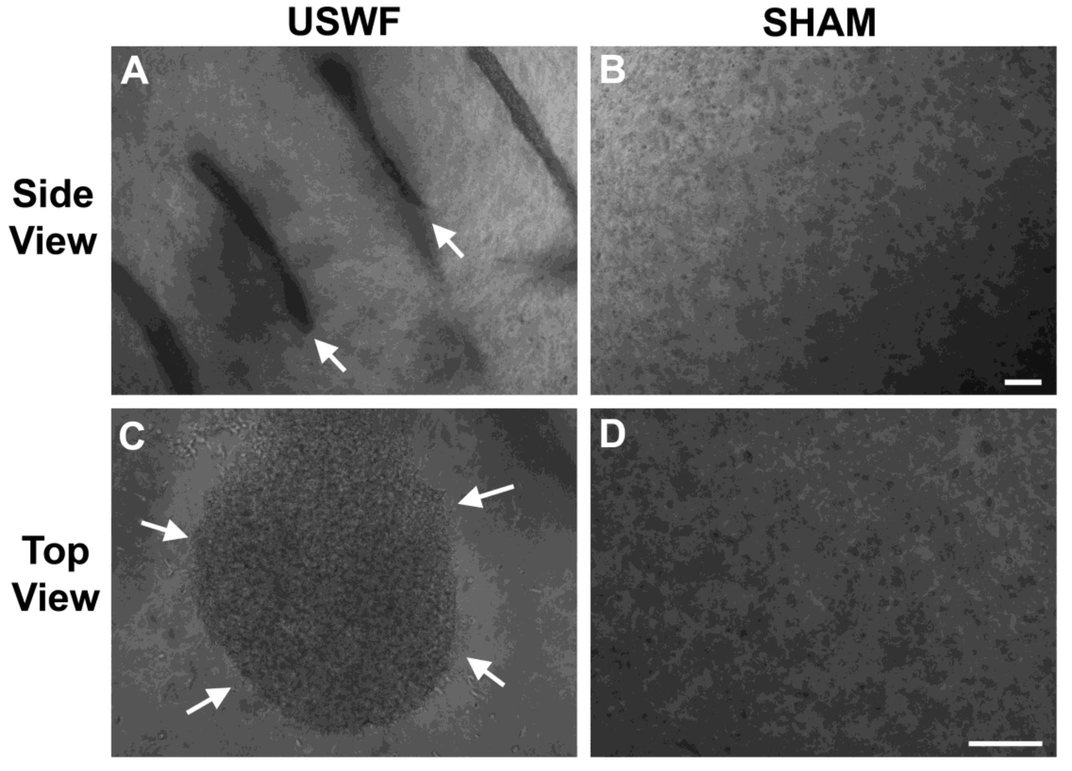
Acoustic Patterning of Cells in Collagen Hydrogels





