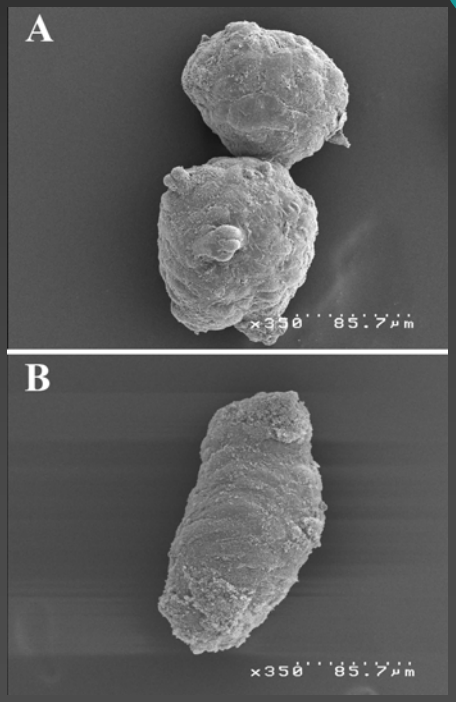
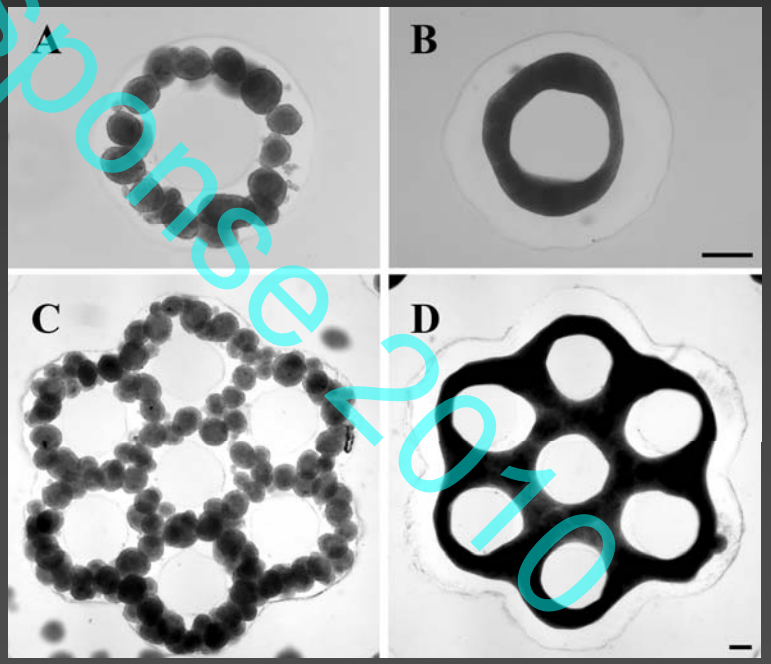
A New Platform Technology for the Self Assembly of 3D Living Microtissues

Jeff Morgan





Brown University

In vitro versus *in vivo* testing

	In vitro	*In vivo*
Ease & cost	+	
Dose response	+	
Human cells	+	
Physiologic		+
Predictive		+
Ethical issues	+	

Needed is something a step closer to *in vivo*

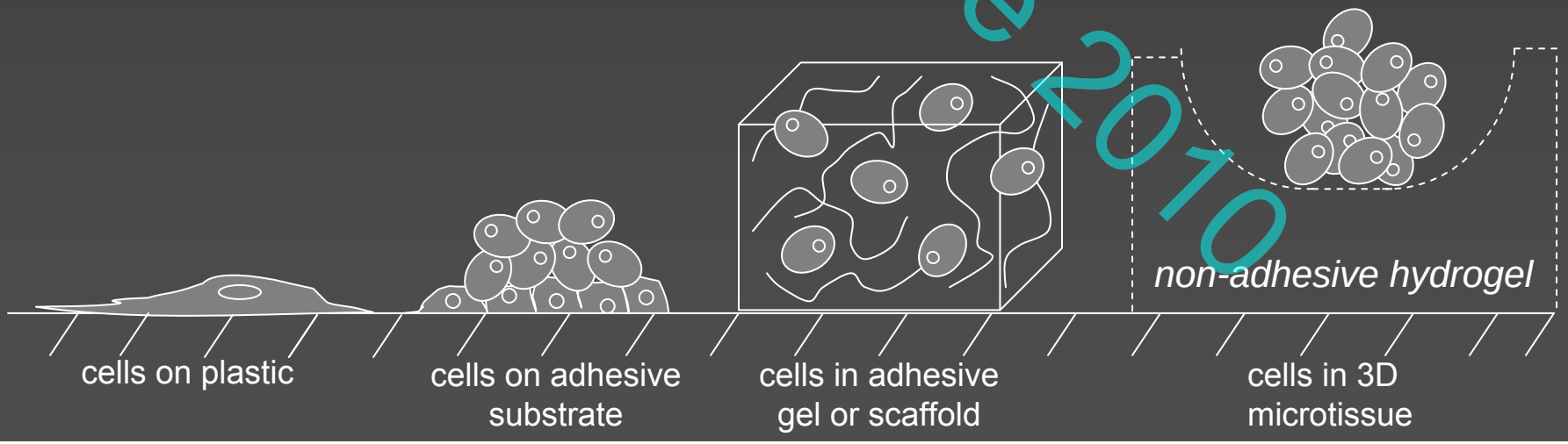
Tissue engineering

- Definition
  - Interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue function or a whole organ
  - Combine living cells with a scaffold, then transplant
- Methods
  - Scaffolds
    - synthetic (PLGA), natural (collagen type 1)
  - Cell sources
    - human cells (skin), stem cells, (iPS)
- New applications
  - *In vitro* 3D models that more closely mimic natural organs and tissues
    - More accurately replicate chemo and radio sensitivity of cancer cells
    - Speed testing of drugs and reduce animal use

Our method: Self-assembly of cells to form 3D microtissues

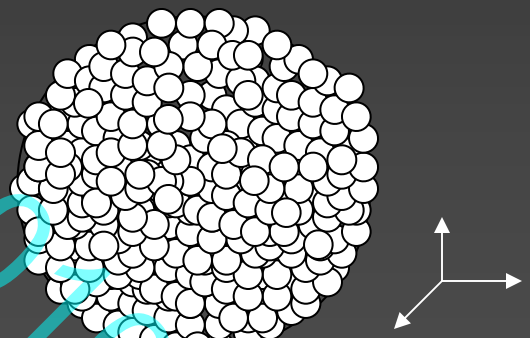
(scaffold-free)

- high cell density, similar to natural tissues
- cell morphology, function, differentiation, more natural
- cells exert adhesive forces on each other
- cell-to-cell interactions/communication are maximized
- cells self organize, form complex 3D tissue units
- step up from individual cells grown on surfaces



