Morphogenesis as a problem of fluid mechanics:

understanding, modeling & exploring tissue fluidity in organ printing

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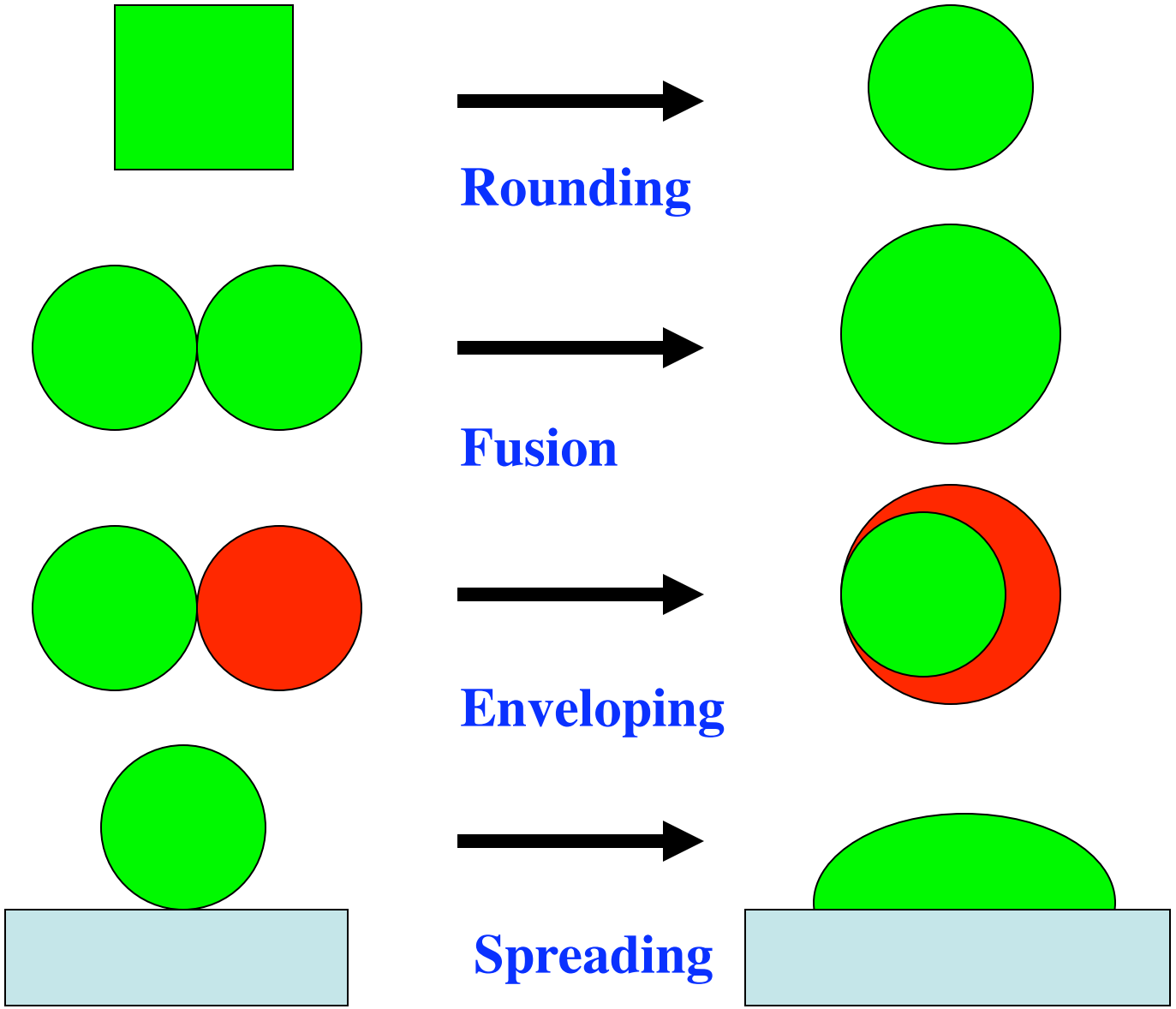
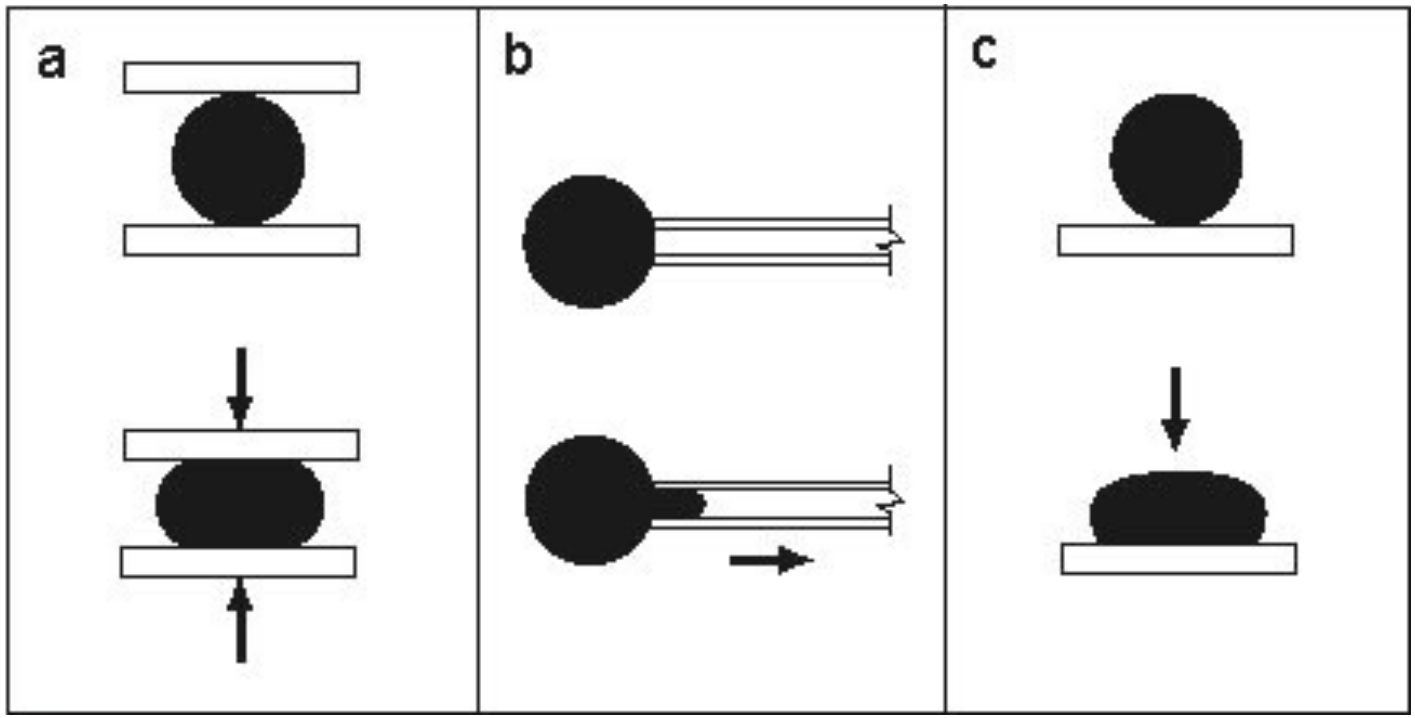
Evidence of Tissue Fluidity

