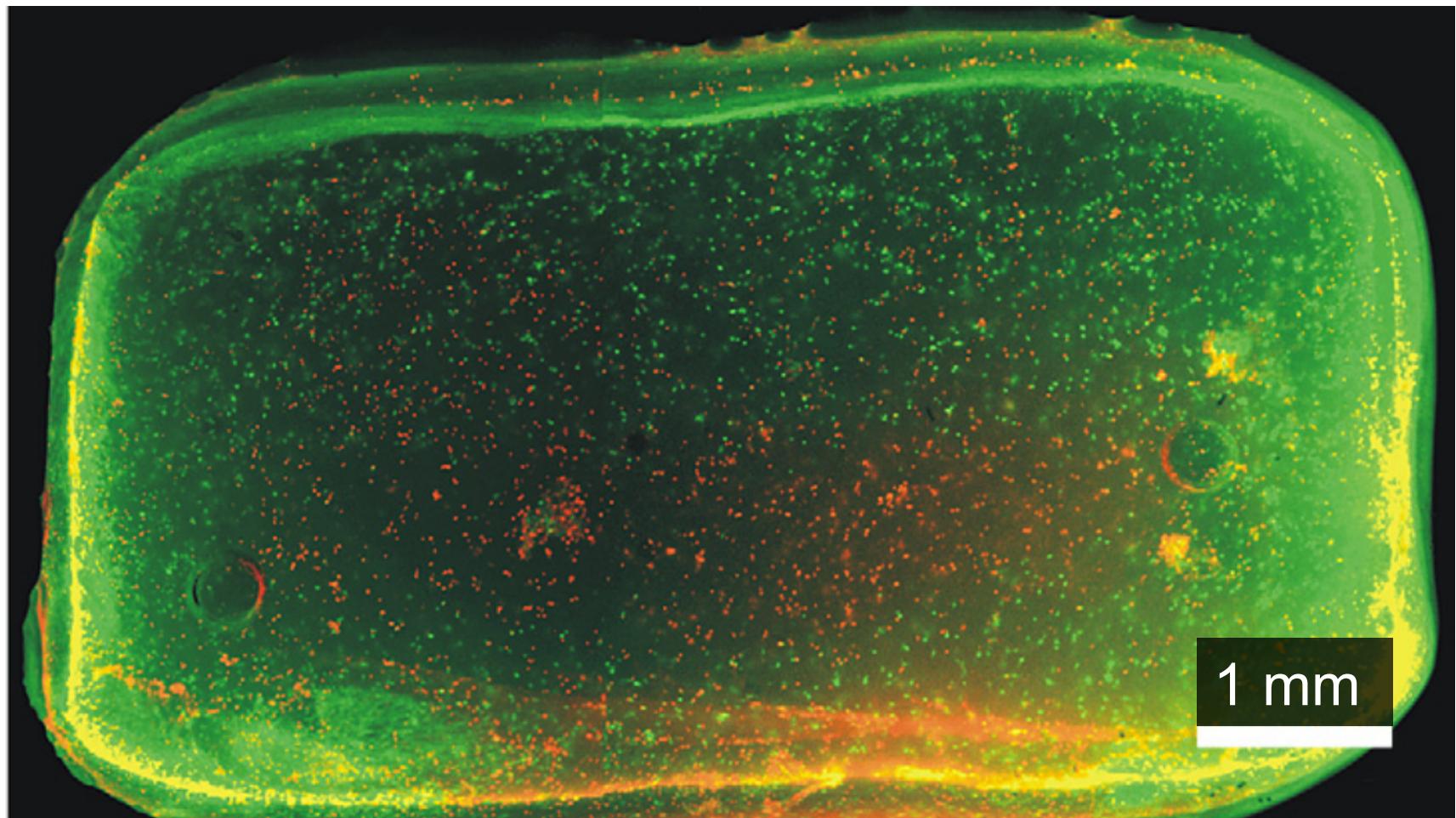


# Enhancing cell viability by controlling the O<sub>2</sub> diffusion-consumption profile via rational cell distribution

Erik Reinertsen  
Wu Lab seminar  
9/13/2011



# $O_2$ diffusion-consumption limits viability of homogeneously seeded implants



1 mm

# The Conservation of Species equation describes mass transport

$$\partial C_i / \partial t + v \nabla C_i = D_i \nabla^2 C_i - R_{Vi}$$

The diagram illustrates the components of the conservation of species equation. It consists of four vertical red lines of equal height, each followed by a label below it:

- The first line is labeled "change with time".
- The second line is labeled "convection".
- The third line is labeled "diffusion".
- The fourth line is labeled "reaction".

# How can oxygen transport to core scaffold regions be improved?

$$\partial C_i / \partial t + v \nabla C_i = D_i \nabla^2 C_i - R_{Vi}$$



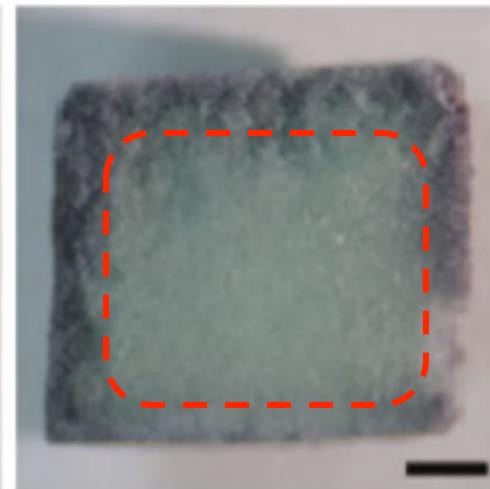
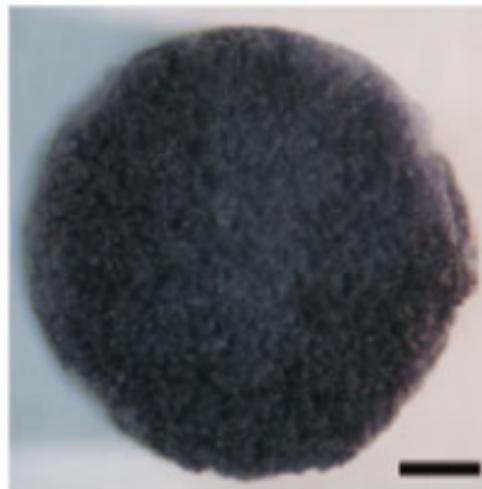
convection

# Bioreactors improve *in vitro* cell viability in scaffolds via convection

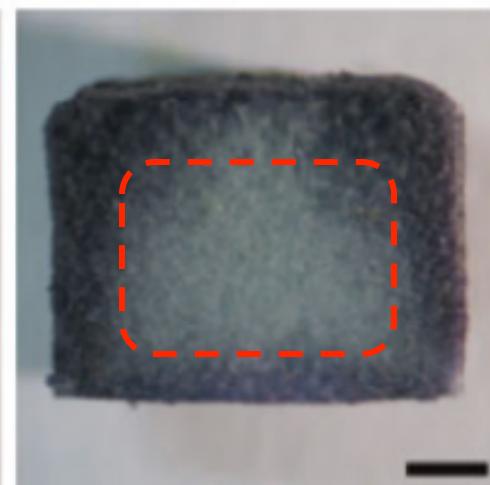
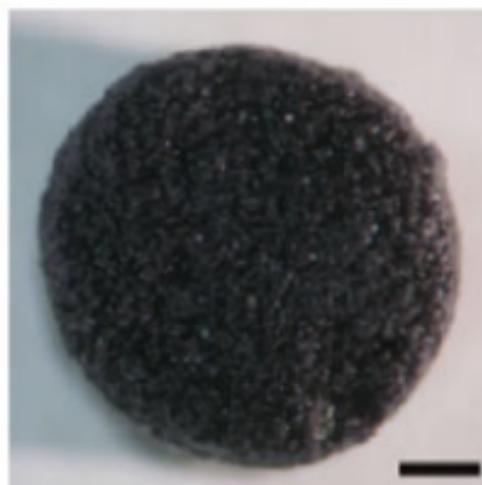


# Bioreactors improve *in vitro* cell viability in scaffolds via convection

Static  
Culture  
Day 7



Dynamic  
Culture  
Day 7



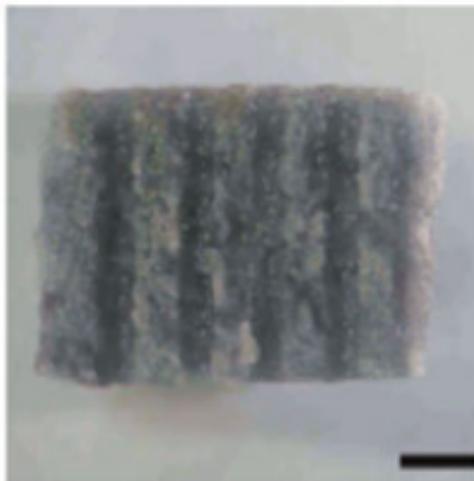
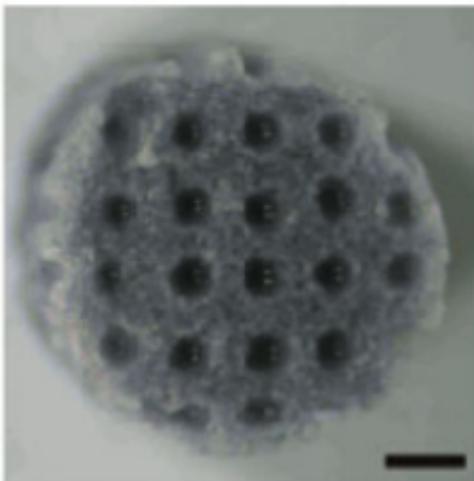
# How can oxygen transport to core scaffold regions be improved?

$$\partial C_i / \partial t + v \nabla C_i = D_i \nabla^2 C_i - R_{Vi}$$

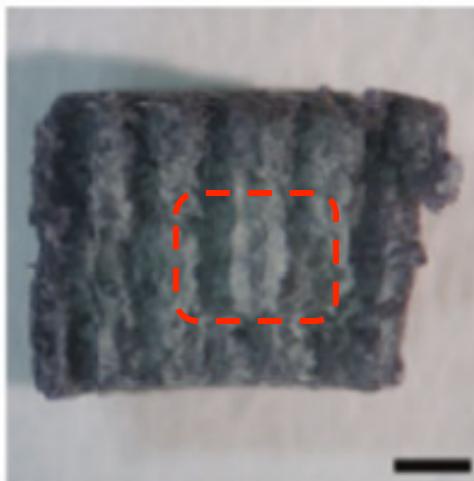
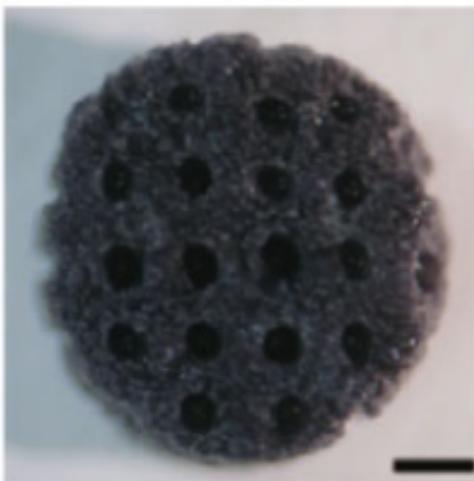
diffusion