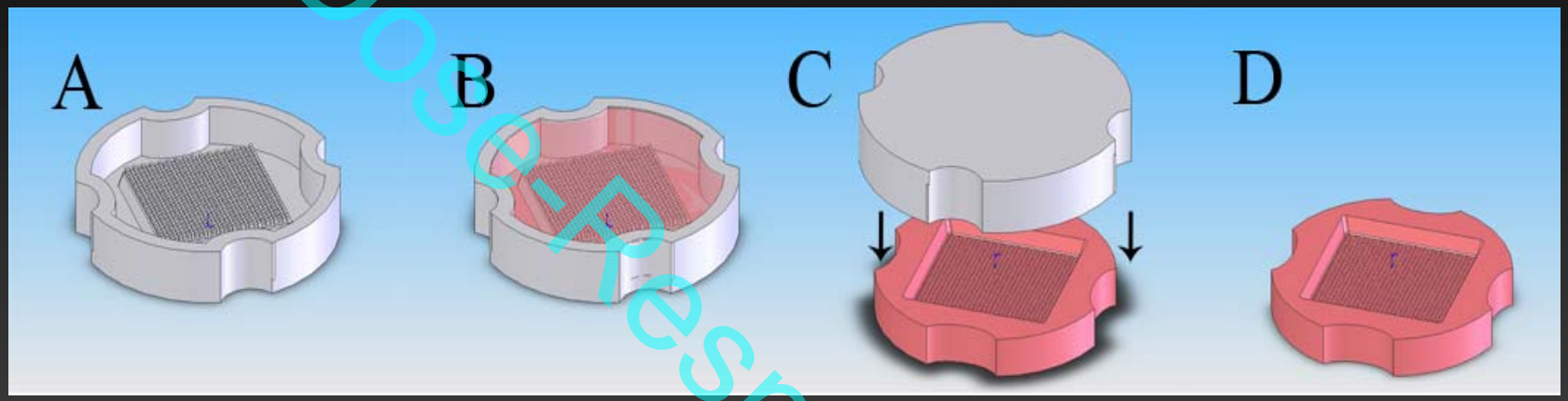
3D spheroids more closely replicates *in vivo*

- Cell biology is more like *in vivo*
  - Cell morphology, function and differentiation
  - Cell-to-cell interactions
  - Cell-to-ECM interactions
  - Co-culture with normal cells (endothelial cells, immune cells, stromal cells)
- Microenvironment is more like *in vivo*
  - Gradient of cell proliferation (proliferating, arrested, necrotic)
  - Gradients of oxygen, nutrients, pH, metabolism and waste products
- Drug transport is more like *in vivo*
  - Diffusion (intercellular, extracellular)
  - Cellular pumps
  - Cellular barriers
  - Influence of micro-environment



- Opportunities for high content
  - Mathematical model for growth is same as for tumor
  - Spherical symmetry provides coordinate system to map microenvironment
  - Spherical symmetry enables modeling of drug penetration, binding, and activity

Technology: Production of micro-molded agarose gels



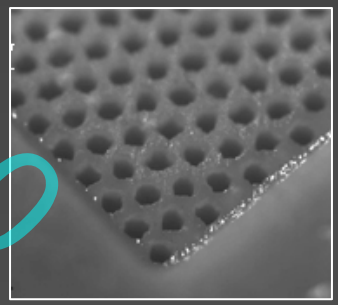
Rubber mold with square array of small feature

Mold filled with molten agarose

Agarose sets and is separated from mold

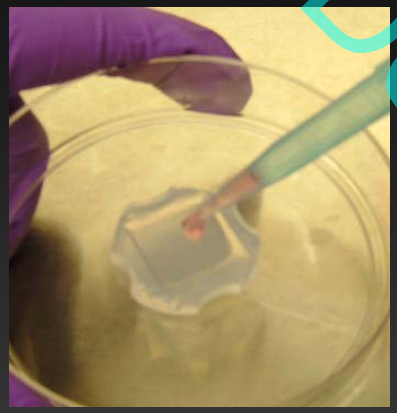
Micro-molded agarose gel

Close up of small features: pillars



Close up of small recesses in agarose

Cells settle into recesses & self-assemble 3D microtissues

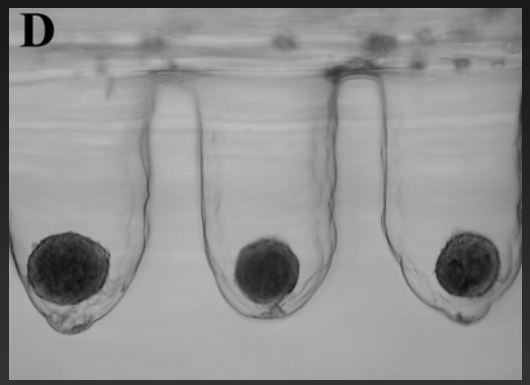


Seed gels with mono-dispersed cells

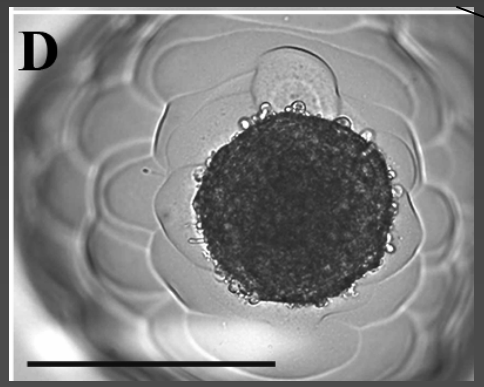
Side view microscopy

Cells settle into recesses

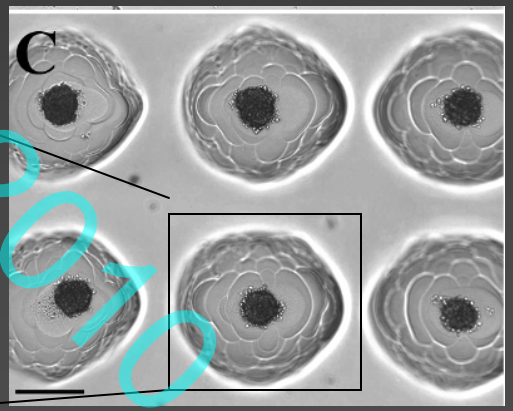
Side view microscopy



Cells self-assemble 3D microtissues (<24 hrs)