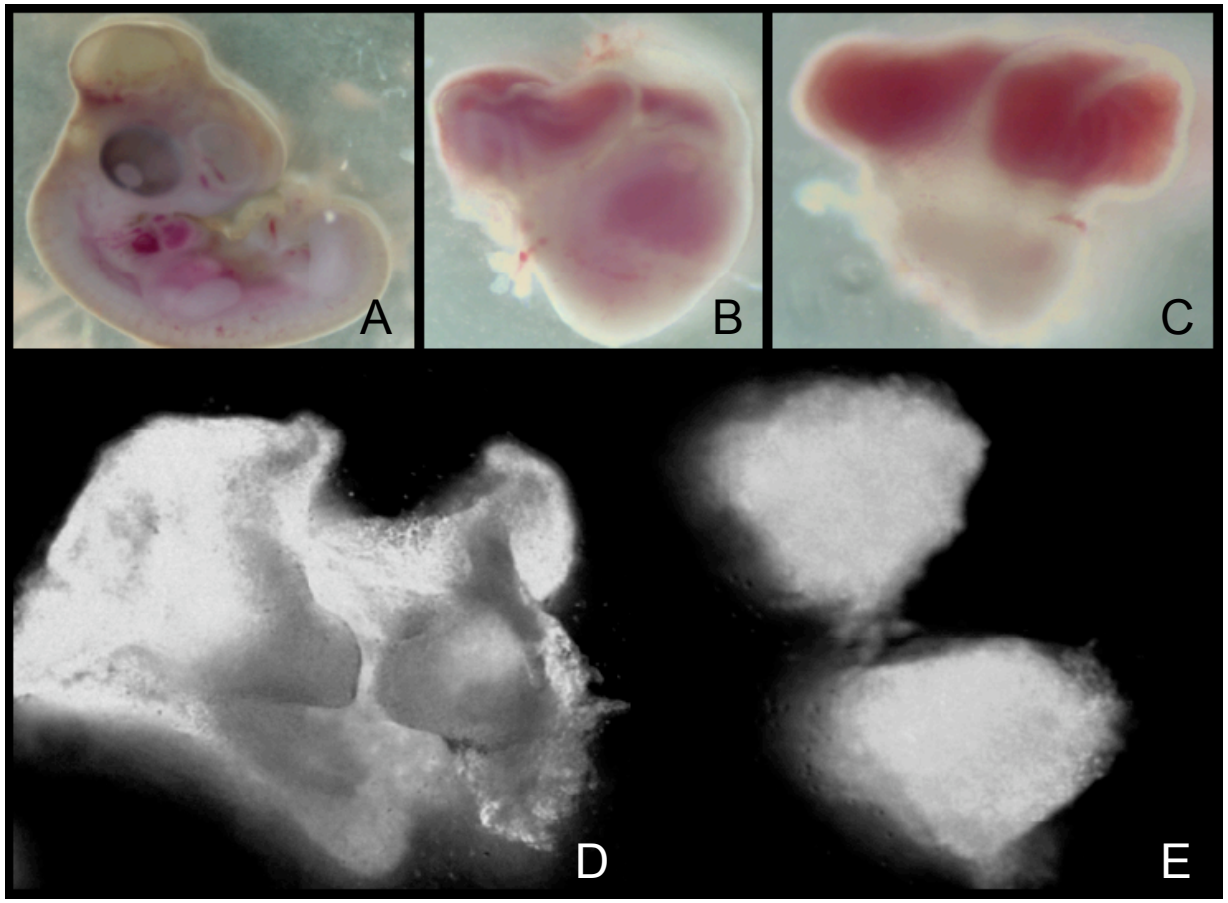


Methods of quantitative evaluation the material properties of tissue spheroids



Tensiometry Aspiration Centrifugation

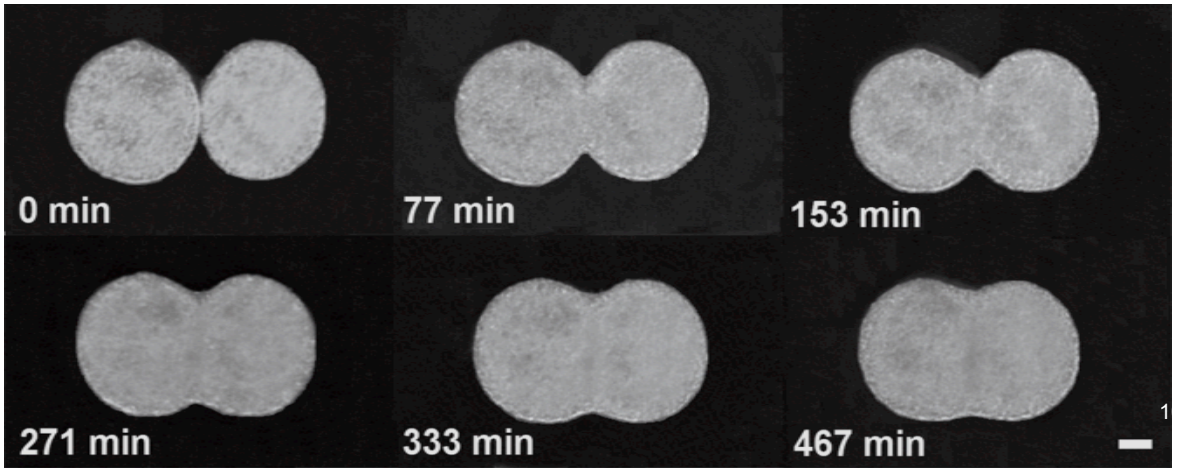
Avian Embryonic Heart Dissection and Cushion Tissue Isolation



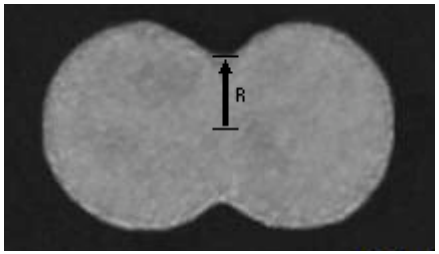
Embryo dissection: **A.** HH27 chick embryo, **B.** isolated heart, **C.** section of AV myocardium containing cushion tissues, **D.** view of unfused AV cushions within the ruptured AV myocardium, **E.** cushion tissue explants.

Cushion Tissue Fusion *In Vitro*