# Improving diffusion via scaffold architecture is also semi-successful

4Hrs  
Post  
Seeding



Static  
Culture  
Day 7



# How can oxygen transport to core scaffold regions be improved?

$$\partial C_i / \partial t + v \nabla C_i = D_i \nabla^2 C_i - R_{Vi}$$

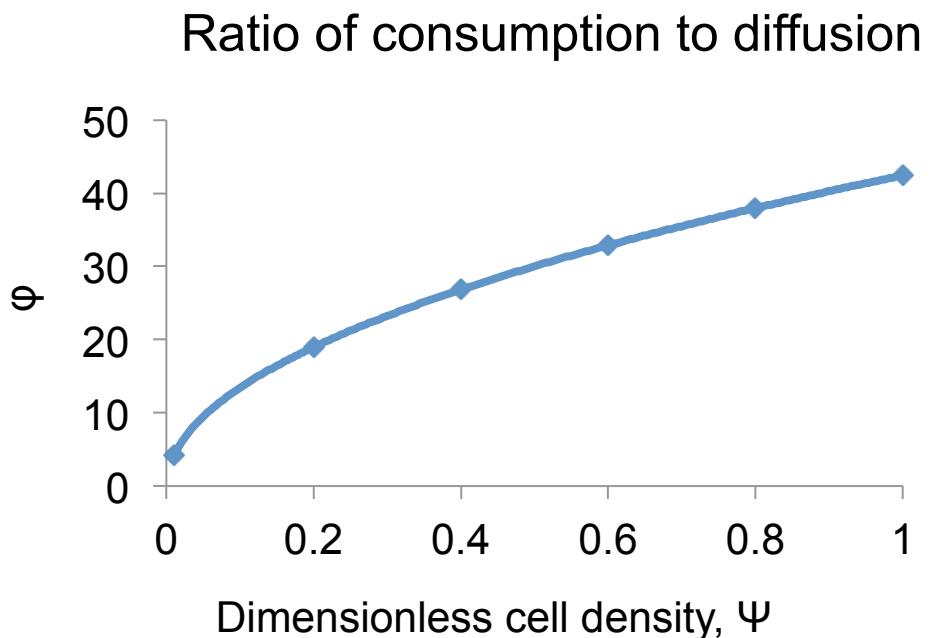
reaction

# Literature values predict O<sub>2</sub> consumption dominates diffusion

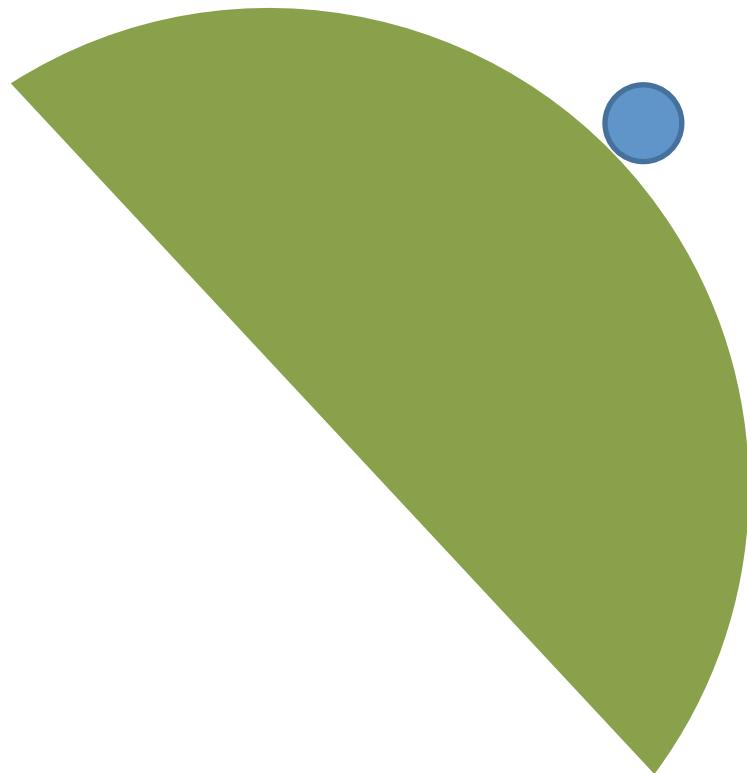
## Thiele modulus

$\varphi \downarrow n = \text{reaction rate}/\text{diffusion rate}$

$$\varphi \downarrow n = \sqrt{V\Psi UmL^2/D \downarrow O_2} = 42.4\Psi^{0.5}$$



# How can oxygen transport to core scaffold regions be improved?



# How can oxygen transport to core scaffold regions be improved?



# Improve O<sub>2</sub> transport by seeding cells in a gradient

transport model

*in vitro* model

tracking cells

a strategy to improve core cell viability through rational cell distribution

# Improve $O_2$ transport by seeding cells in a gradient

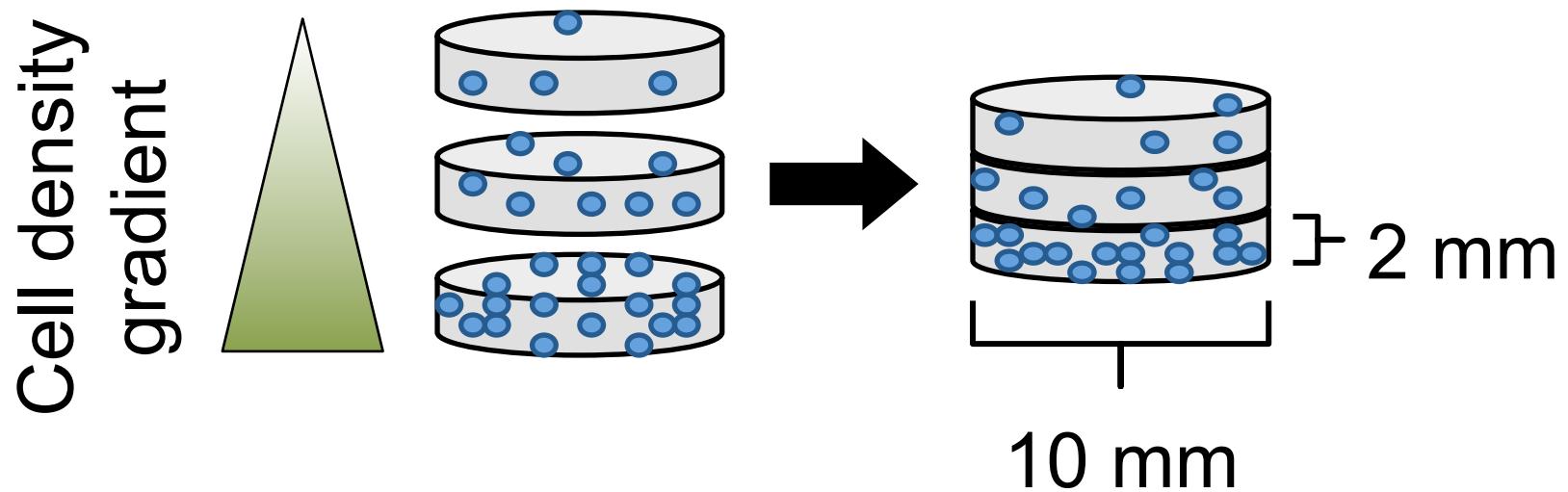
transport model

predict effect of cell seeding distribution on  $O_2$  and cell growth

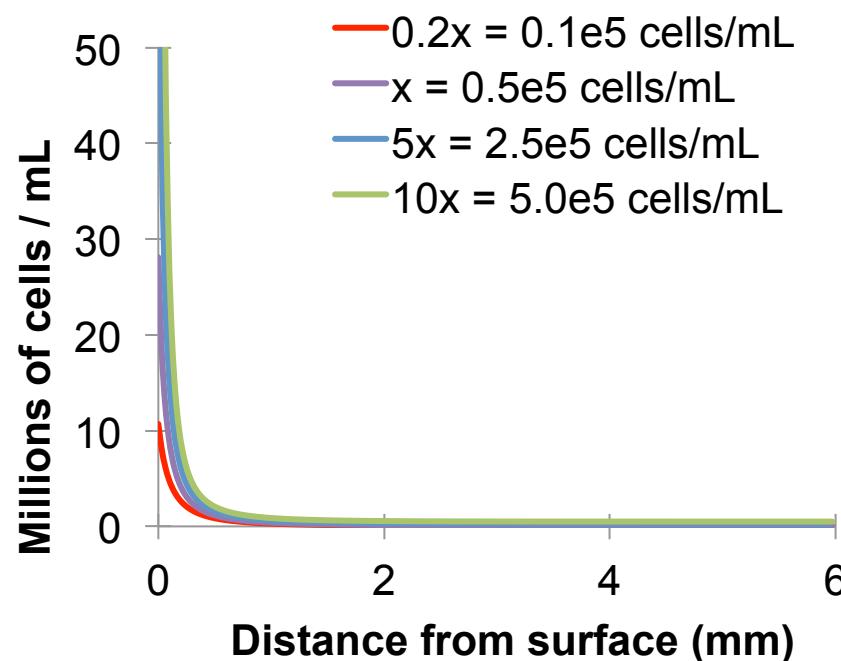
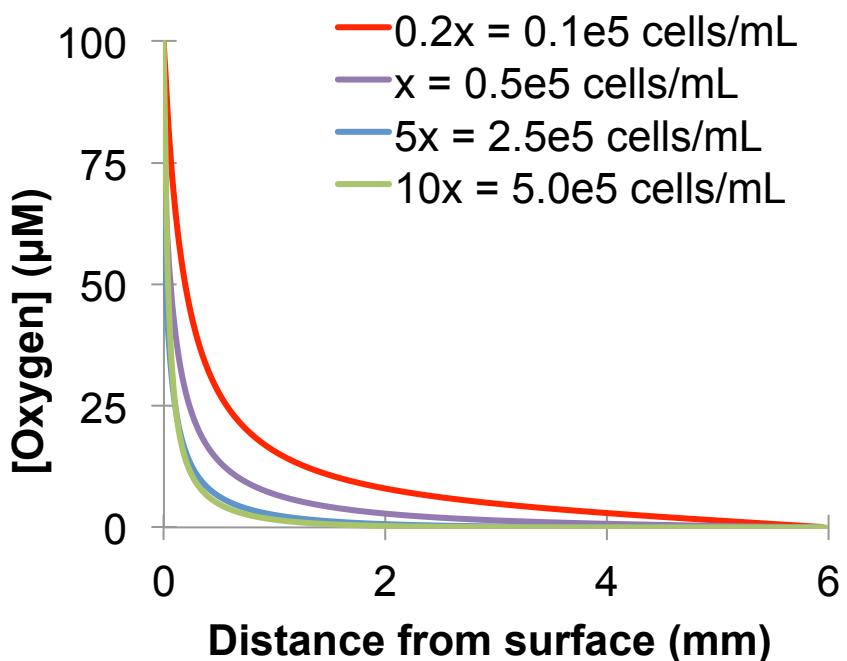
*in vitro* model