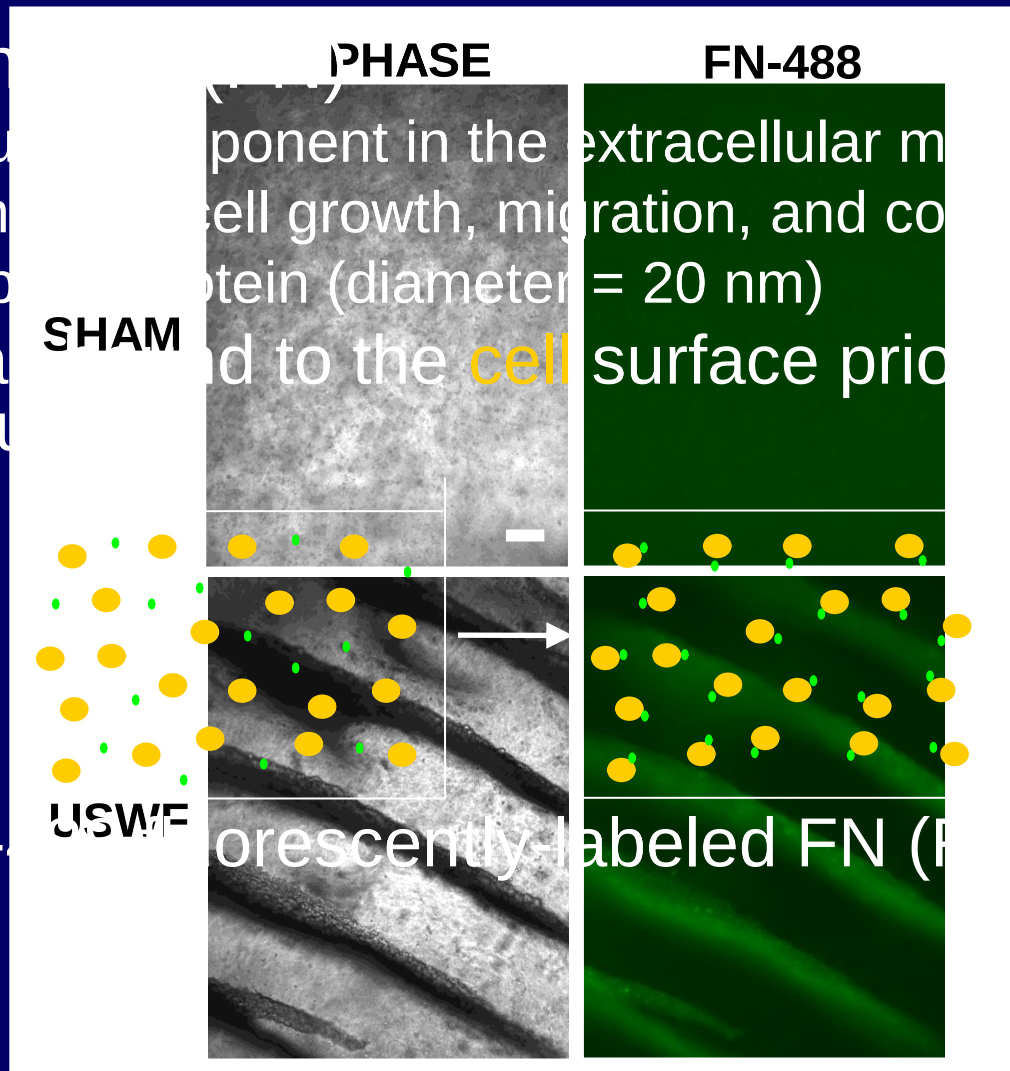
Multicellular planar bands of cells

Scale Bars, 200 μm

Garvin, KA et al., Ultrasound Med Biol 37:1853, 2011

Acoustic Patterning of Cell-bound Proteins

- Fibron … (FN)
  - Natu … ponent in the extracellular m … ix
  - Stim … cell growth, migration, and co … actility
  - Glob … otein (diameter = 20 nm)
- FN wa … d to the cell surface prio … o USWF exposu …



- Alexa- … orescently-labeled FN (F … -488)

Garvin et al., 2010

Scale Bar, 200 μm

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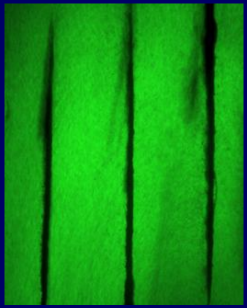
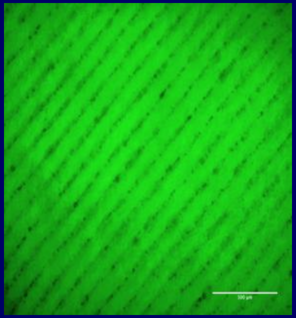
Applications of USWF Patterning