Spherical explants of HH27 cushion tissue were closely placed in a droplet of DMEM, **the time evolution of the fusion process was photographed**. The fusion length parameter was graphed as a function of time and fit to a linear equation describing a standard model of liquidity [Frenkel 1945].



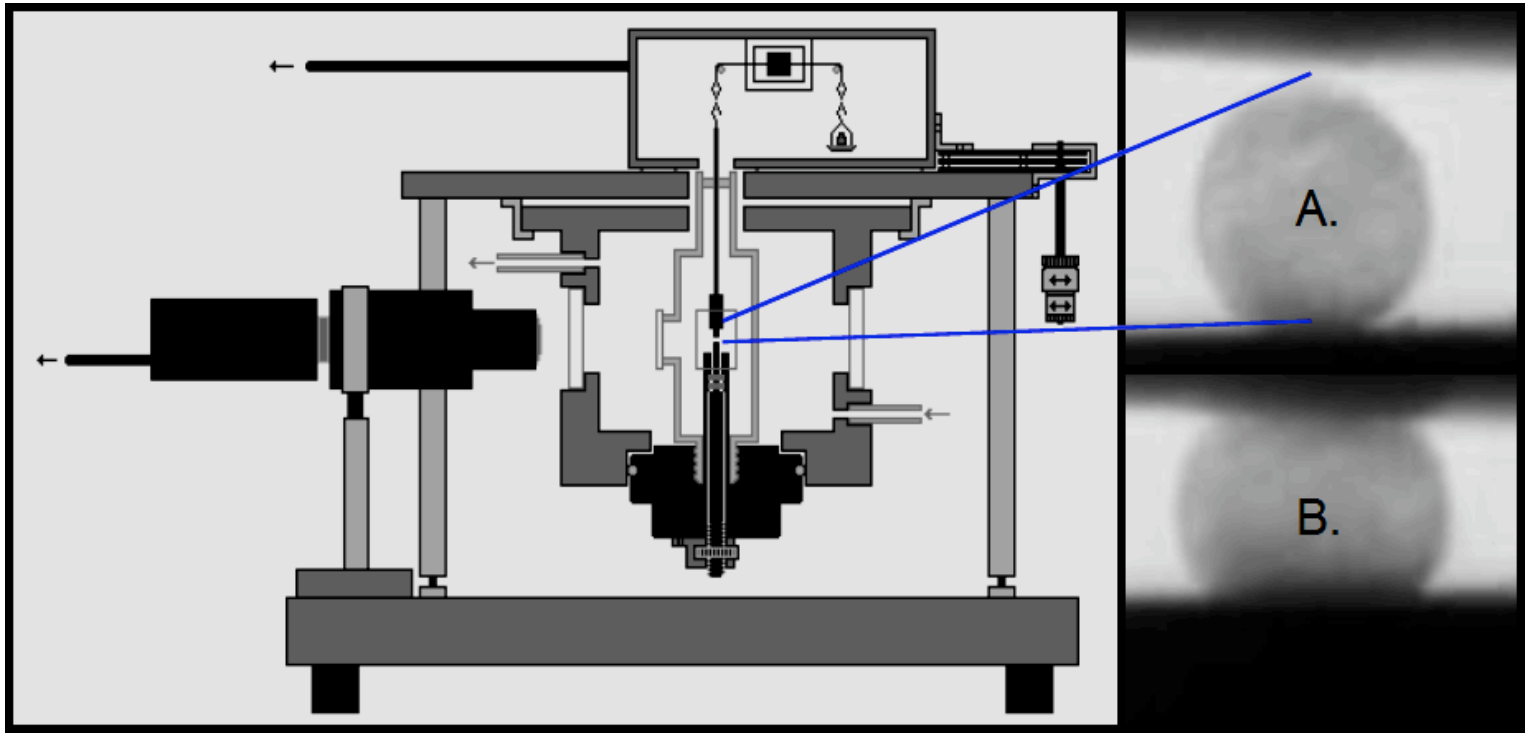
The time evolution of the fusion of two opposing aggregates of cushion tissue suspended within a hanging drop of DMEM, allows the calculation of the ratio of the tissues viscosity and surface tension.