tracking cells

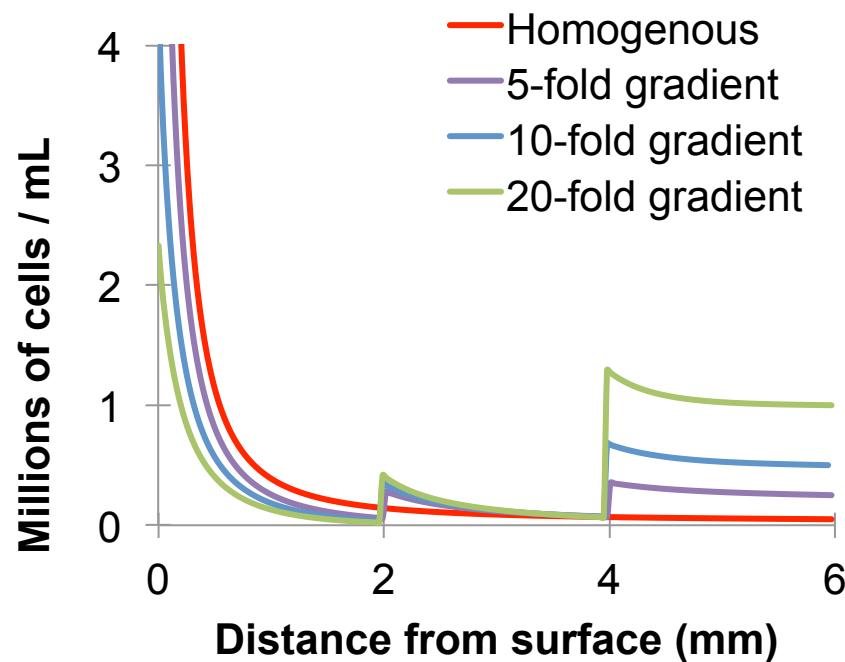
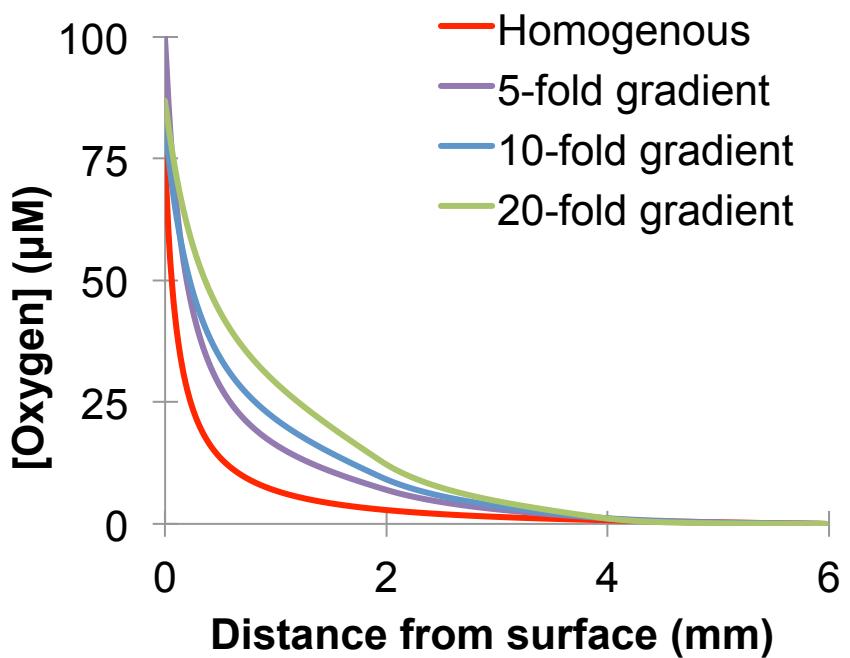
# Cylindrical model simplifies O<sub>2</sub> transport to one dimension



# Decreasing initial cell density does not improve theoretical core cell growth



# Seeding cells in a density gradient improves theoretical cell growth



# Modeling – discussion

hypothesis

seeding cells in a gradient can reduce peripheral  $O_2$  consumption

methods

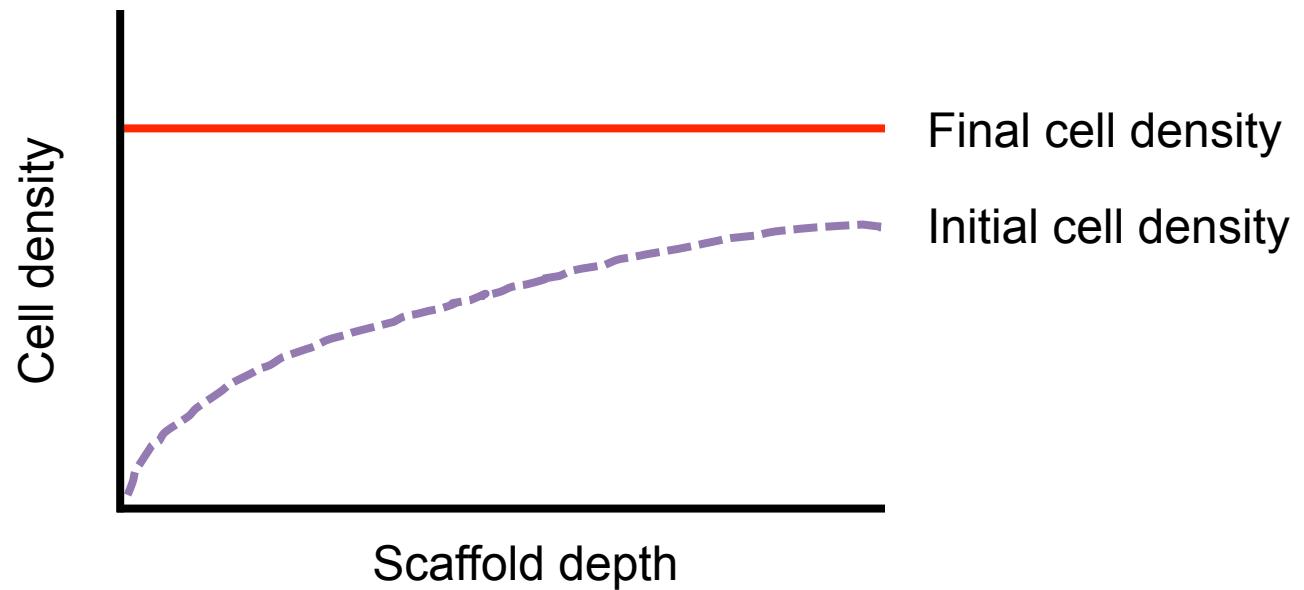
modeling of  $O_2$  transport and cell density

results

hypothesis is theoretically effective

# Modeling – next steps & paper

- Test model feasibility *in vitro*
- Use model to predict specific cell densities that will result in maximum core cell viability



# Improve O<sub>2</sub> transport by seeding cells in a gradient

transport model

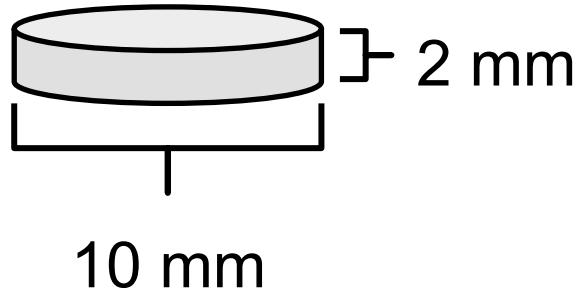
*in vitro* model

tracking cells

test model predictions  
with cells in a scaffold

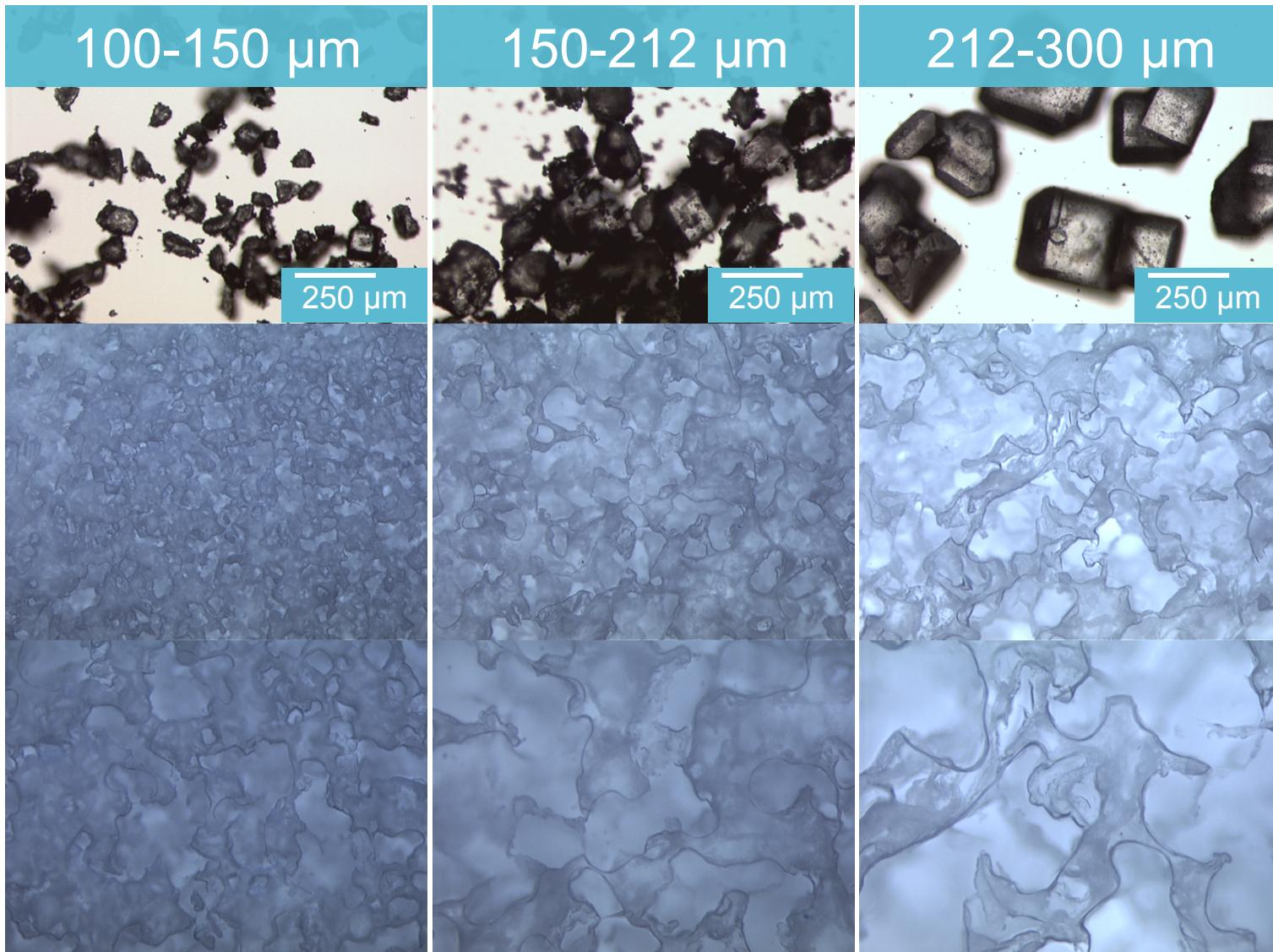
# Goal: fabricate PCL scaffolds with controllable shape and pore size

- Pore size affects transport, cell behavior<sup>4-6</sup>
- Following protocol from Lee et al. 2007
- Slurry = PCL + chloroform + sugar
- Teflon ring mold controls dimensions