- Microparticles
  - Acoustic frequency controls planar band spacing

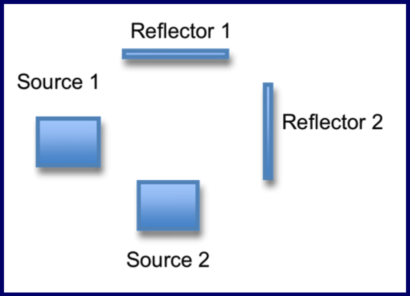


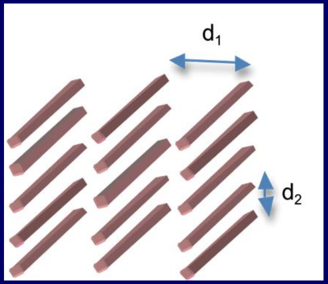
1 MHz

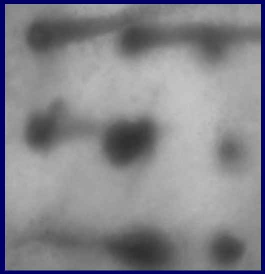


5.7 MHz

- Multiple transducer systems for more complex geometries

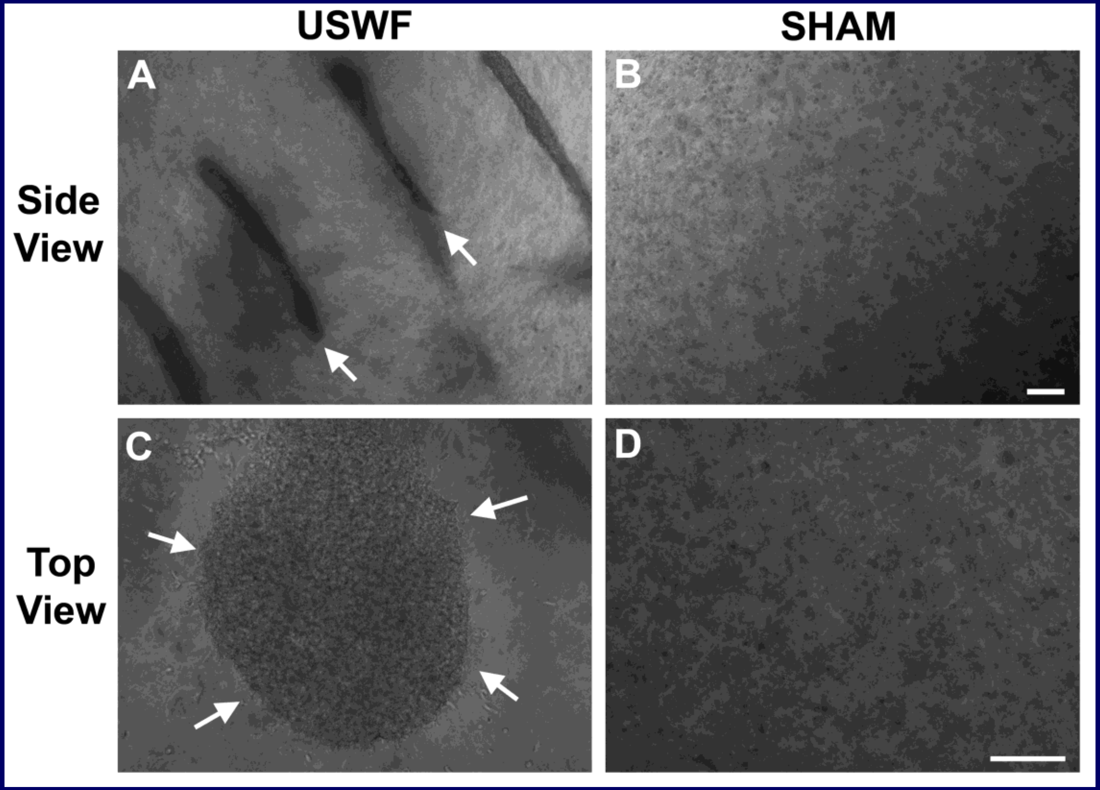






Acoustic Patterning of Endothelial Cells

Human umbilical vein endothelial cells