Close up view of single microtissue



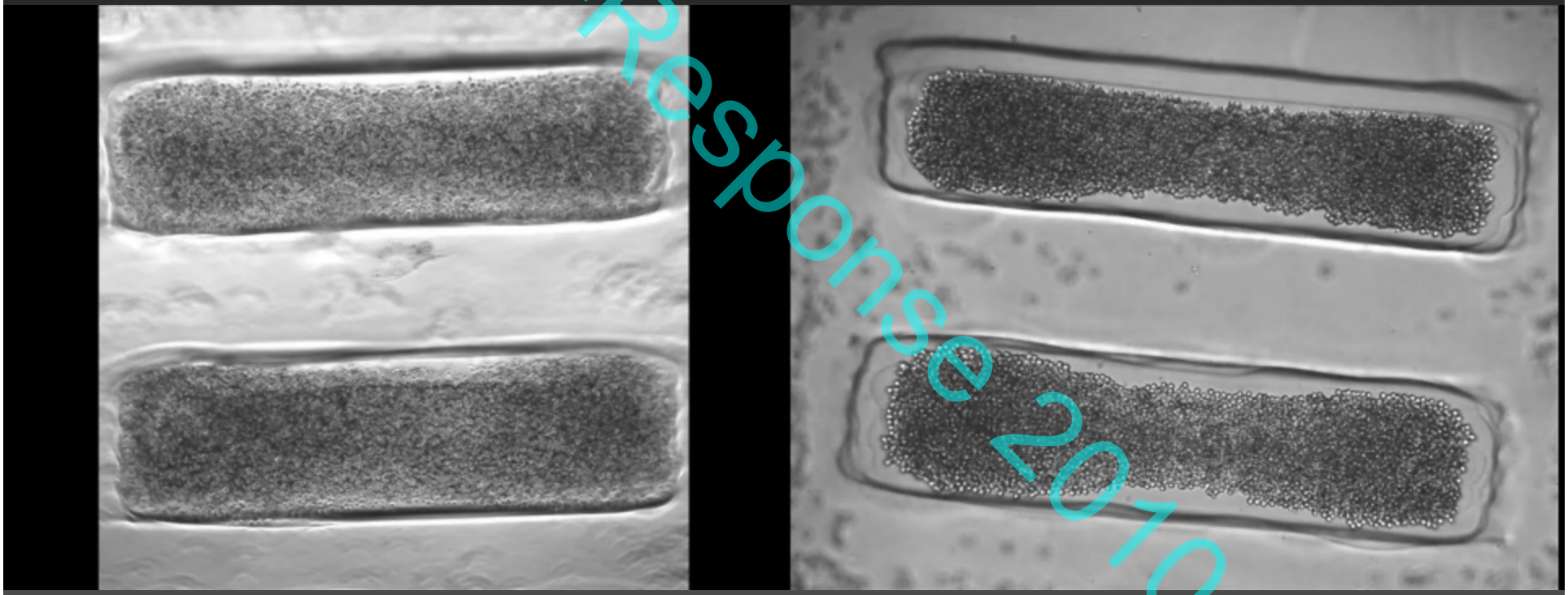
Conventional view microscopy

Napolitano, *Tissue Eng*, 2007

Time lapse microscopy of self-assembly

NHF: Human fibroblasts

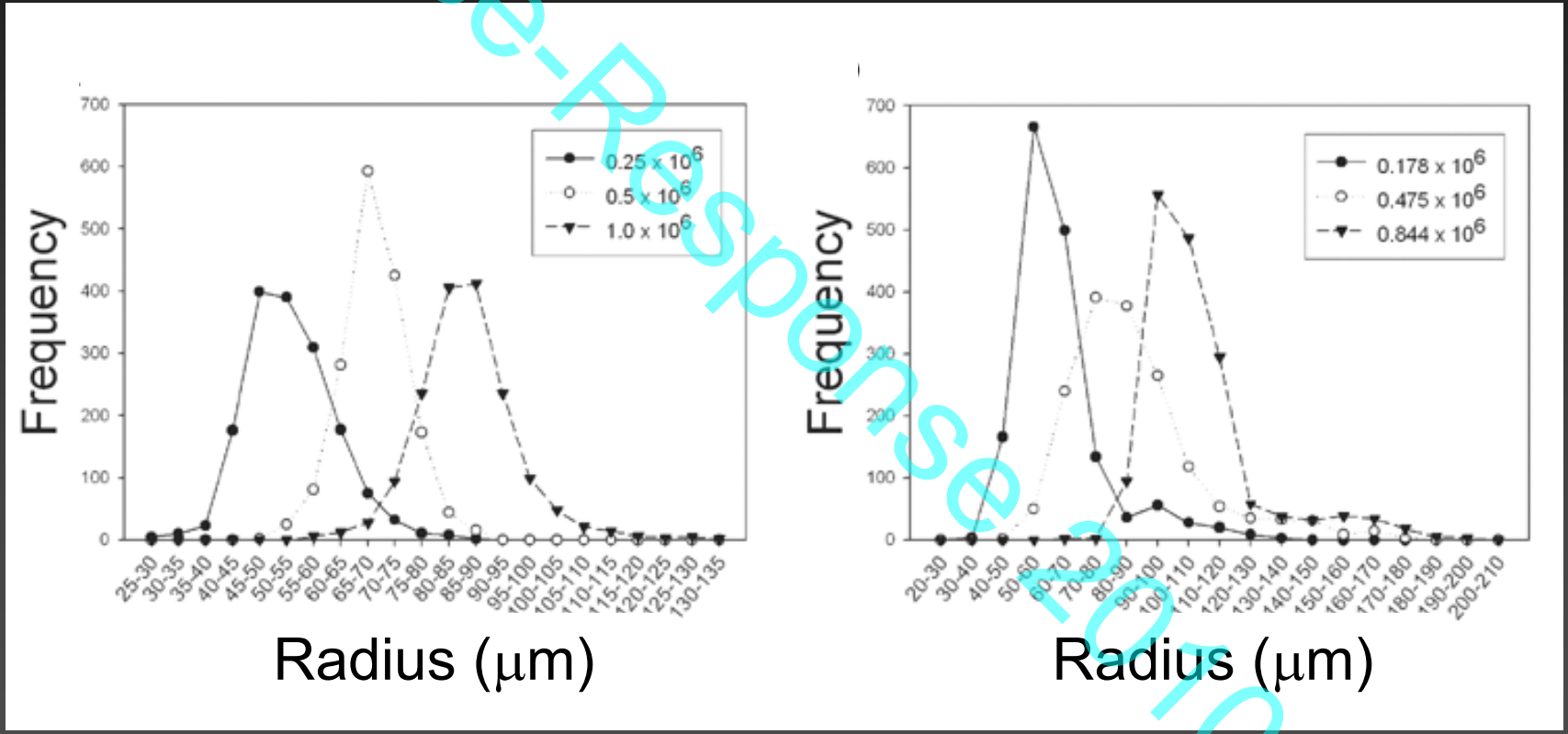
H35: Rat hepatoma cell line



Dean, *FASEB J*, 2007

Microtissue size is controlled by the number of cells seeded

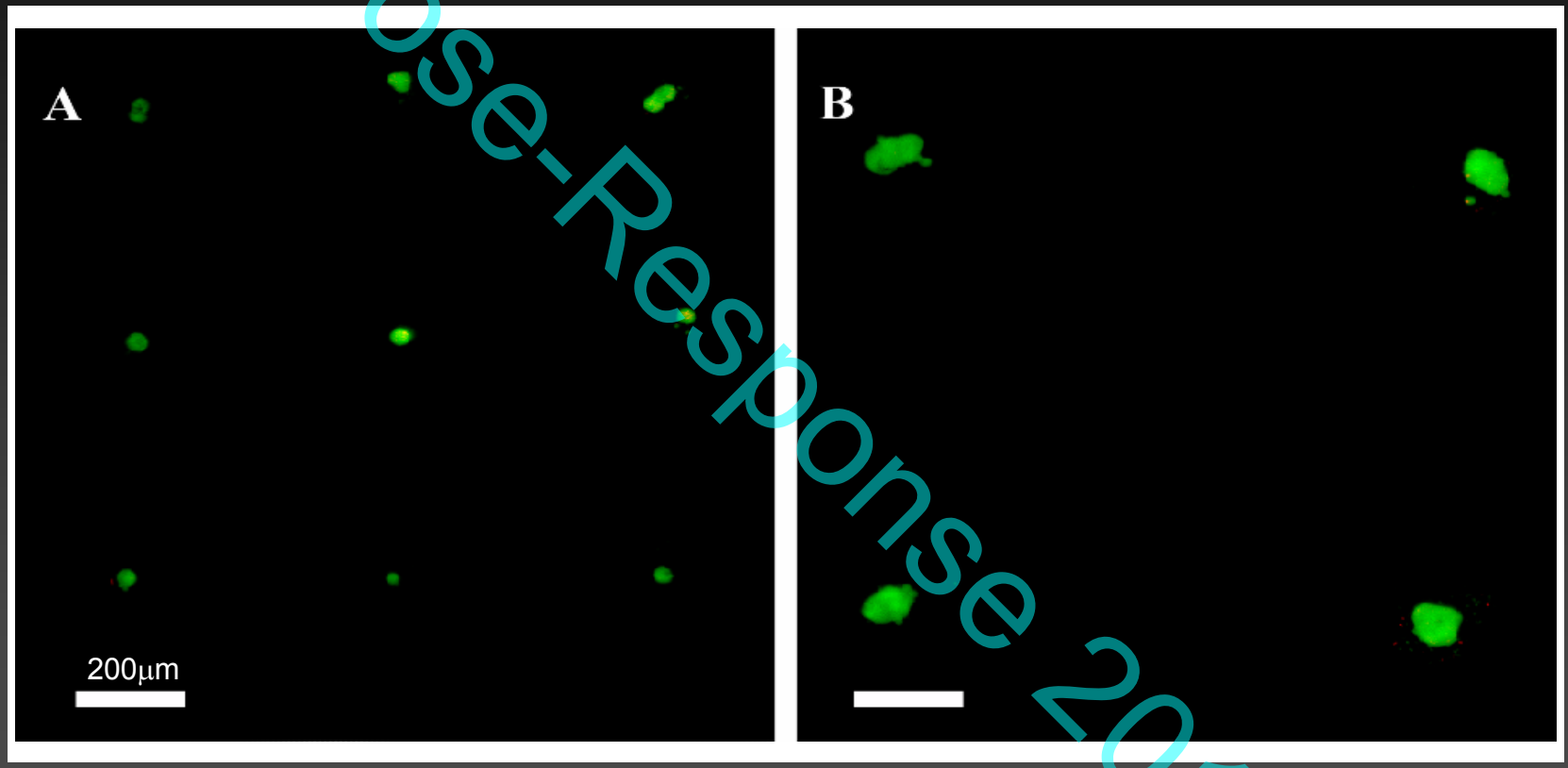
Distribution of 3D microtissue radii as a function of cell seeding number