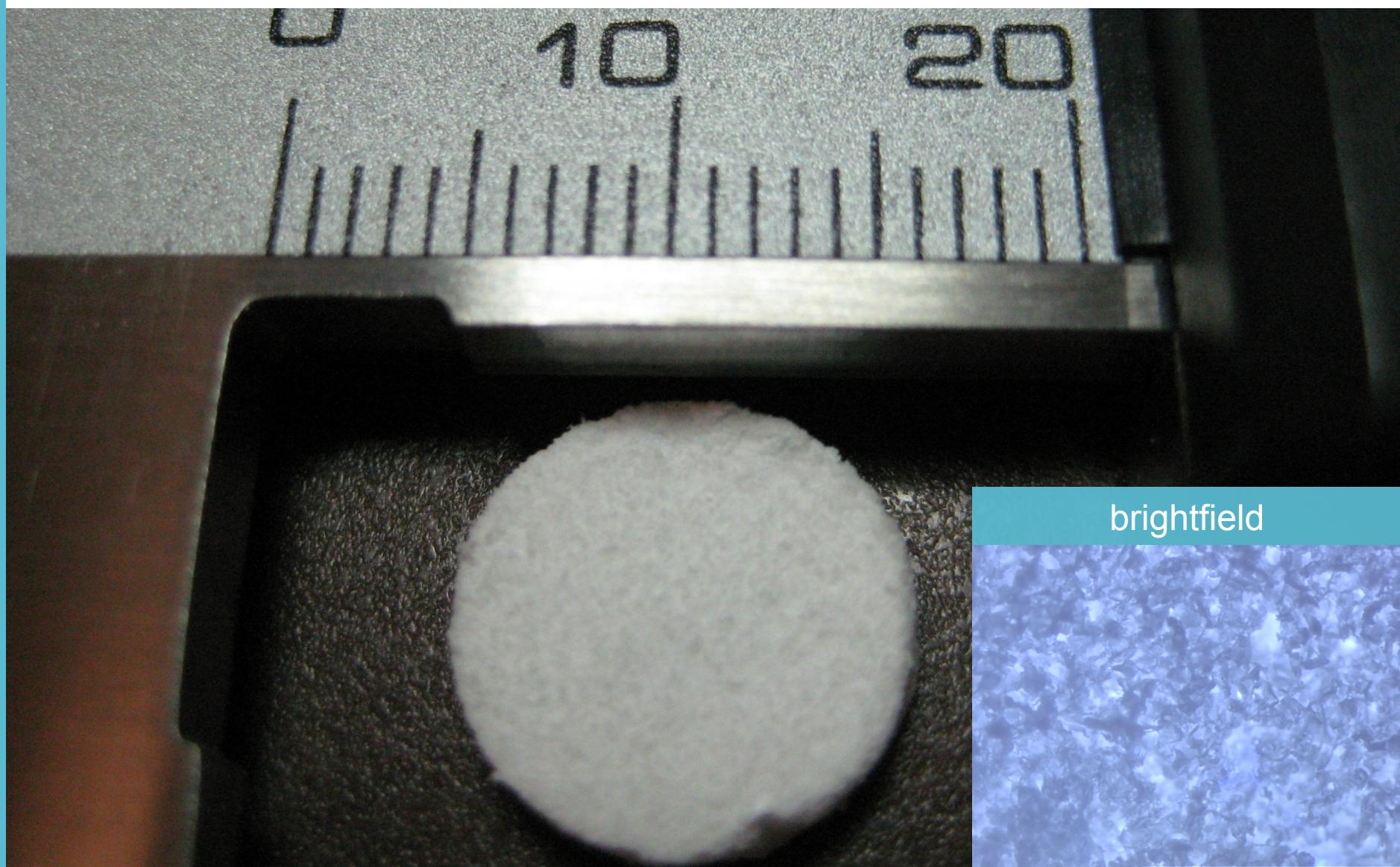


# Sugar size controls PCL pore size

PCL, 10x      PCL, 5x      Sugar

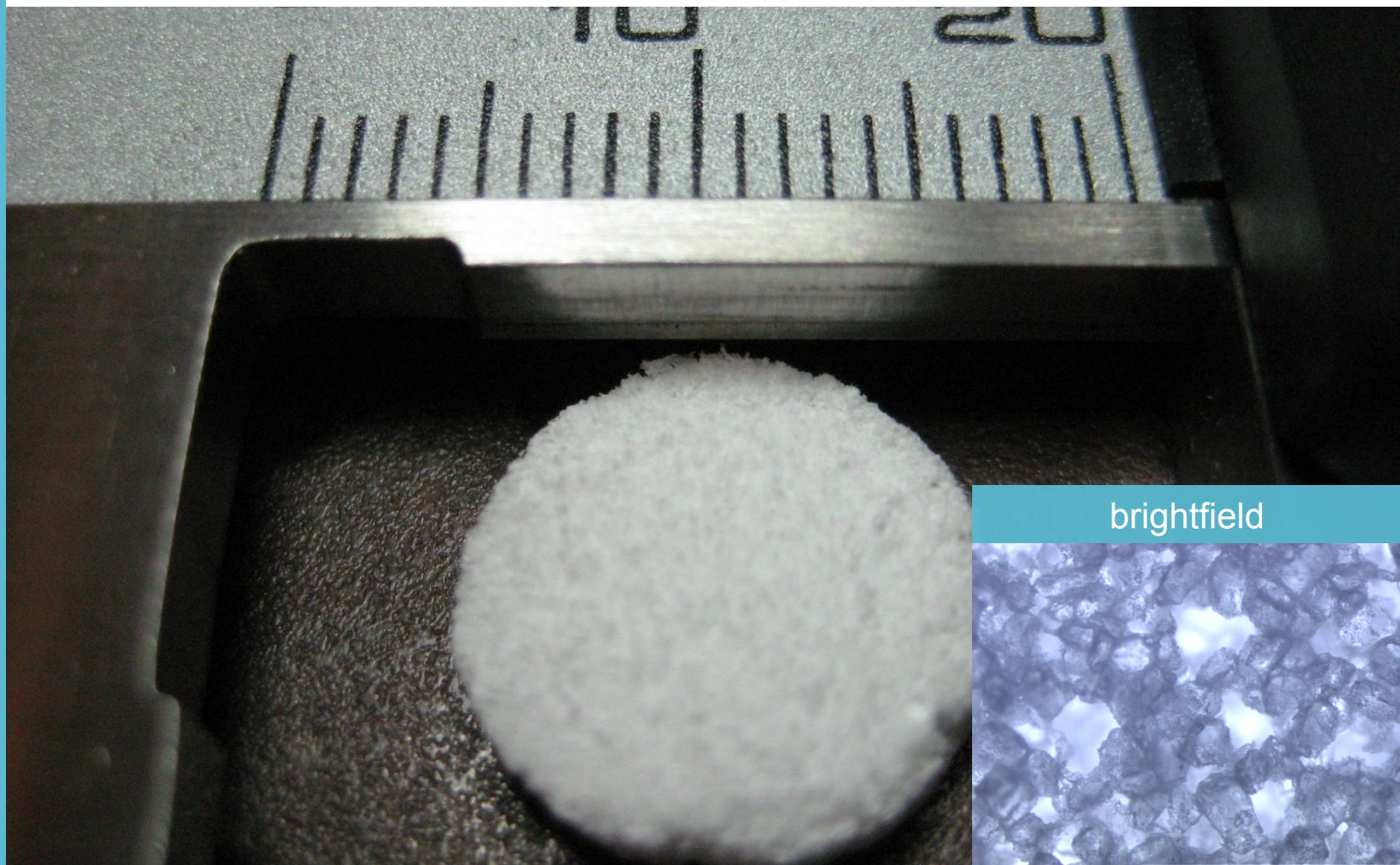


50-100  $\mu\text{m}$  sugar



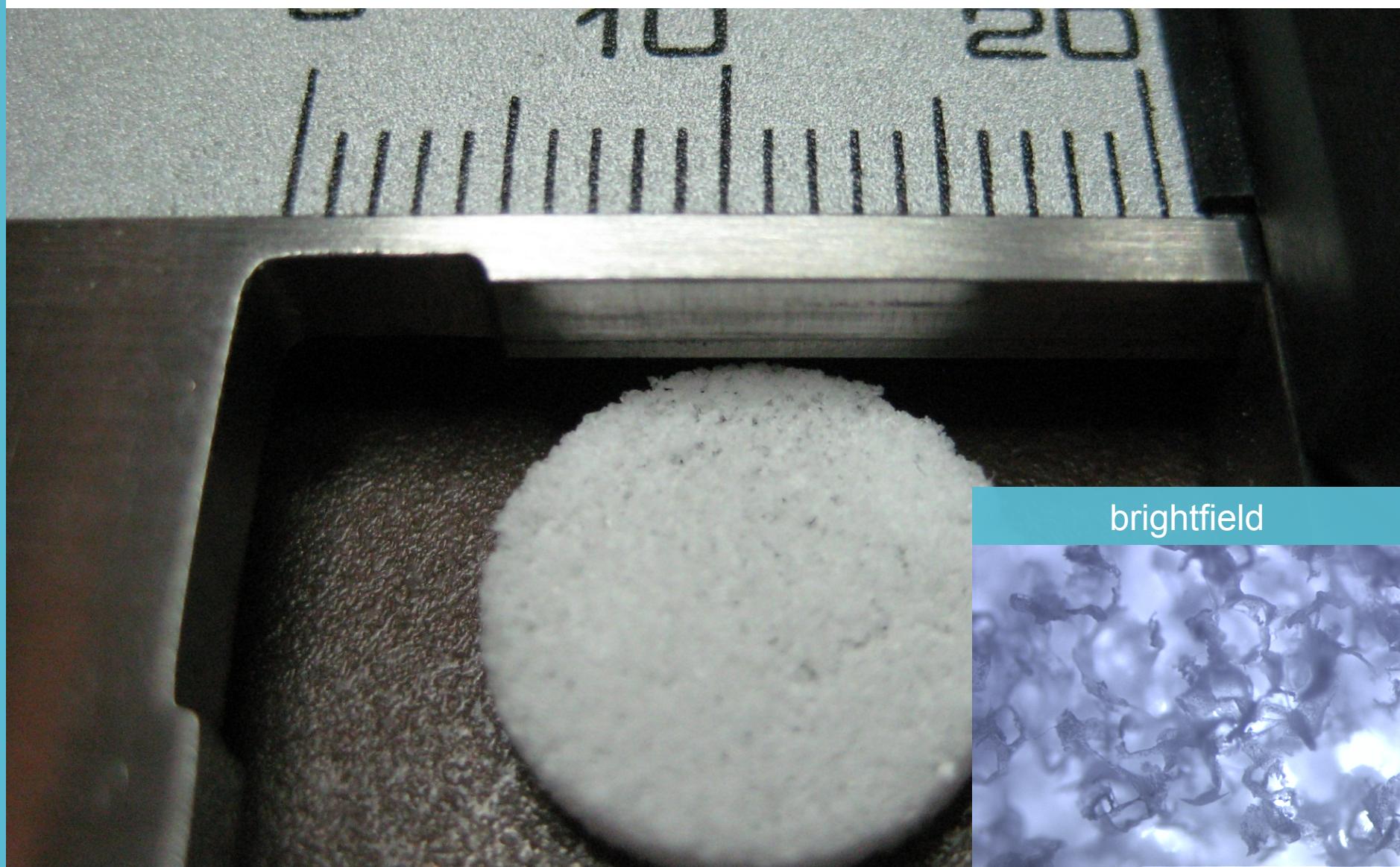
brightfield

150-212  $\mu\text{m}$  sugar



brightfield

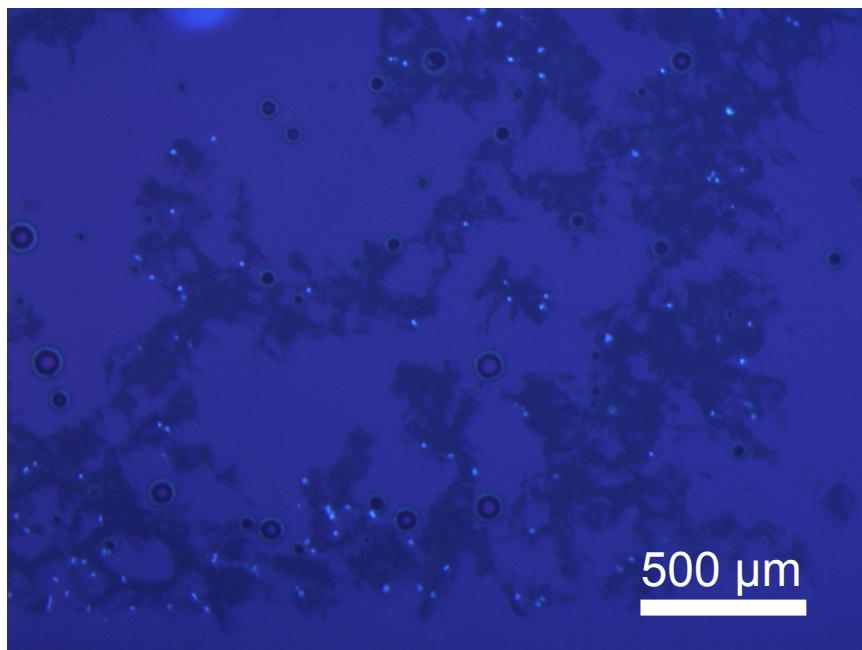
# 212-300 µm sugar



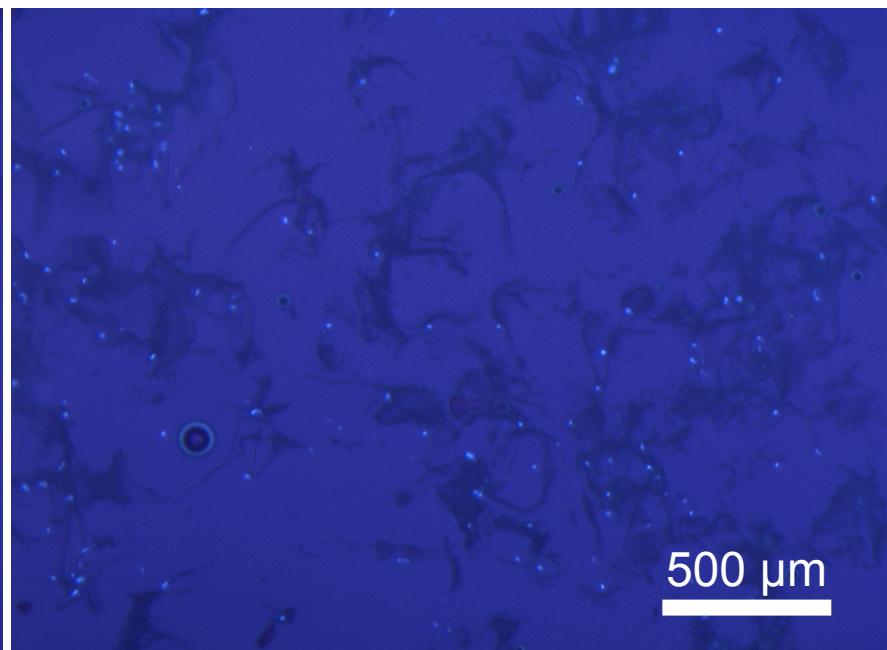
brightfield

# PCL scaffold porosity is sufficient for initial MC3T3 cell infiltration

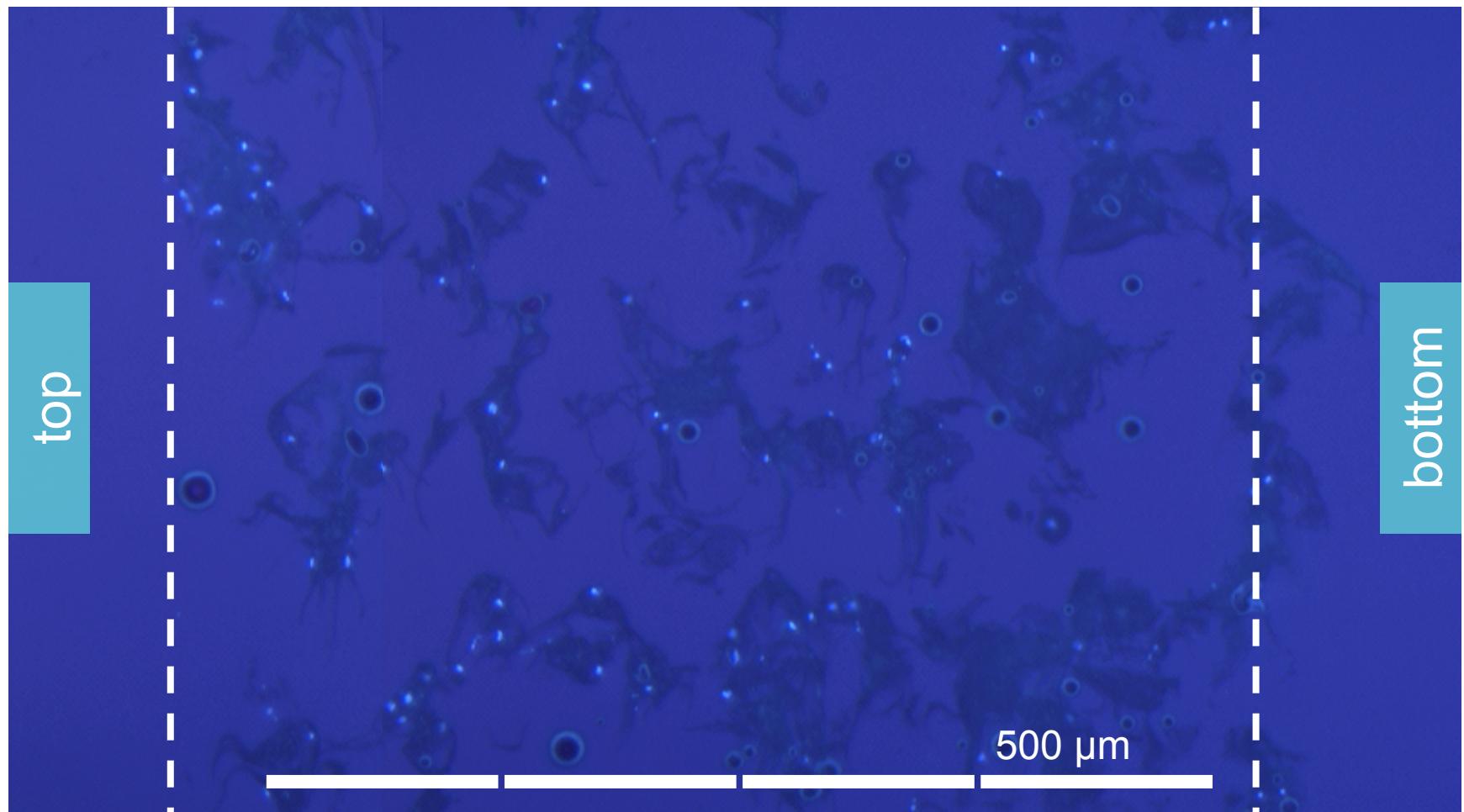
150-212  $\mu\text{m}$  sugar



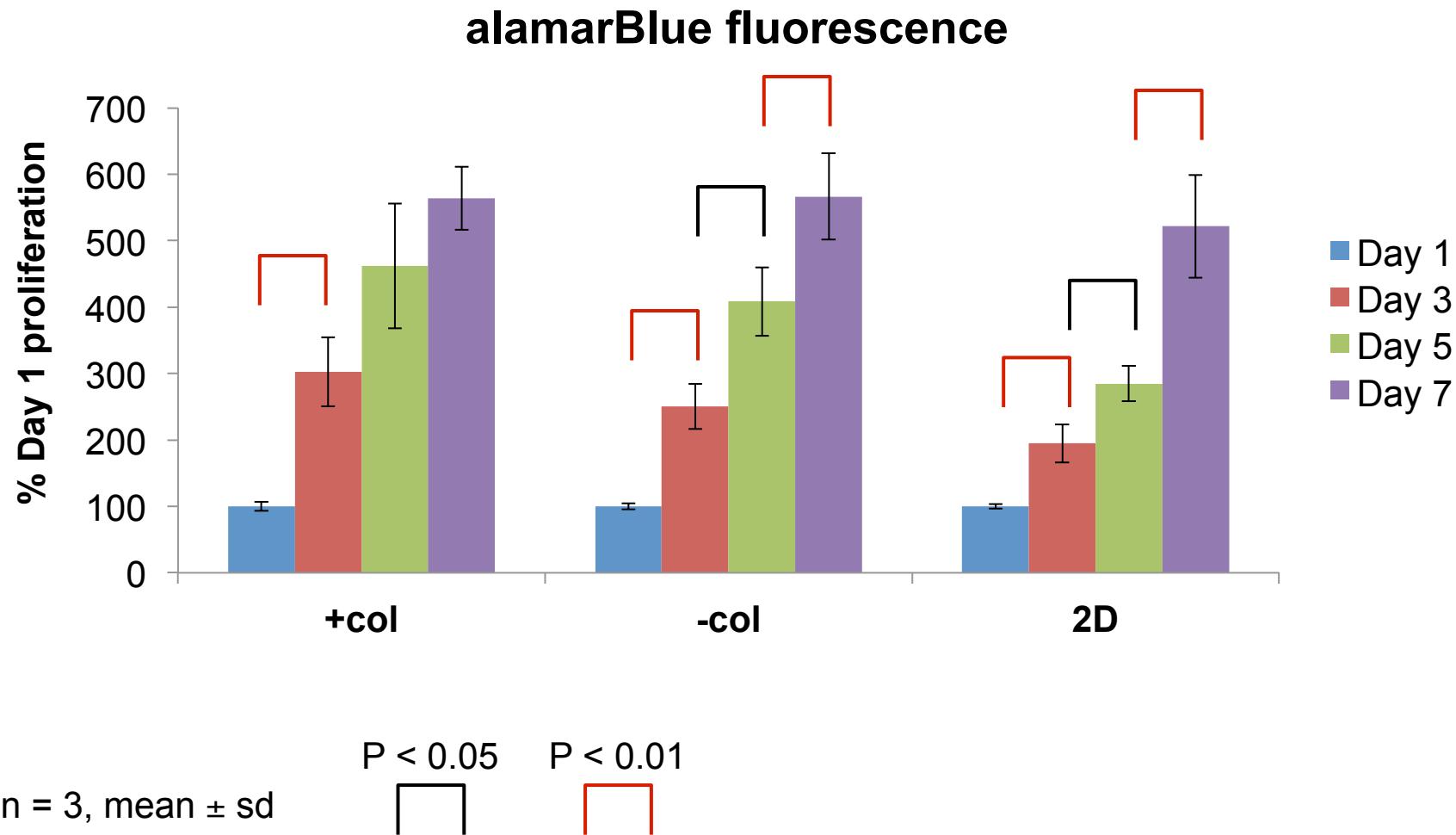