Two fusing aggregates of cushion tissue with the radius of the surface area of contact R connecting the two fusing aggregates illustrated. The length parameter L may be calculated from the radius R using the equation: L = (2/3)p(R2/Ro) [Frenkel 1945], where Ro is the initial radius of the two identical spherical aggregates.

Tensiometry Measurements Yield Tissue Surface Tension and Viscoelastic Properties

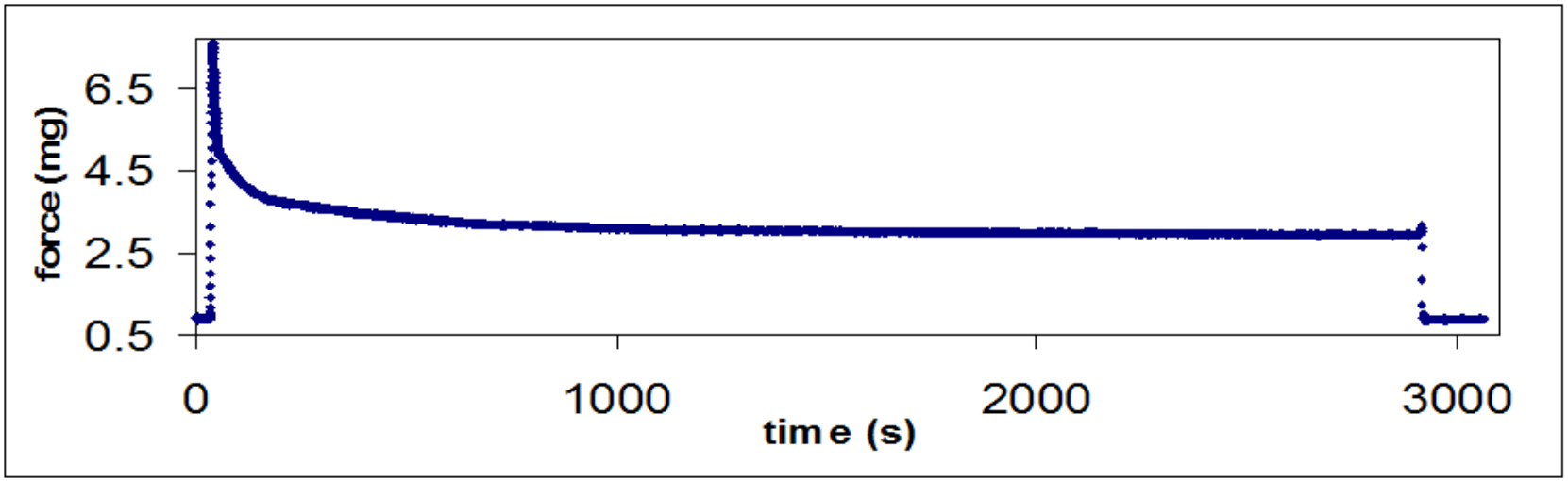
A Cahn 2000 microbalance has been modified to allow for the measurement of the response function of a spherical explant of living tissue due to an applied compressive load (mg), using this apparatus, **the geometric properties and constant force response function** of the tissue are recorded.



Schematic of Tensiometer: **A.** Uncompressed tissue explant, **B.** Compressed tissue explant.