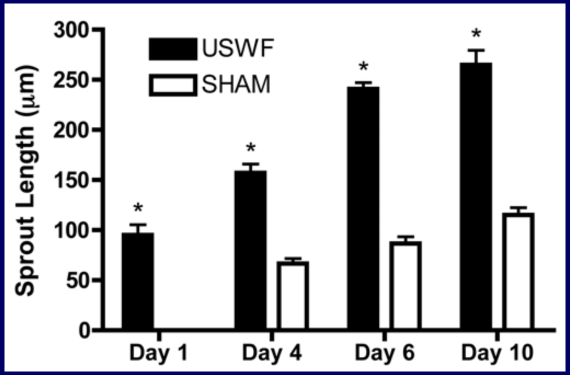


Garvin et al., 2011

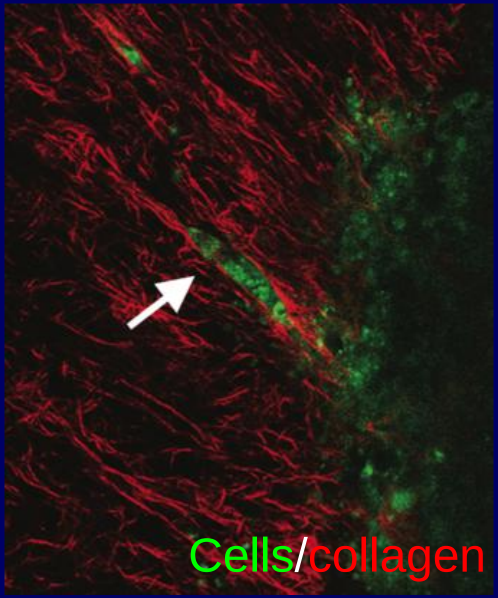
Multicellular planar bands of cells

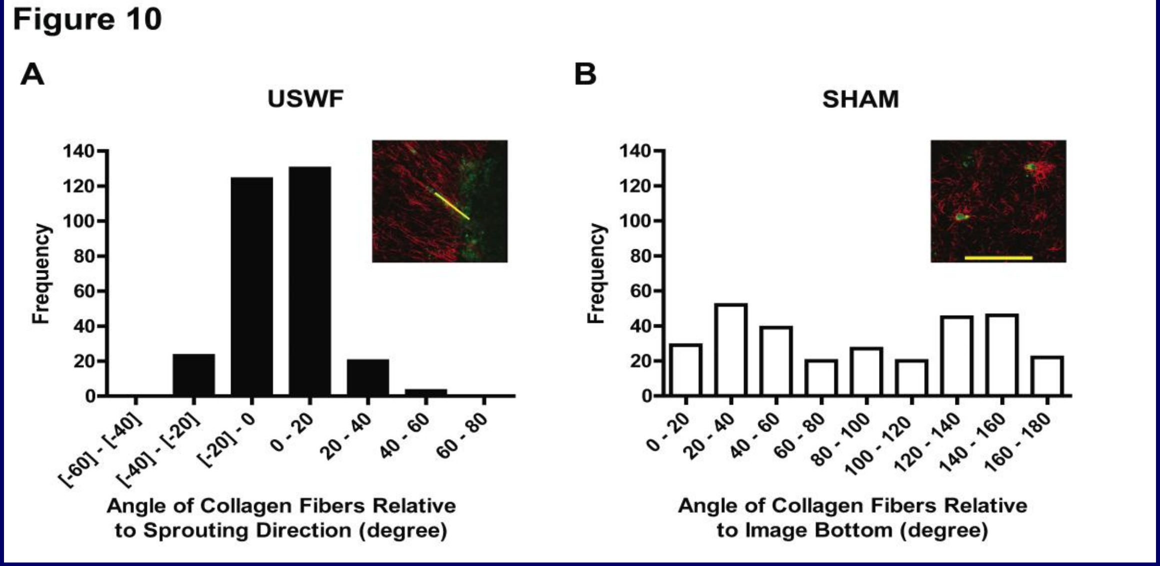
Scale Bars, 200 μm

USWF-induced Microvessel Formation

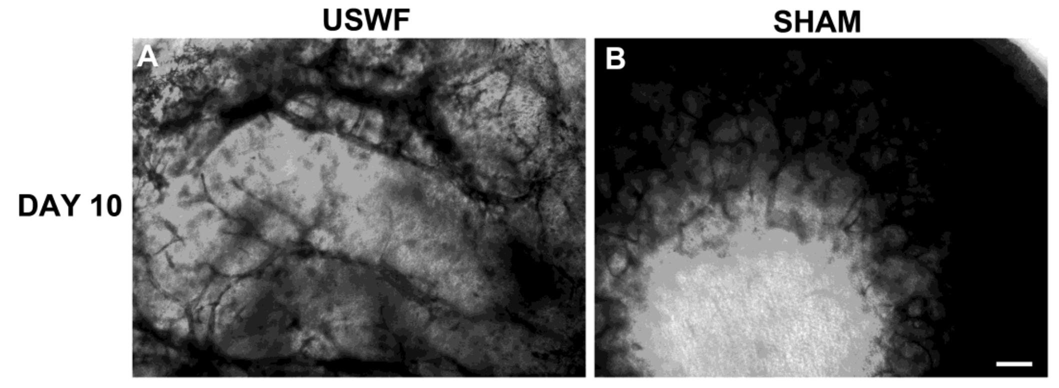


- *Rapid vessel sprouting and elongation*
- Collagen alignment in the direction of microvessel outgrowth