Microtissues with as few as ~25 cells and as many as 1000 cells have been formed

Napolitano, *BioTechniques*, 2007

Microtissues are viable during extended culture

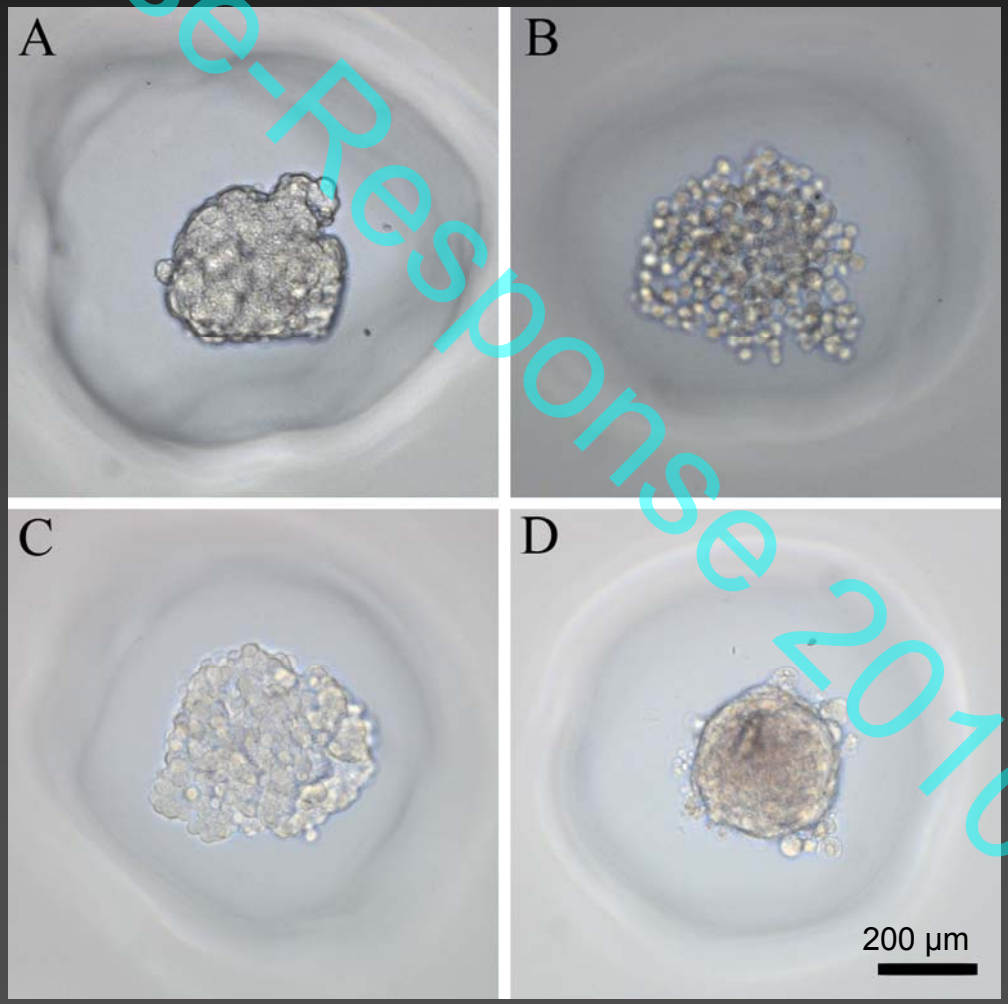


Calcein AM stain after 2 weeks in culture

Cells differ in spheroid formation

Four human breast cancer cell lines

MCF-7

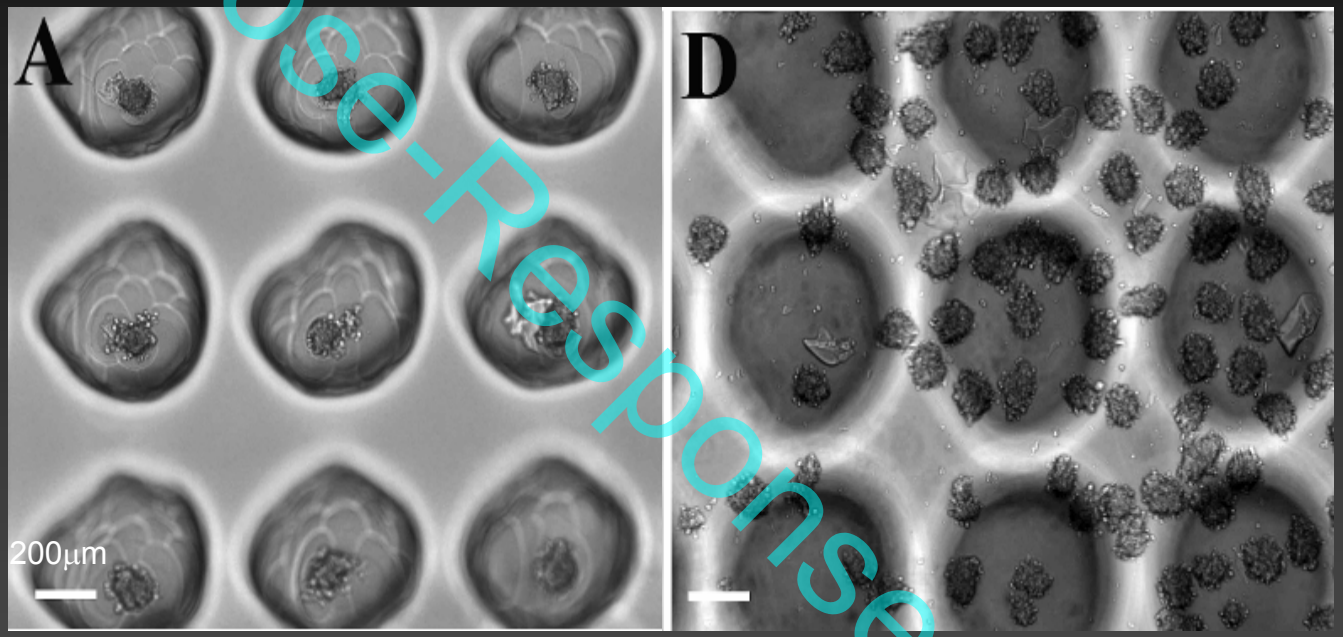


MDA-MB-231

T-47D

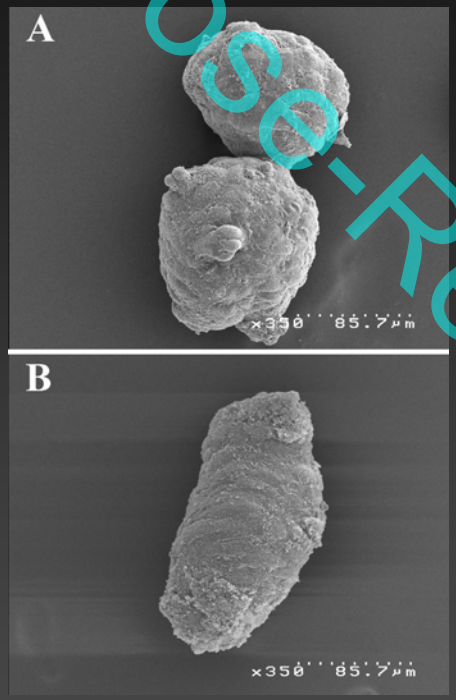