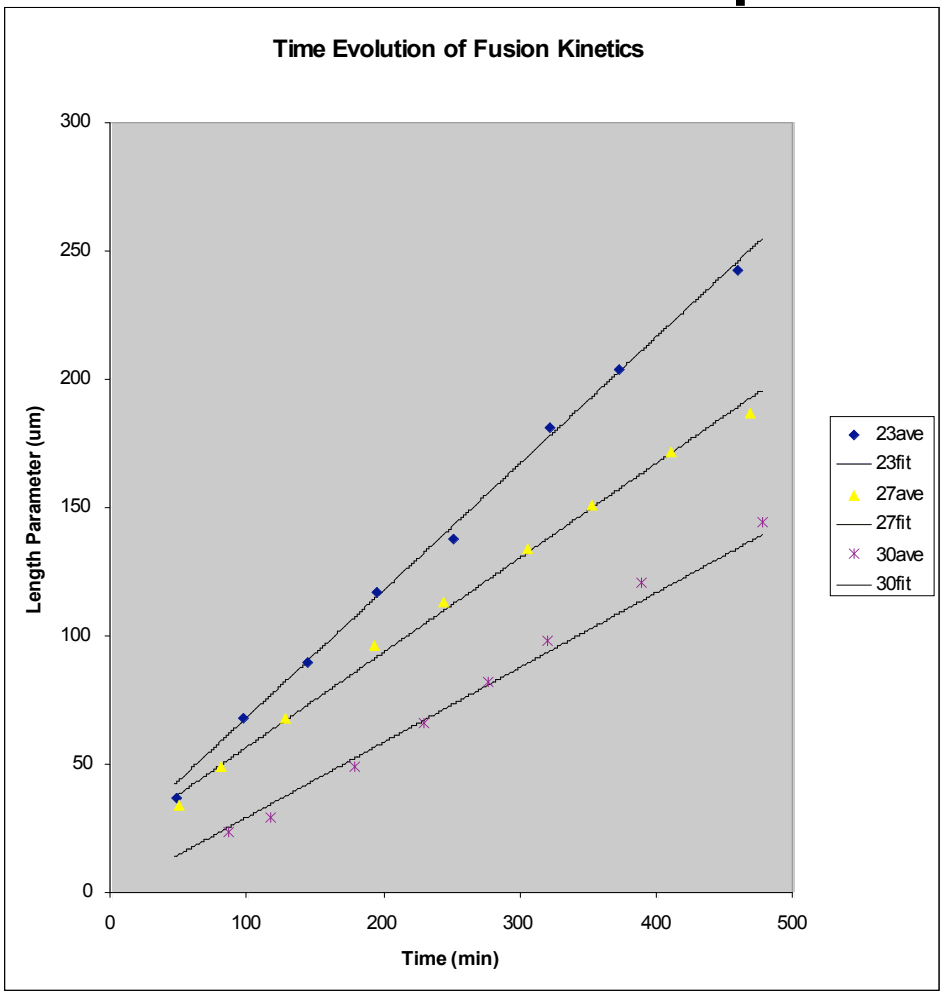
The Constant Force Response Function

The biomechanical physical properties of the tissue explants were measured by **compressing the spheres of tissue** (350mm in diameter) in the compression apparatus **and measuring the ensuing force relaxation.**



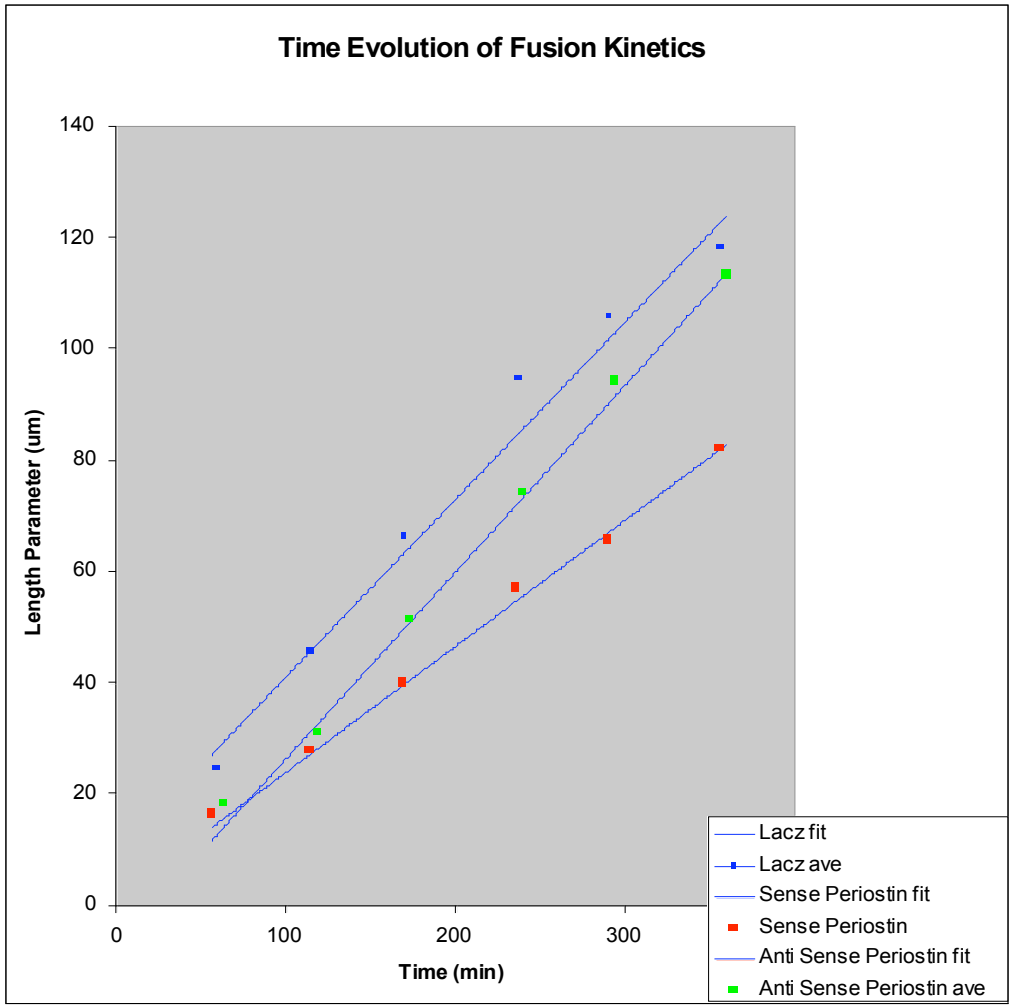
Force response function, **tissue surface tension is determined from the equilibrium plateau** using Laplace's equation [Fung 1993], additionally the viscoelastic characteristics may be evaluated from the relaxing portion of the above curve using standard models of viscoelasticity, i.e. Kelvin model [Forgacs 1998].