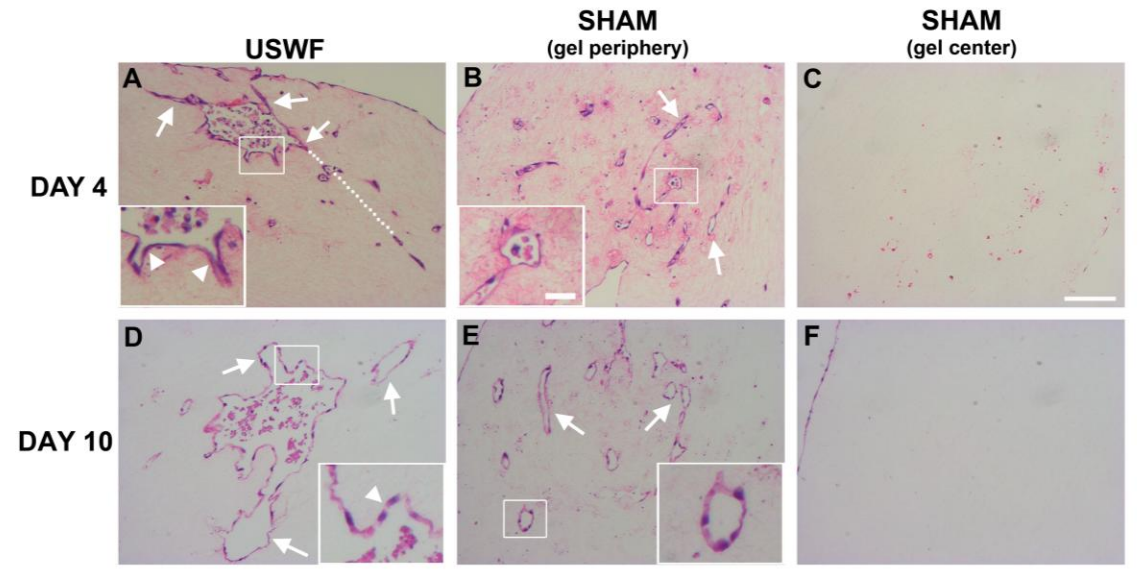




Anastomosing, Lumen-Containing Networks Form Throughout USWF-Exposed 3D Collagen Gels

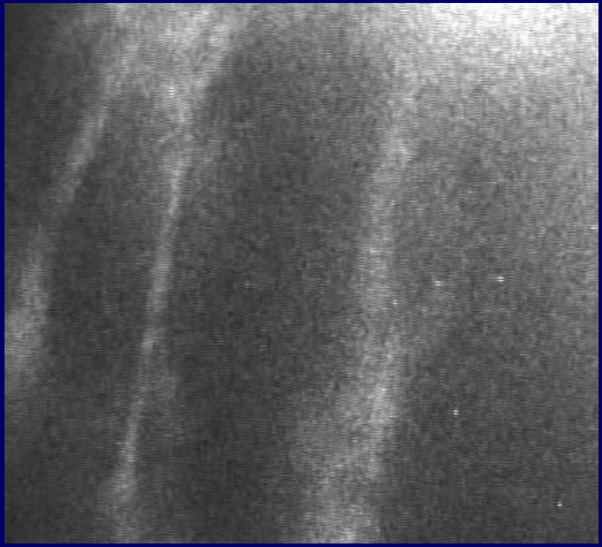


Scale Bar, 200 µm



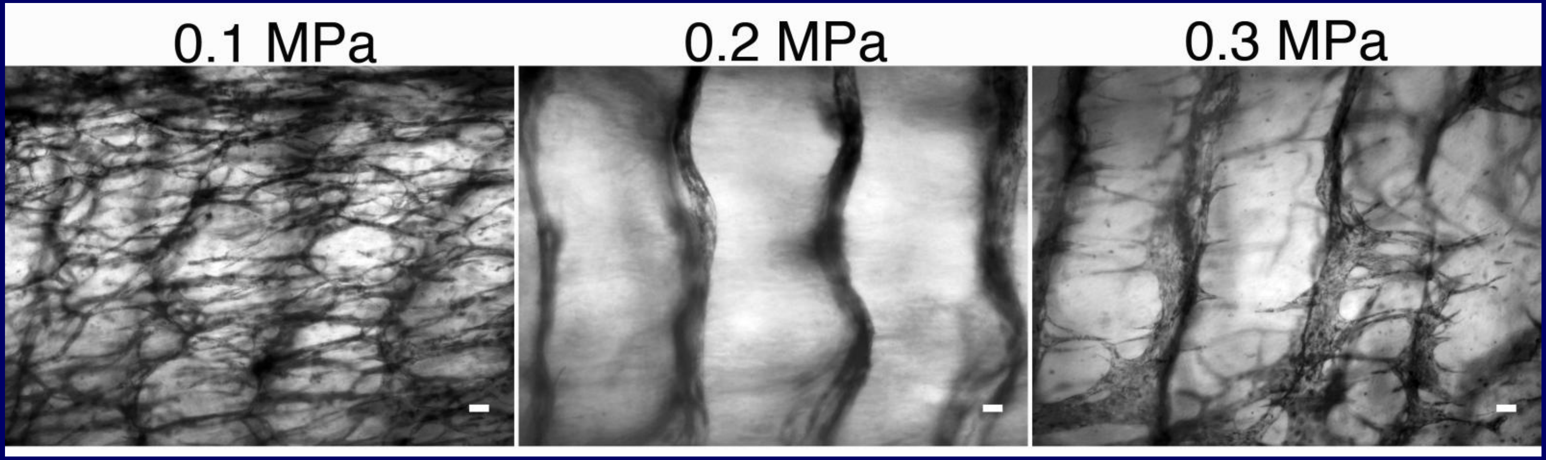
Scale Bar, 100 µm (Inset, 20 µm)