212-300  $\mu\text{m}$  sugar



# PCL scaffold porosity is sufficient for initial MC3T3 cell infiltration



# MC3T3s proliferate well in PCL discs regardless of collagen coating



# *In vitro* – discussion

hypothesis

seeding cells in a gradient can reduce peripheral O<sub>2</sub> consumption

methods

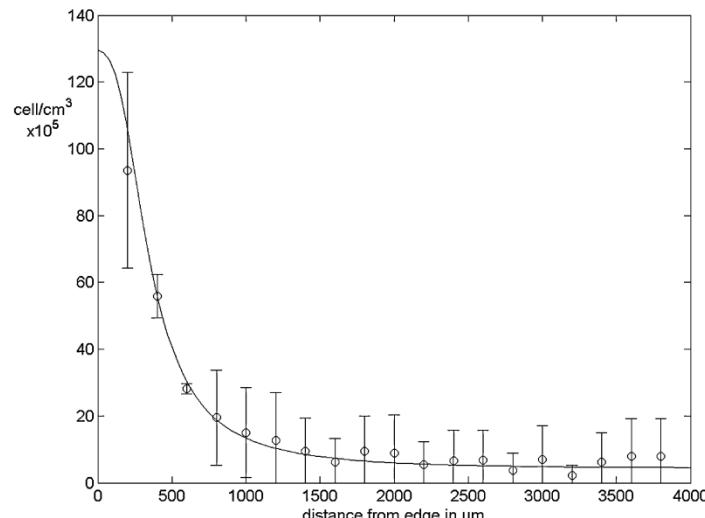
seeded MC3T3s on PCL scaffolds

results

scaffolds support MC3T3 infiltration and proliferation

# *In vitro* – next steps & paper

- Must show agreement between mathematical model and experimental results
- Quantify histological sections