Tissue Fusion Kinetics (viscoelastic properties) of Explants from Different Developmental Stages



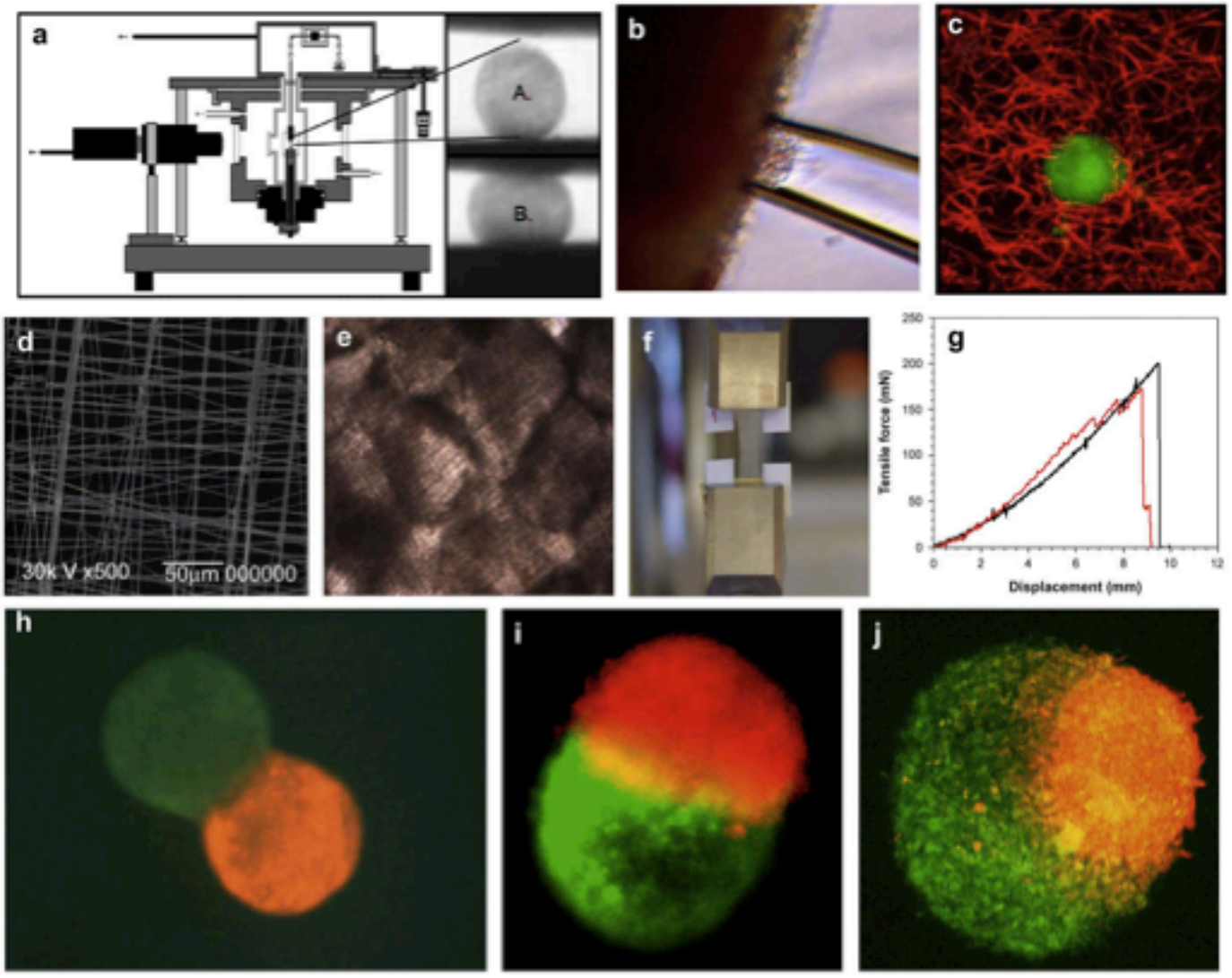
A graph of the **fusion kinetics for HH stages 23, 27, and 30 AV cushion tissue explants** averaged over 5 measurements and fit to a line, the **values for the slopes represent the ratio of the surface tension and the viscosity σ/μ (μm/min).**