Perfused with FITC-Dextran



Garvin, KA et al., Ultrasound Med Biol 37:1853, 2011

Vessel morphology can be controlled using USWF technologies



Scale bars = 100 microns

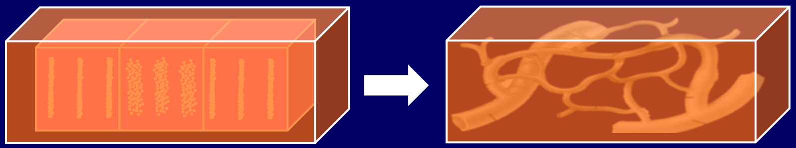
Rate of vessel formation and morphology of resulting microvessel networks can be controlled through design of USWF exposure.

Garvin, K.A. et al., JASA, 134:1483, 2013

Developed a suite of image analysis and 3D visualization tools to rapidly analyze and quantify differences in microvessel networks.

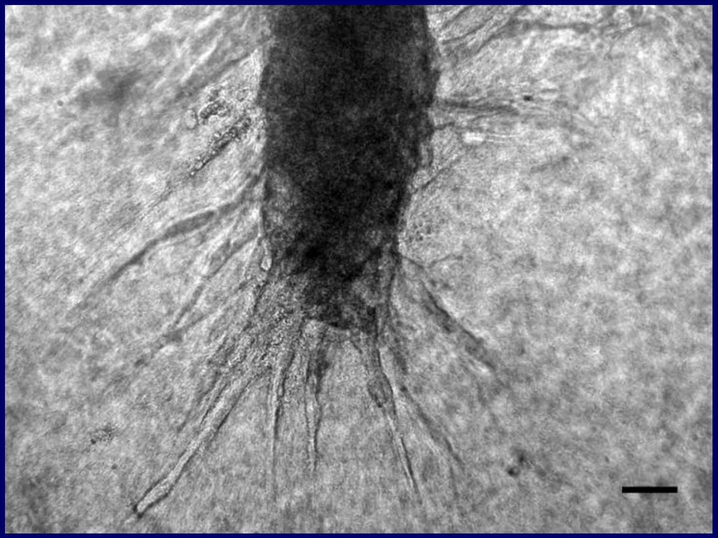
Mercado, K.P., et al. Annals BME 42:1292, 2014