# Improve O<sub>2</sub> transport by seeding cells in a gradient

transport model

*in vitro* model

tracking cells

gold nanoparticle labeling to track cells with microCT

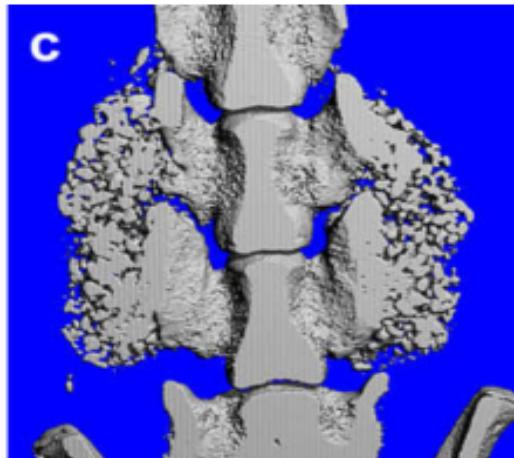
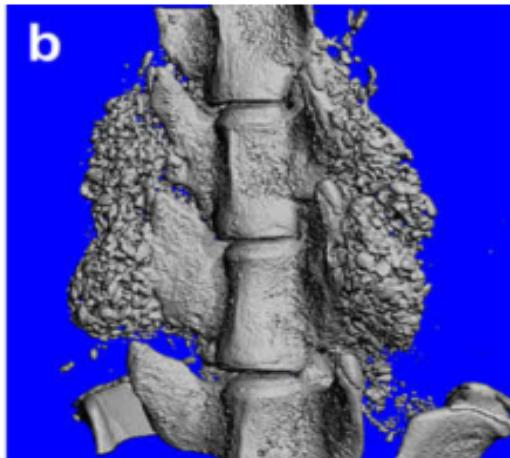
# MicroCT to image cells in 3D scaffolds could overcome issues from histology

- Histology suffers from sampling error and only looks at slices
- Difficult to analyze data quantitatively

X-ray



MicroCT

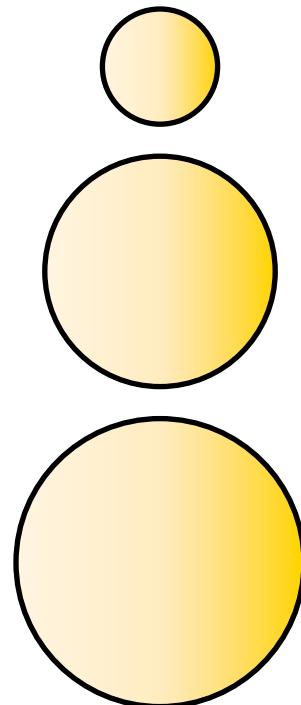


# Why MRI?

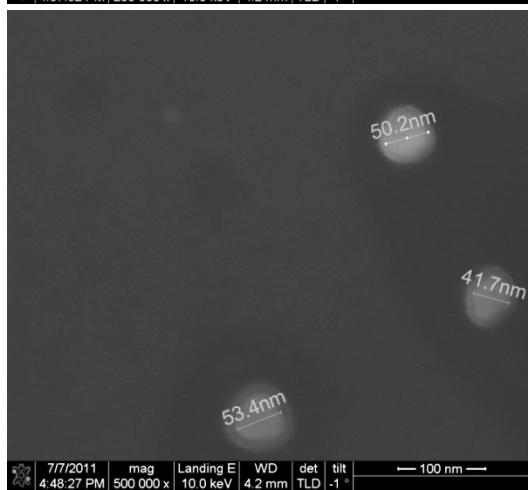
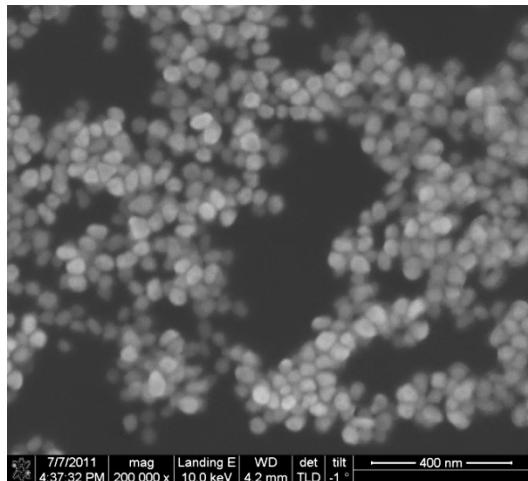
- Stuff

# Gold nanoparticles (AuNPs) could be internalized to provide cellular contrast

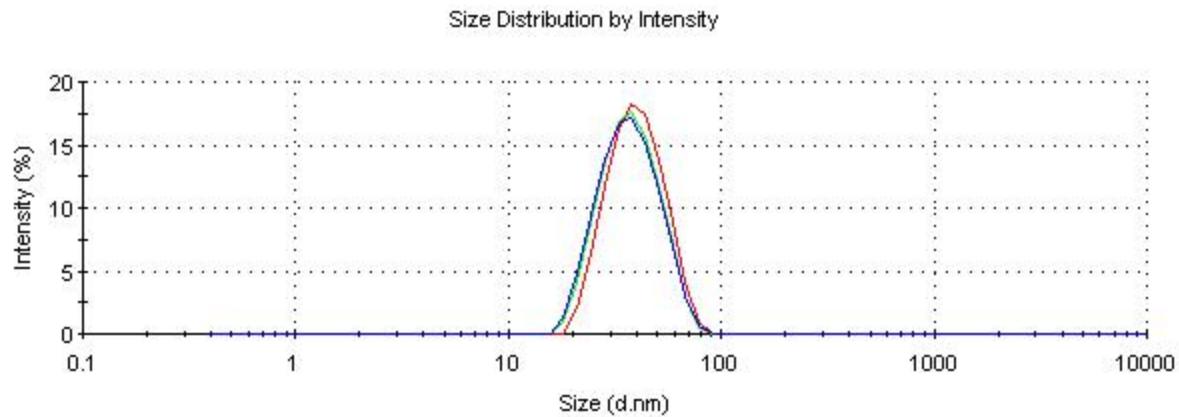
- What we test:
  - Cytotoxicity
  - Cellular uptake
  - X-ray contrast
- What we change:
  - AuNP size
  - AuNP incubation time



# Synthesized AuNPs are moderately uniform in size and shape: DLS & SEM



- 50 nm
- PDI 0.24



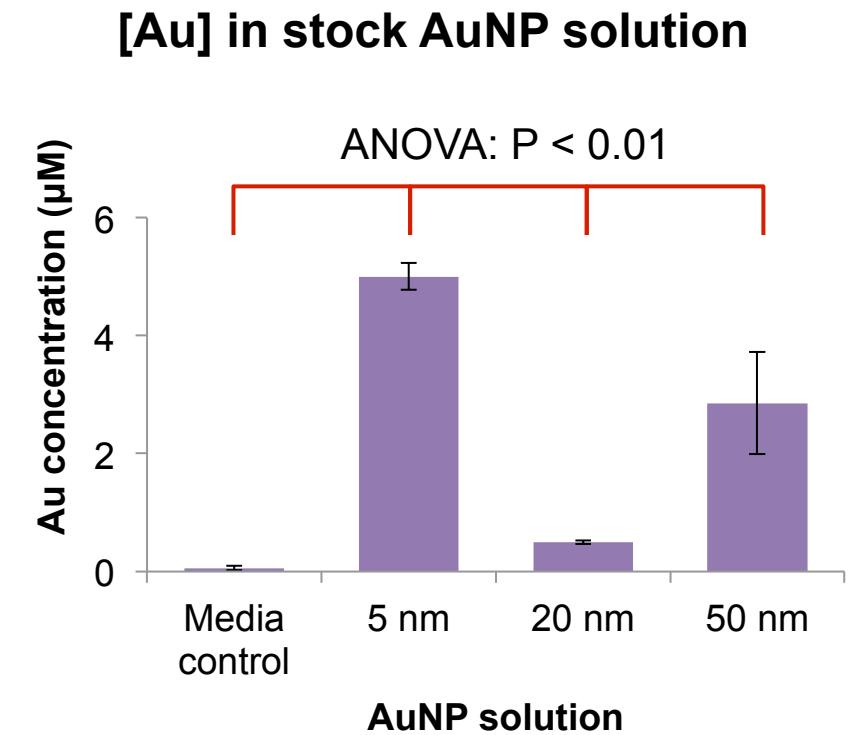
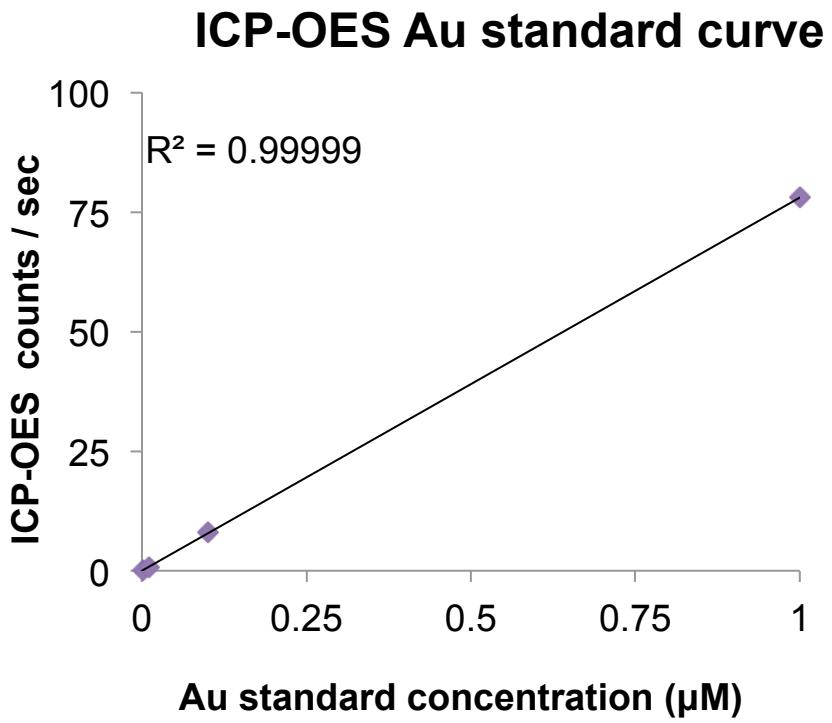
# A variety of AuNPs were used