Tissue Fusion Kinetics of Periostin Transfected AV Explants

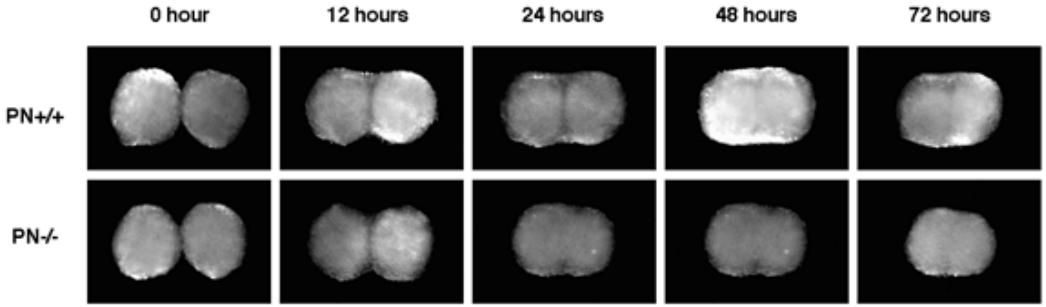


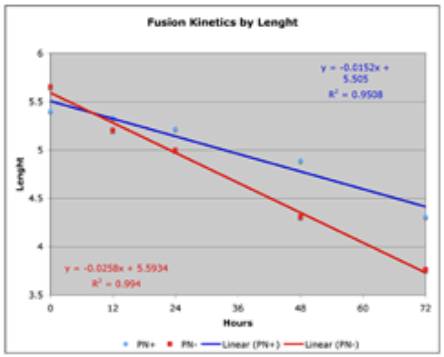
A graph of the fusion kinetics averaged over 5 measurements for **HH 27 AV cushion tissue explants** that has been infected with adenovirus containing **Lacz, sense Periostin, and antisense Periostin DNA**.

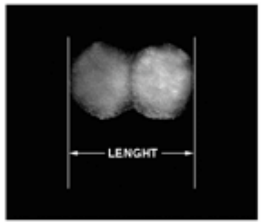
Evaluation of material properties of tissue spheroids

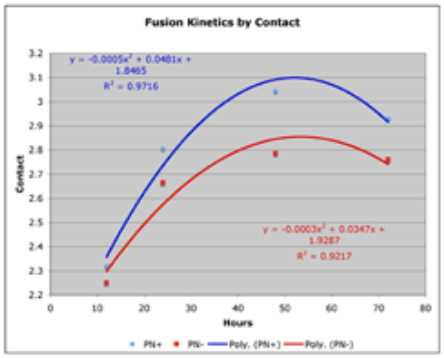


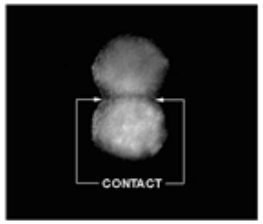
Fusability of PN+/+ and PN-/- Fibroblast Spheroids