Hs-578T

Microtissues can be harvested

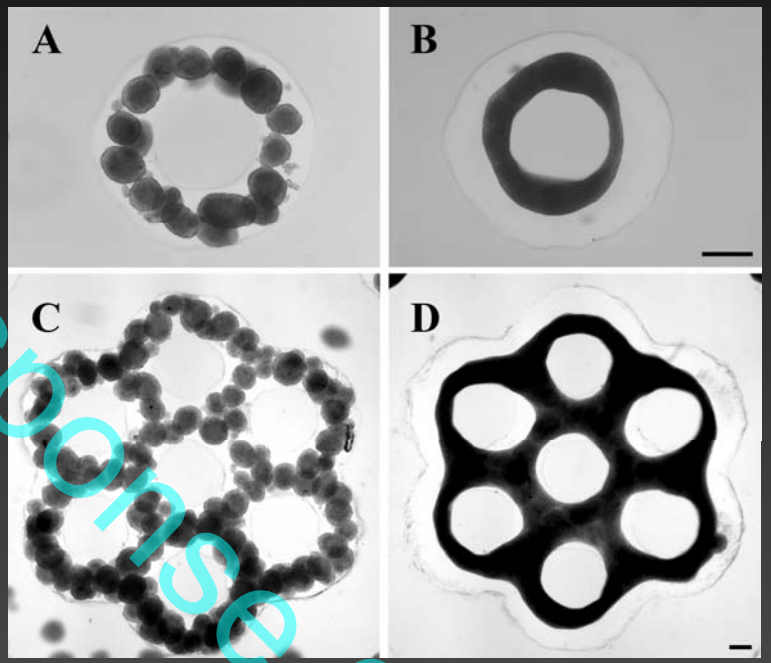


Inverted gel showing
released 3D microtissues

Microtissues can be used as building blocks



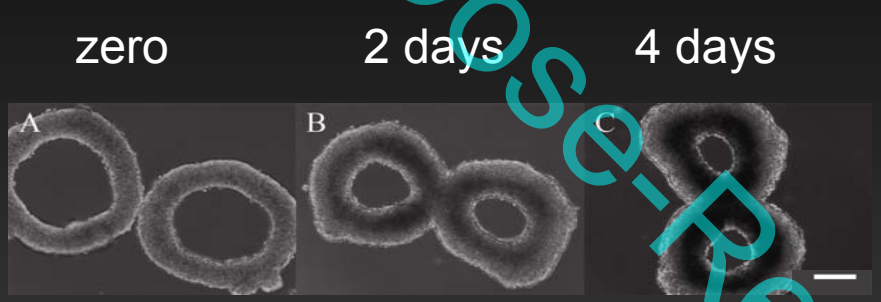
SEM of fusion

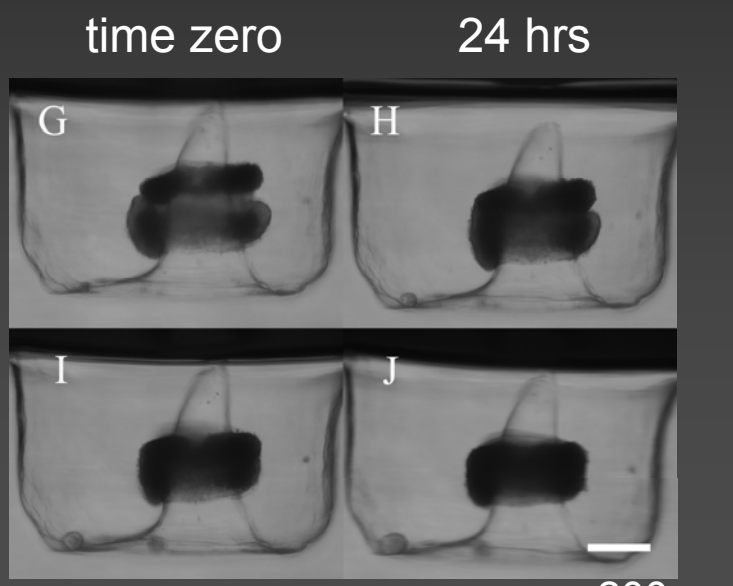


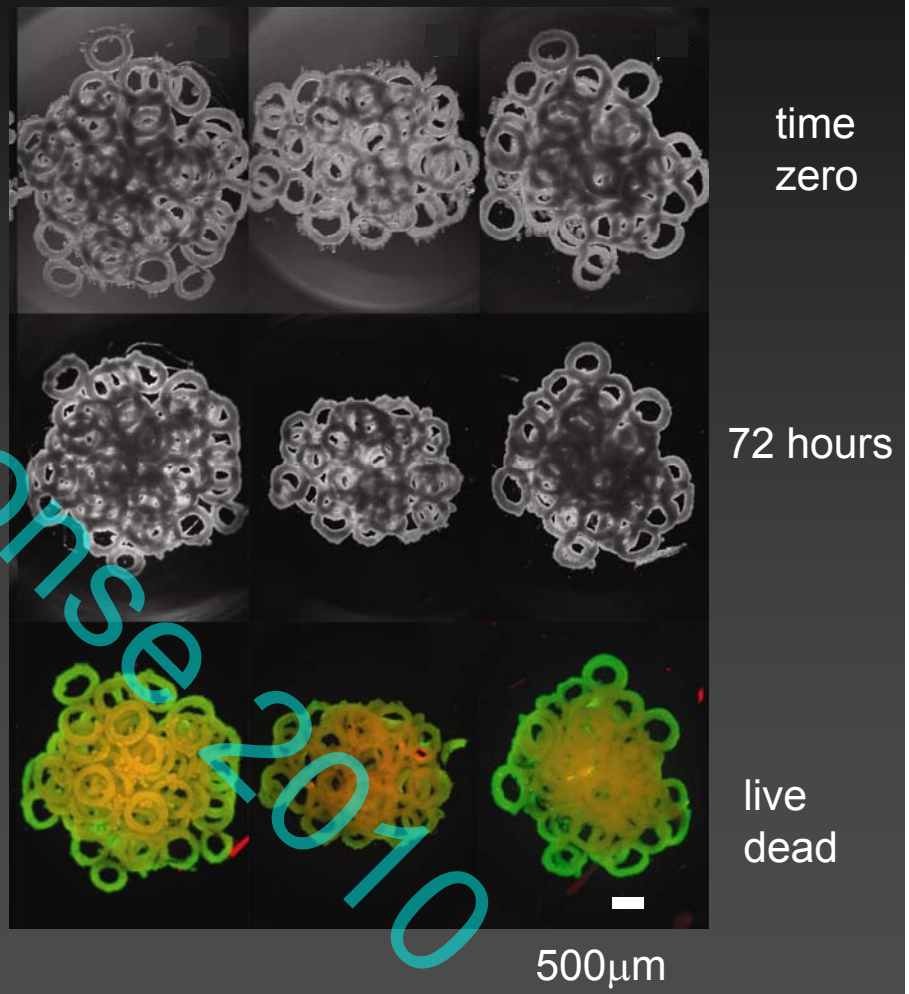
Building blocks can be used in secondary molds

Rago, *Biotech & Bioeng*, 2009