5 nm dextran-coated, commercial

20 nm dextran-coated, commercial

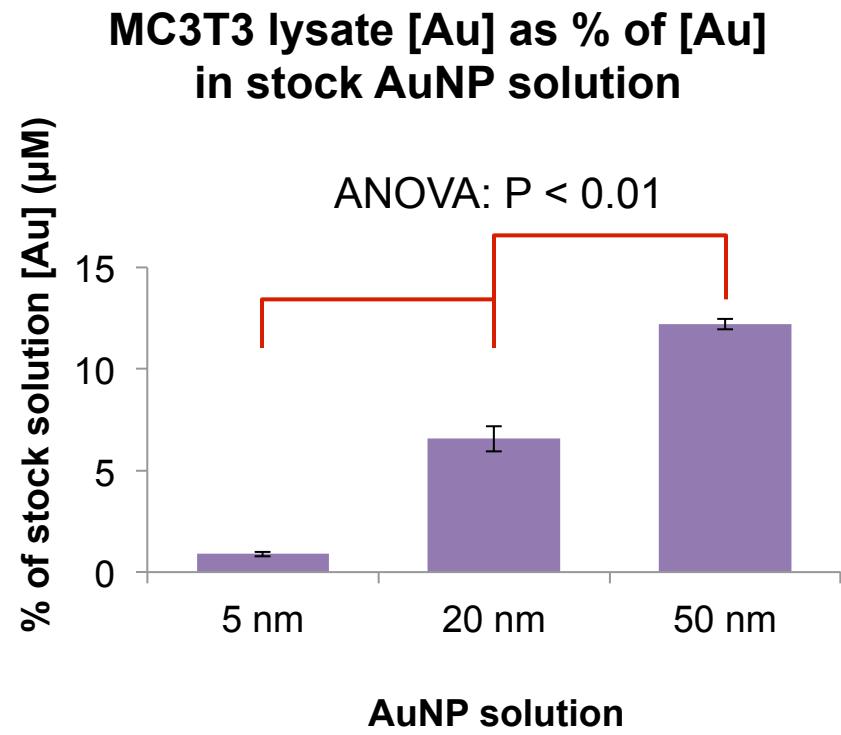
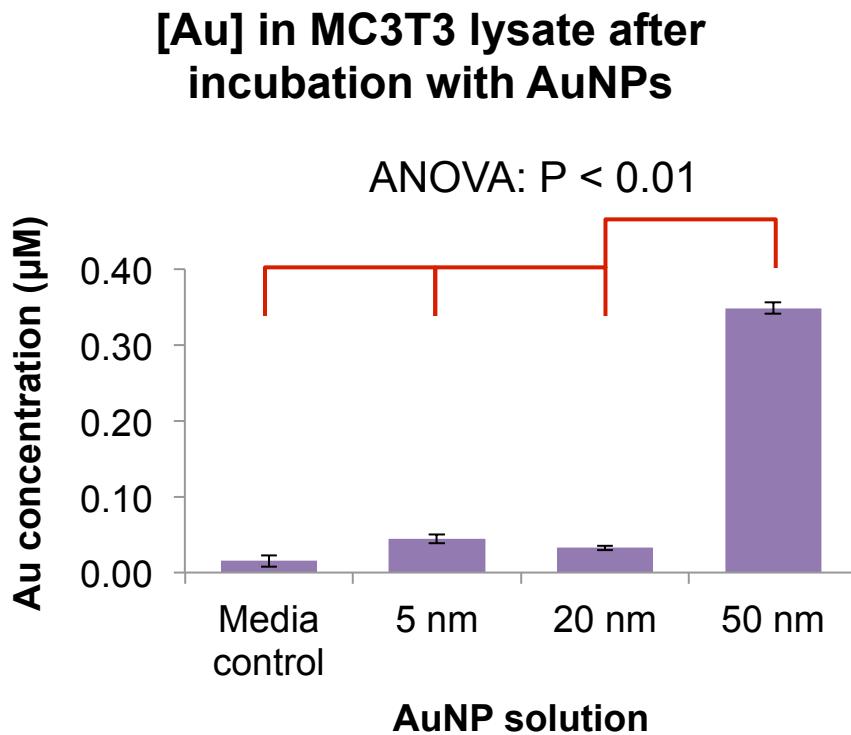
50 nm citrate-coated, synthesized

# ICP-OES shows [Au] is different for each AuNP solution



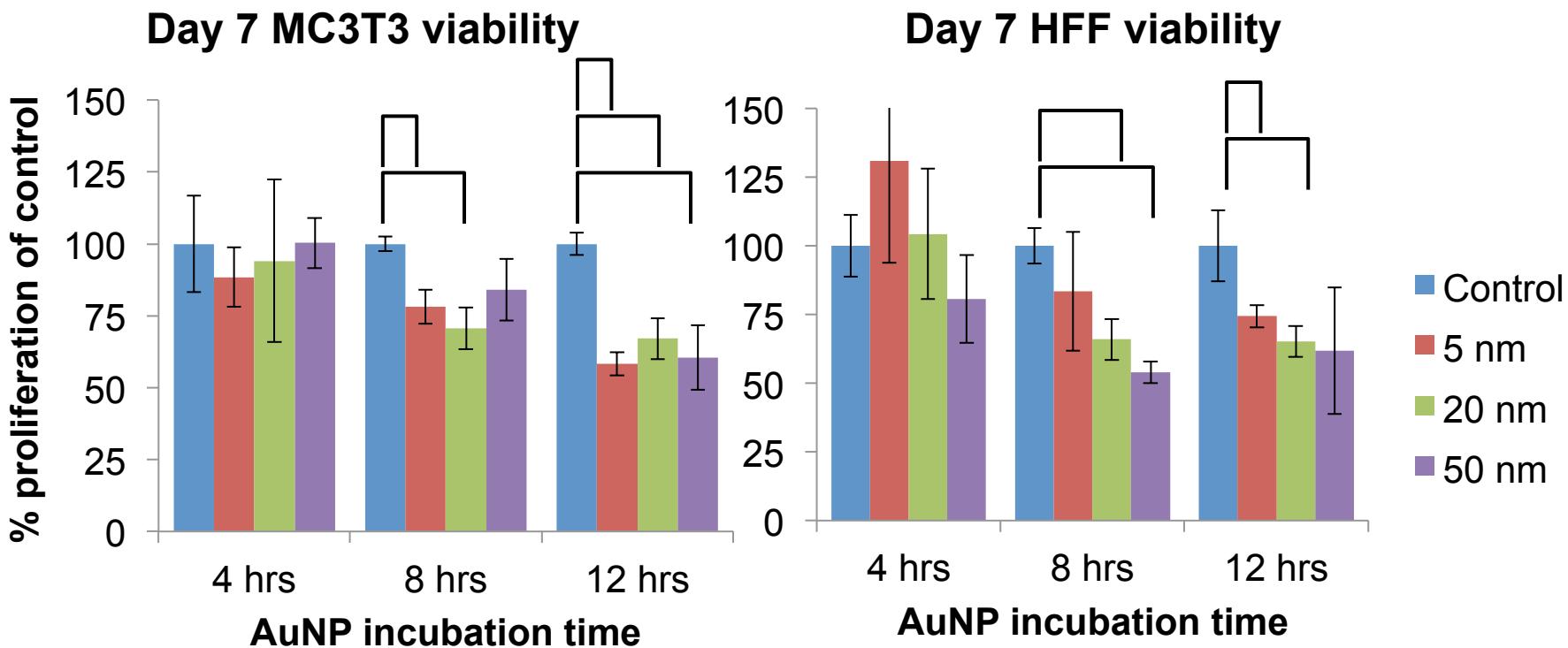
$n = 3$ , mean  $\pm$  sd

# ICP-OES shows AuNP uptake is size-dependent and maximized for 50 nm



n = 3, mean  $\pm$  sd

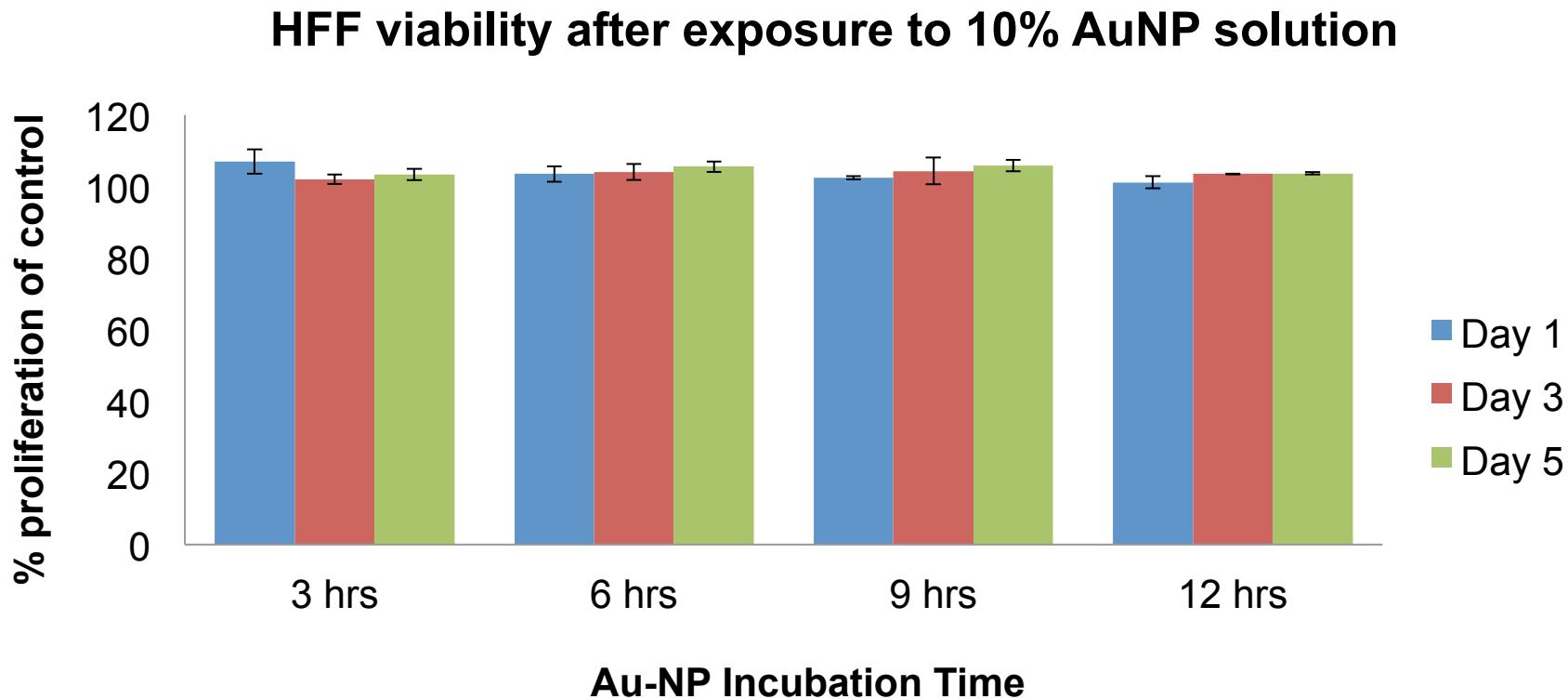
# AuNP viability decreases to 60% with greater incubation time, not AuNP size