Versatility of Acoustic Patterning

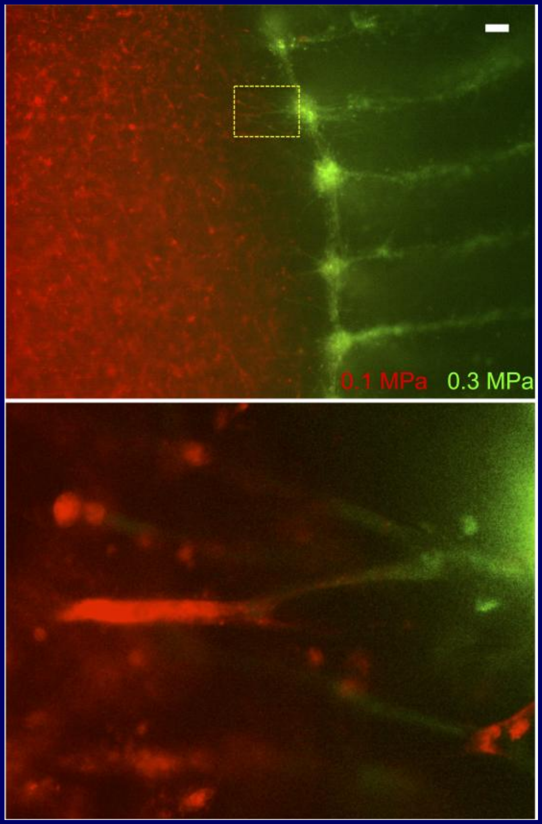


Fabrication of a composite construct

Lymphatic endothelial networks

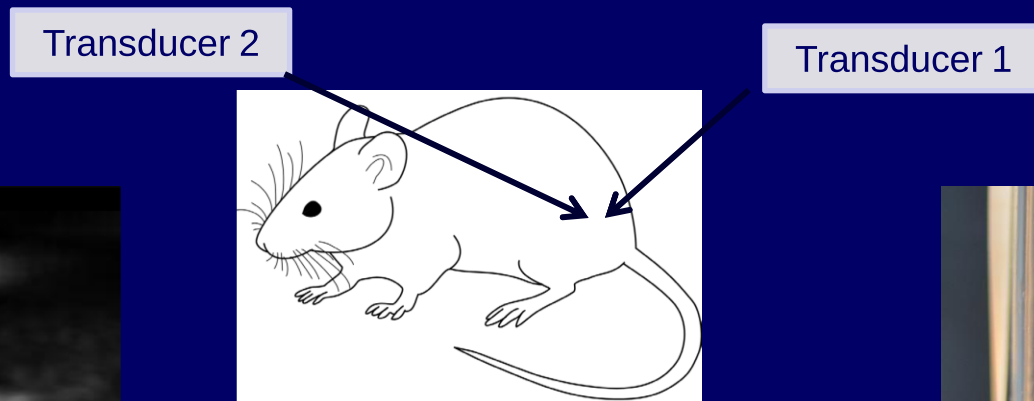


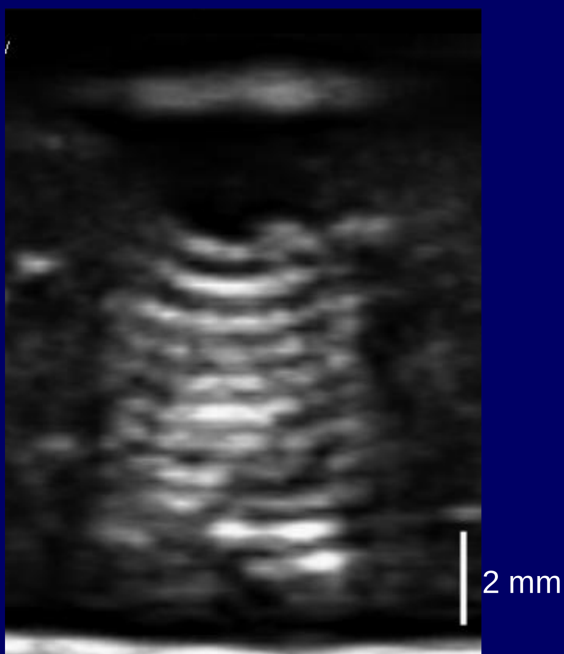
Scale bar = 50 microns



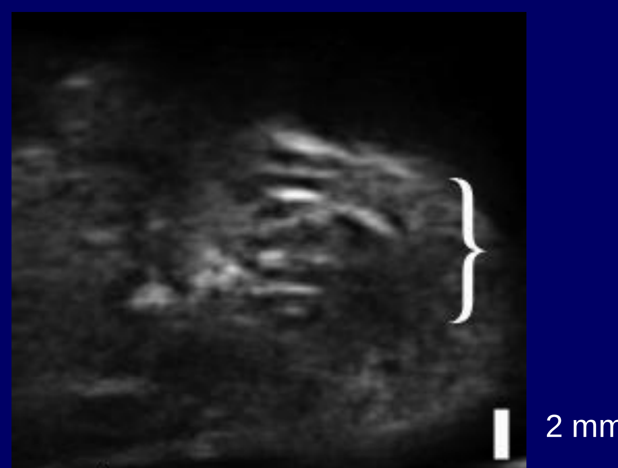
Scale bar = 100 microns

Acoustic Patterning
for Tissue Engineering *in situ*

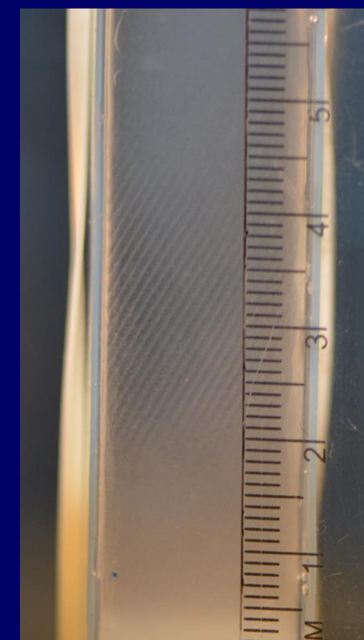




Ex vivo liver section



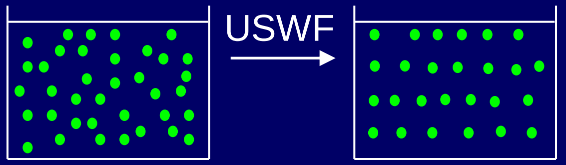
Rat hind limb

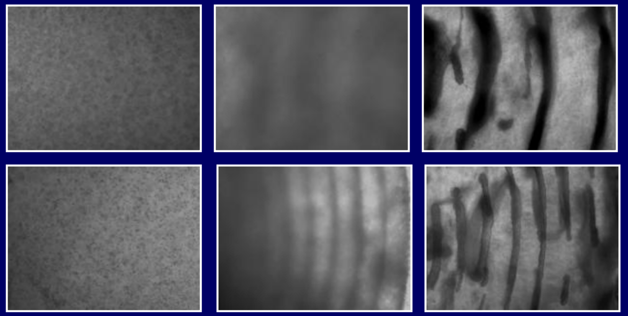


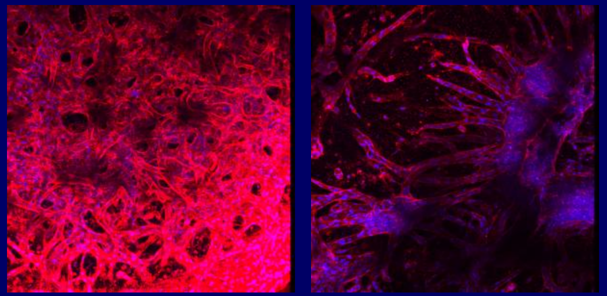
Dual transducer system
for in situ exposures

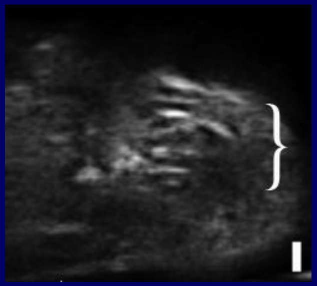
Summary

- Acoustic radiation forces associated with USWF can rapidly and non-invasively pattern cells and/or microparticles within 3D collagen-based hydrogels
- Acoustic exposures can be programmed to create various spatial patterns
- Varying acoustic exposure parameters produces different vessel morphologies; sequential or multiple acoustic exposures can be used to create hierarchical vasculatures
- Dual transducer systems may be used for patterning cells and/or particles directly in situ









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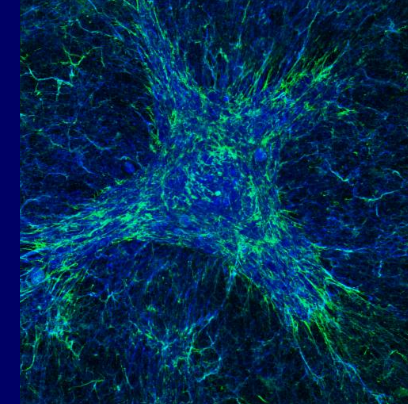
Conclusion

USWF technology provides a rapid, noninvasive approach to pattern endothelial cells in hydrogels in 3D and to direct vascular network formation and morphology within engineered tissue constructs.

Key Advantages of USWF Technology:

- Noninvasive
- Rapid
- Applicable to different cell types and hydrogels
- No loss in cell viability; enhanced cell function
- Exposure parameters allow for control of 3D patterning capabilities
- Unique capacity to pattern cells and particles directly in situ

Acknowledgements



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