Toroid building blocks can be fused



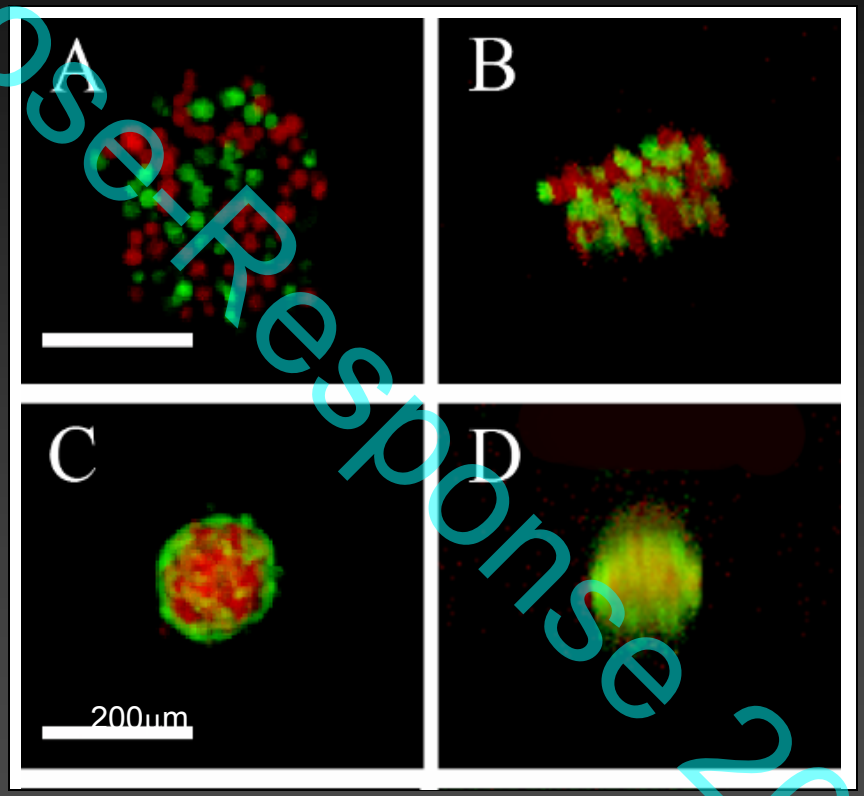




Livotti, *Tissue Eng*, 2010

Cells will self assemble & self-sort in microtissues

Time zero



NHF (red)

HUVEC (green)

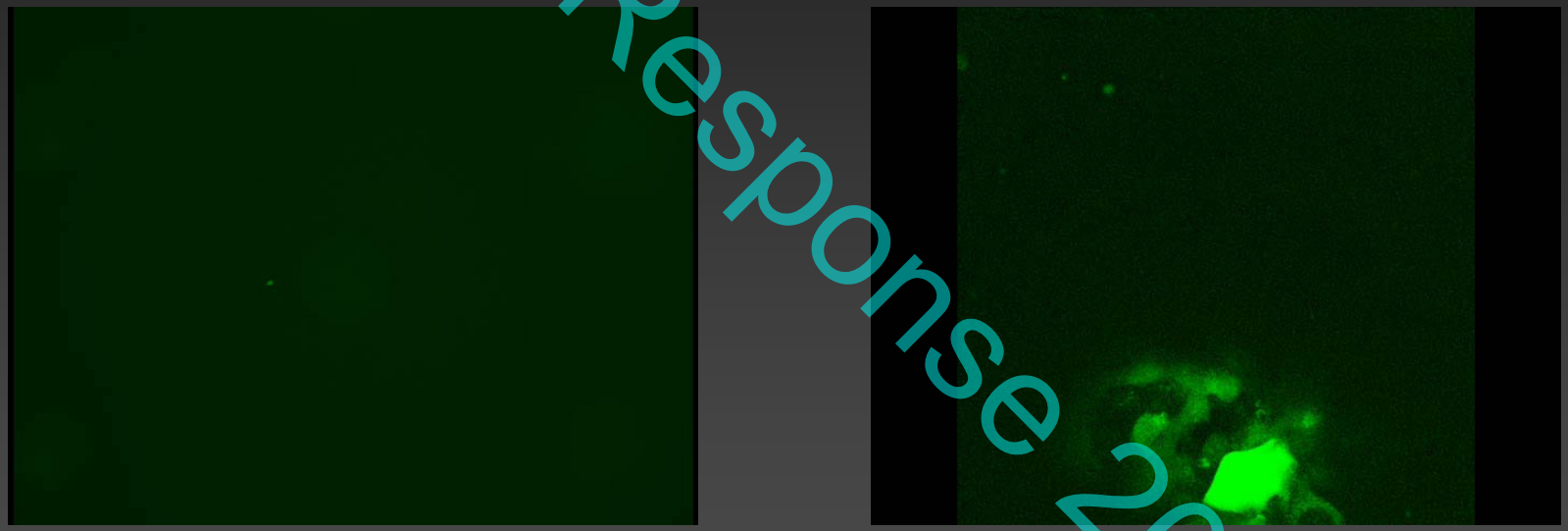
24 hrs

1:1 mix of NHFs (red) and HUVECs (green) seeded onto gel

Replicate complex tissue units such as the vascular wall

Microtissues can sense inflammation (TNF-α)