n = 3, mean  $\pm$  sd

$P < 0.05$

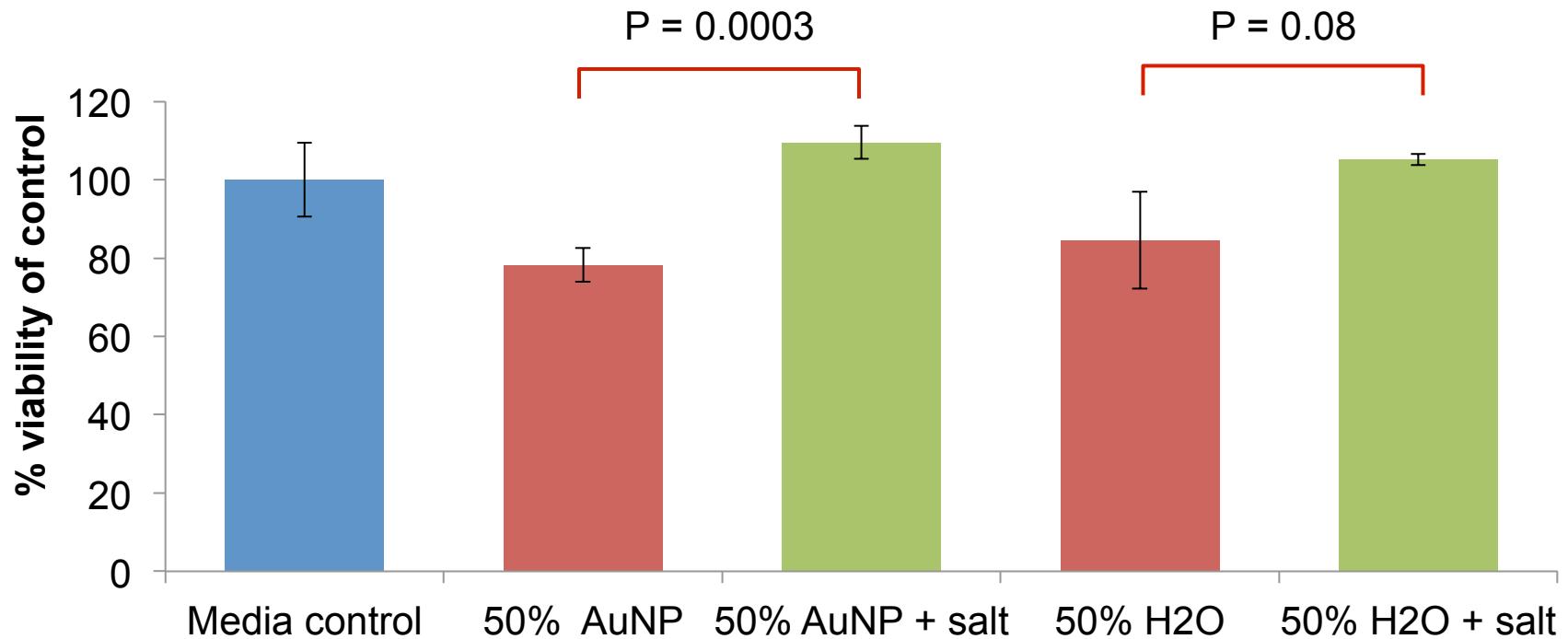
# HFFs are not harmed by 12 hr incubation in 10% AuNP solution



n = 3, mean  $\pm$  sd

# Adding PBS to AuNPs rescues MC3T3s from hypotonic cytotoxicity

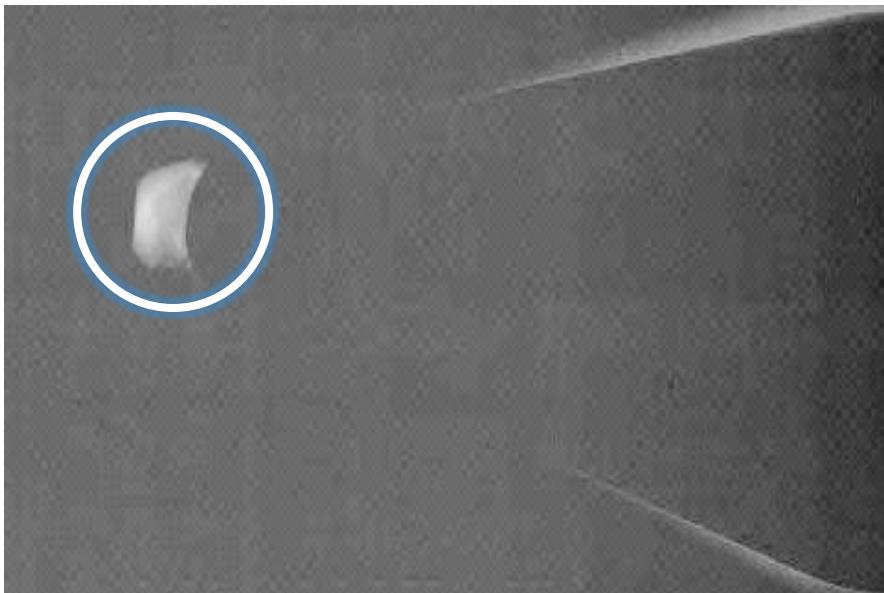
MC3T3 viability assessed via alamarBlue



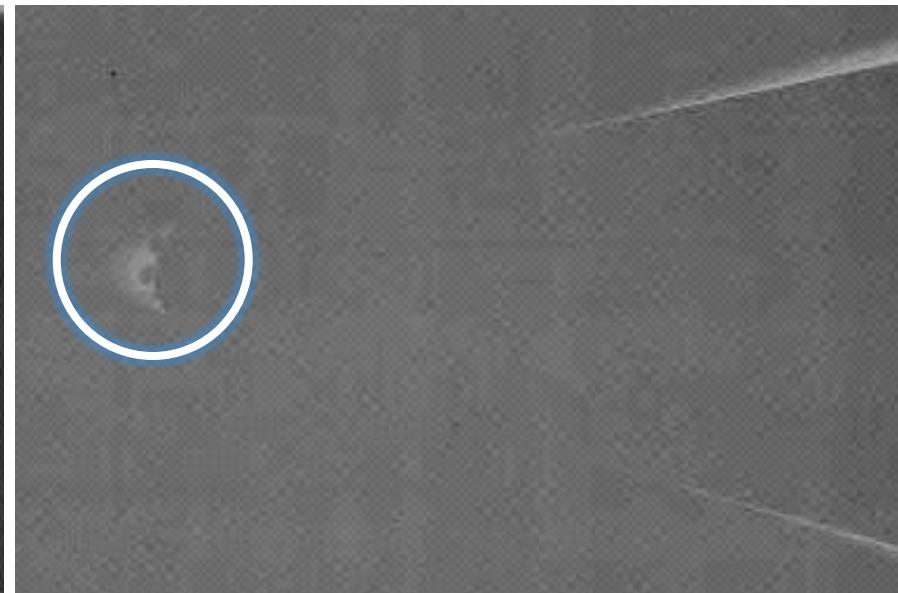
n = 3, mean  $\pm$  sd

# AuNP-labeled HFFs spun in a pellet provides X-ray contrast

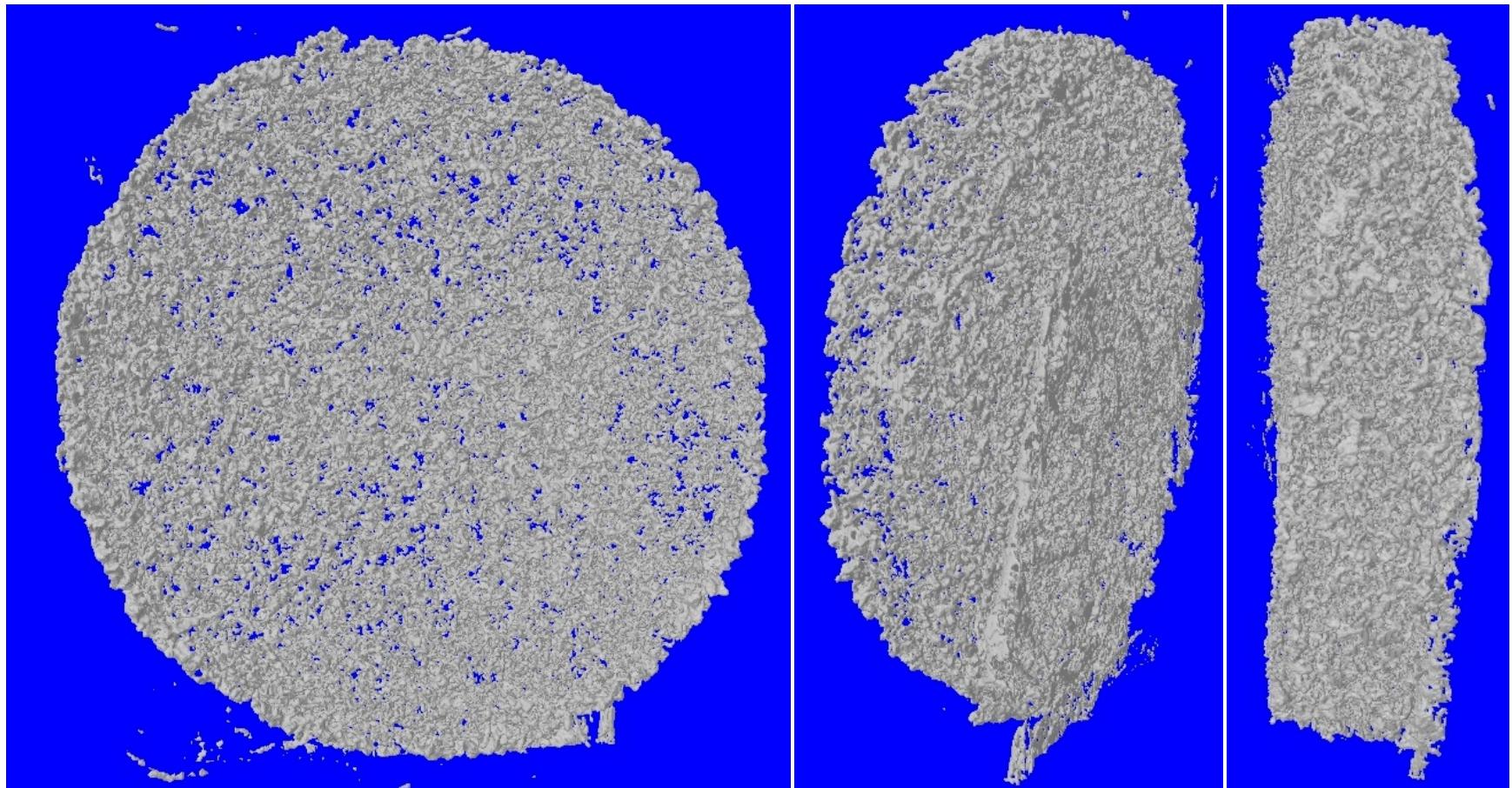
Cells labeled with AuNP



Unlabeled cell control



# MicroCT scans high-resolution volumetric renders of PCL scaffolds



# Tracking cells – discussion

hypothesis

AuNP-labeling can provide safe cellular contrast for microCT

methods

- labeled cells with AuNPs
- assessed cytotoxicity and uptake
- assessed X-ray contrast

results

- AuNP uptake is size dependent
- AuNP cytotoxicity is rescued with PBS
- AuNP labeling may produce X-ray contrast

# Tracking cells – next steps & paper

- Repeat uptake and cytotoxicity with AuNPs + PBS
- Quantify labeling distribution: does 90% of the gold go to 10% of the cells?
- Are labeled cells visible in microCT 3D scans?
- Must show agreement between histology and microCT

# Improve O<sub>2</sub> transport by seeding cells in a gradient

transport model

*in vitro* model

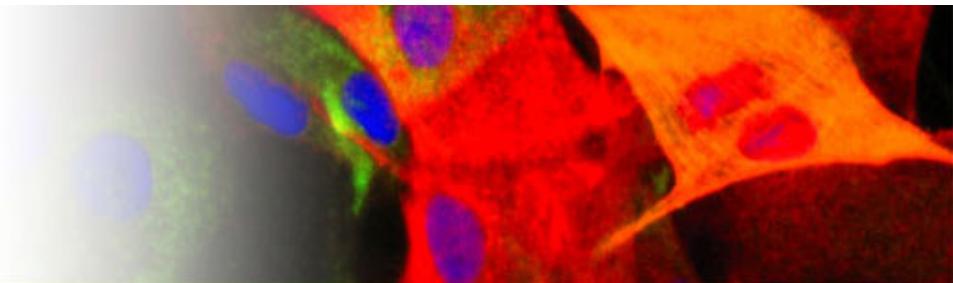
tracking cells

a strategy to improve core cell viability through rational cell distribution

# Acknowledgements

- Dr. Benjamin Wu
- Dr. Sergey Prikhodko
- Capstone team
- Christina Liu
- Ting Lab
- Wu Lab

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# References

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