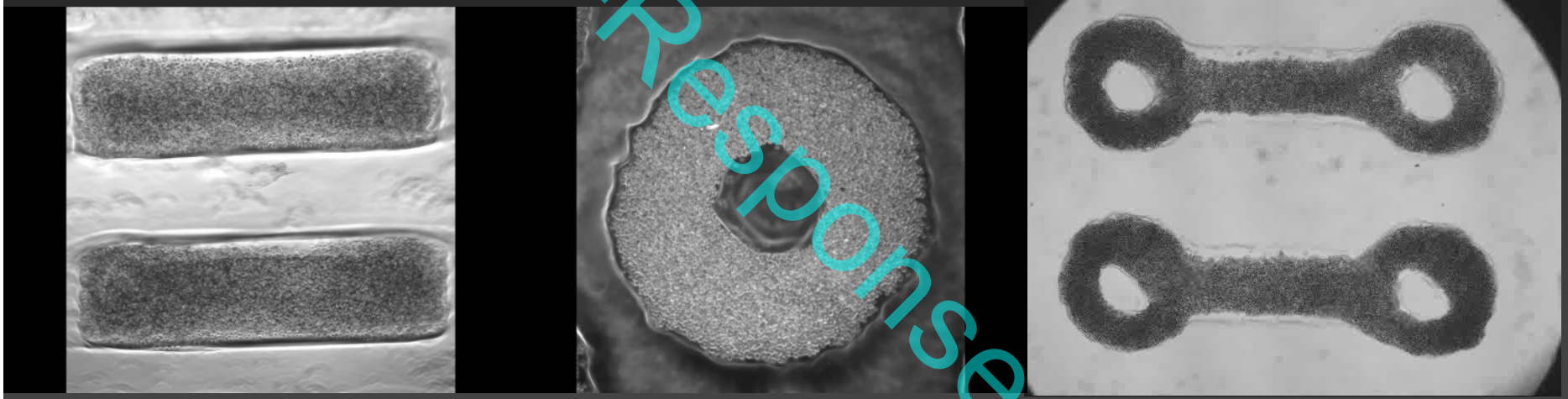
[*GGGAATTTCCGGGAATTTCCGGGAATTTCCGGGAATTTCC*]$_4$



Time course: 60 hours, single exposure to TNF-α,

200μm spheroids, 1μm confocal sections, ~50μm

Microtissues are biomechanically active



rod in trough

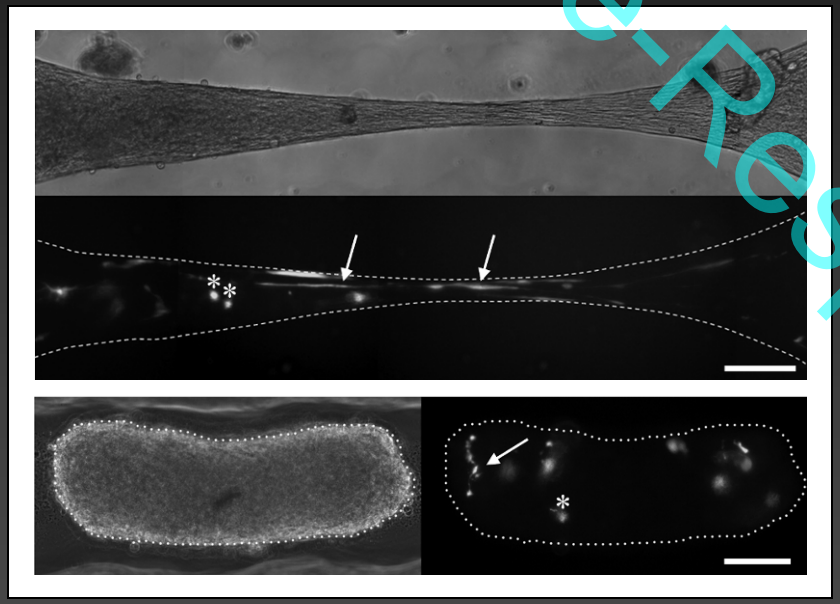
toroid in cone

constrained dogbone

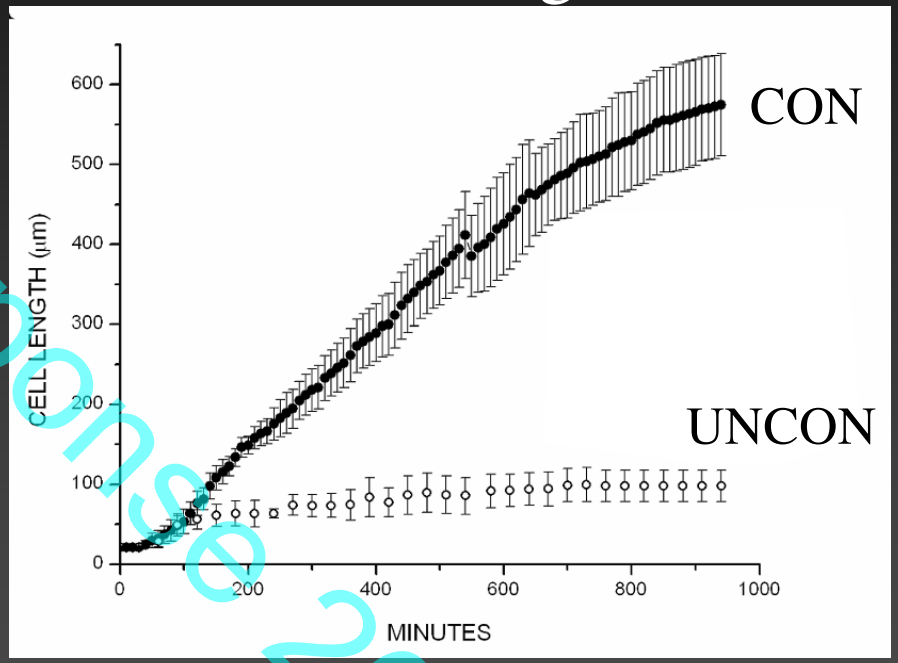
NHFs, time lapse <12 hours

Cells elongate in response to tension

GFP+ cells



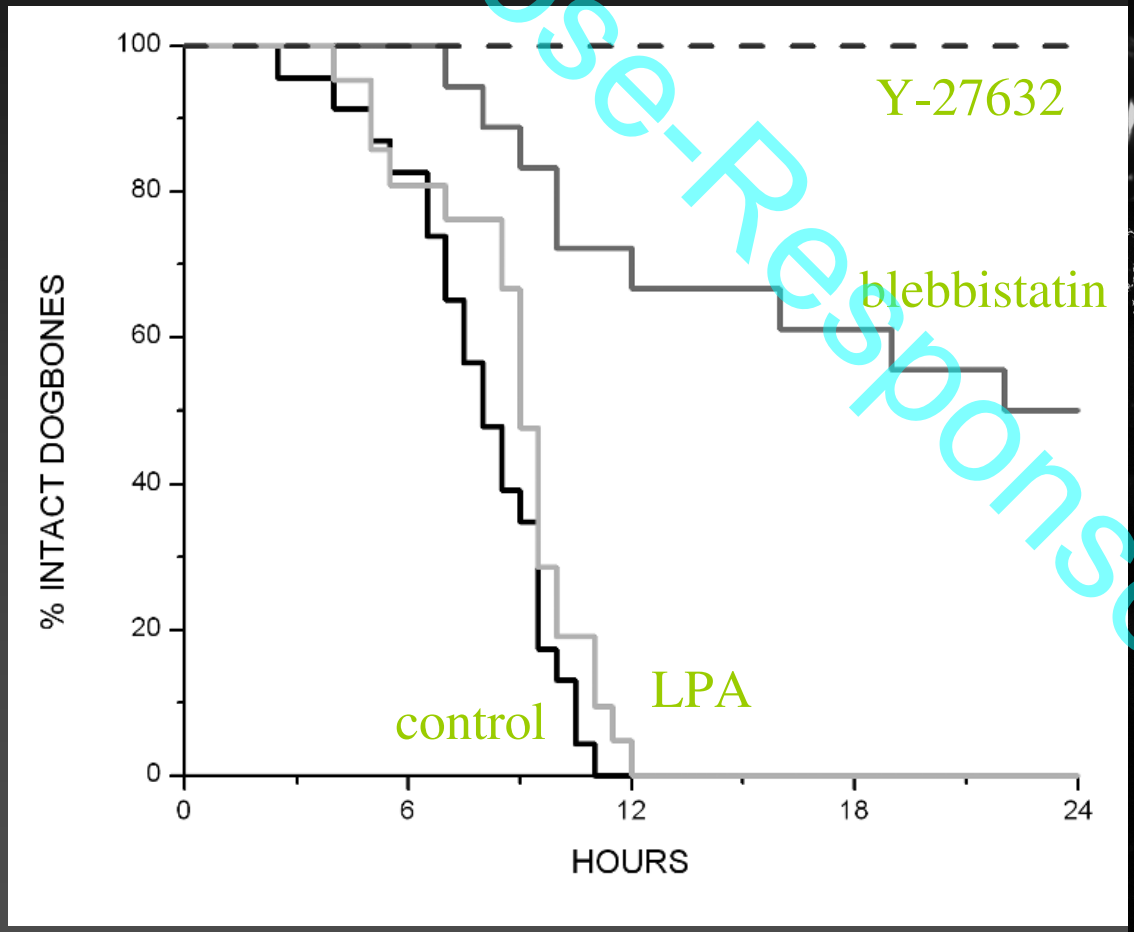
Cell elongation



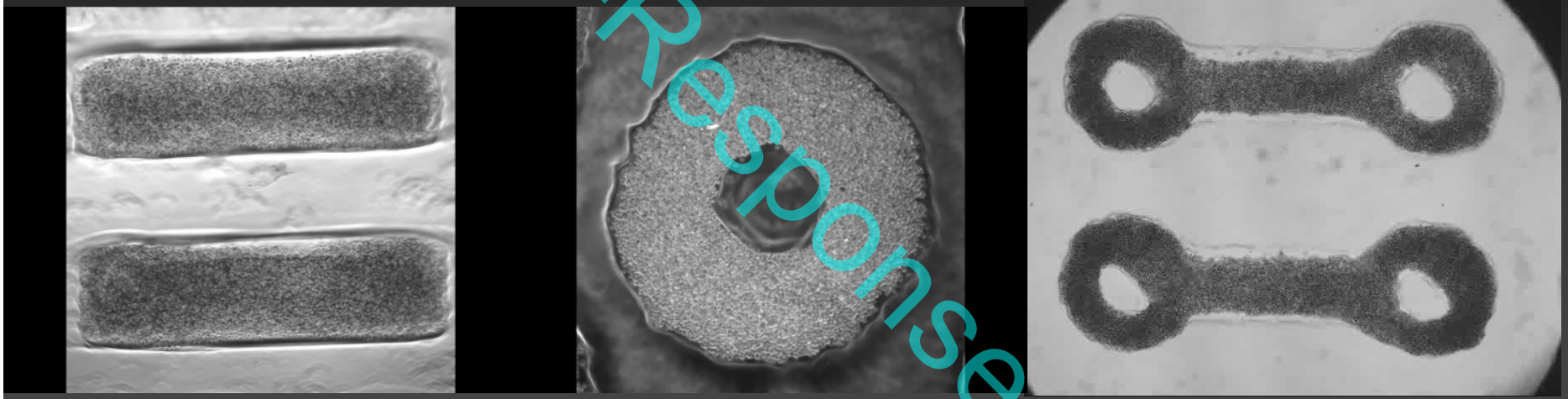
Elongation is greatest in constrained (dogbone) versus unconstrained (rod) structures

Dean, *Cell Motility & Cytoskeleton*, 2009

Drugs to block cyto-skeletal mediated tension



Microtissues are biomechanically active



rod in trough

toroid in cone

constrained dogbone

NHFs, time lapse <12 hours

Microtissues can be formed from many cell types

- Fibroblasts
  - Human dermal fibroblasts
  - Murine fibroblasts
- Cardiac cells
  - Rat cardiac myocytes
  - Rat cardiac fibroblasts
- Endothelial cells
  - Human: HUVEC
  - Calf: CPAE
- Breast cancer cell lines
  - Human: MCF-7
  - Human: T47D
  - Human: MDA-MB-231 cells
  - Human: Hs-578T
- Other epithelial cells
  - HeLa,
  - A431
- Liver cell lines
  - Rat: H35
  - Human: HepG2
- Mesothelioma
  - Human: M28
  - Human: REN
- Reproductive system
  - Human: Granulosa cells
  - Human: TCL trophoblast cells
  - Human: Theca cells
- Neuronal cell lines
  - Rat: RG2, neuroblastoma
  - Rat: 9L, glioma
  - Rat: A7; astrocytes
- Musculoskeletal cell lines
  - Human: C28/I2, chondrocytes
  - Human: CRL 11372 osteoblasts

Some attributes of the technology and why it might be useful

- Rapid production of large numbers of uniform sized microtissues
- Control of microtissue size
- Control of microtissue shape
- Microtissues made of human cells
- Microtissues that replicate complex tissue units,
  - heterotypic cell interactions
- Microtissues that sense drugs and biological response modifiers

Acknowledgements

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