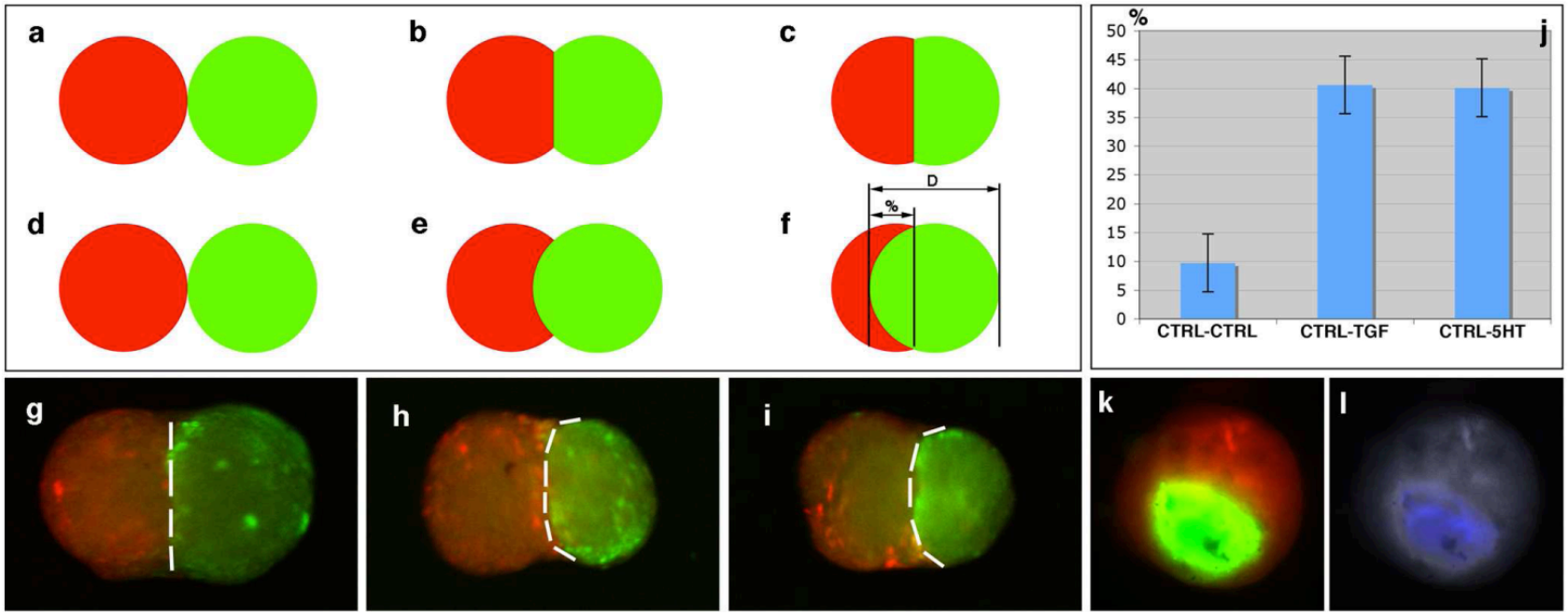


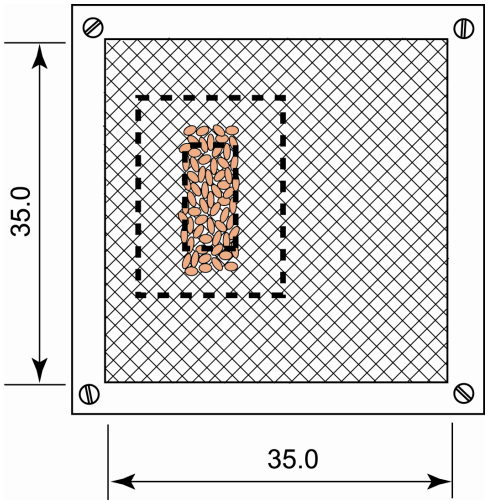


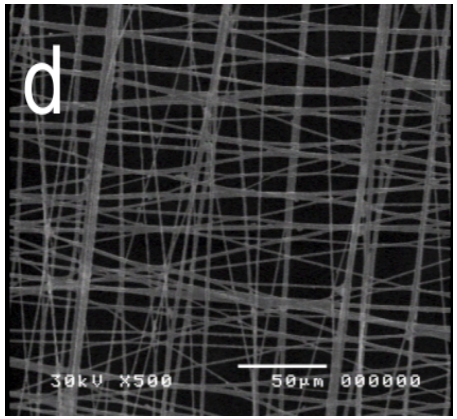


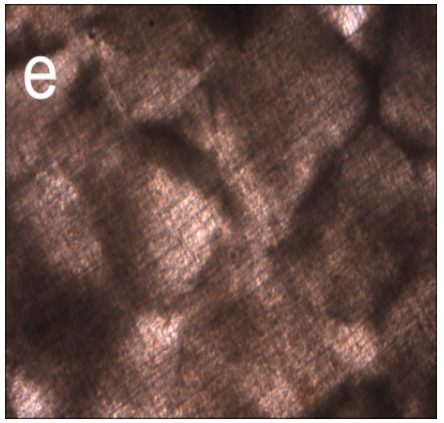


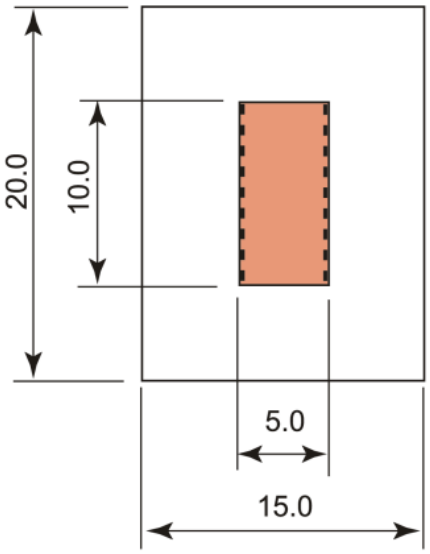
Tissue Spheroids Enveloping Assay

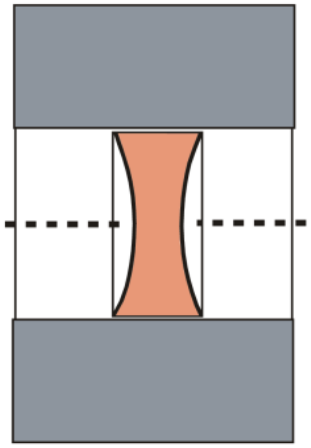


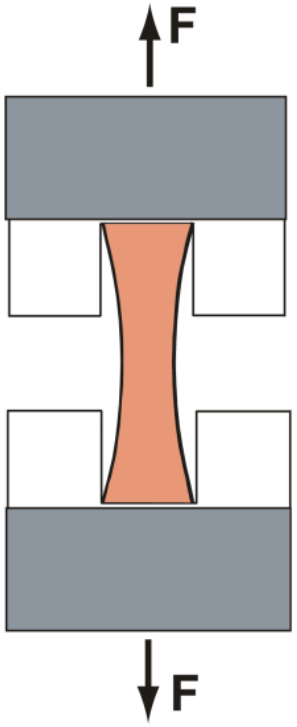


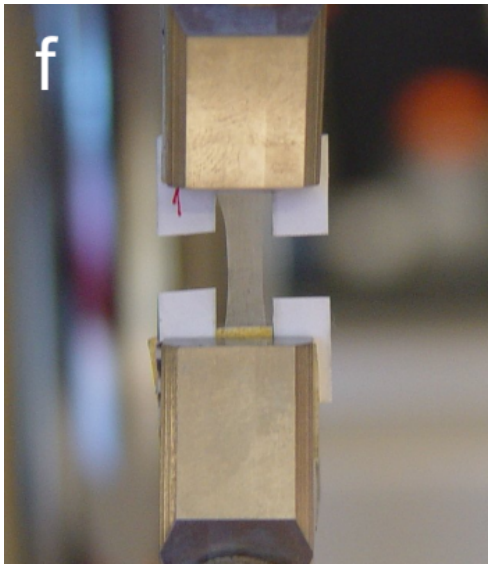












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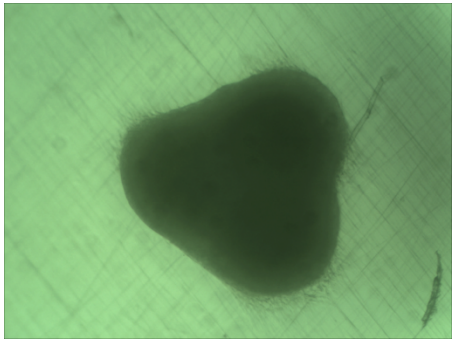
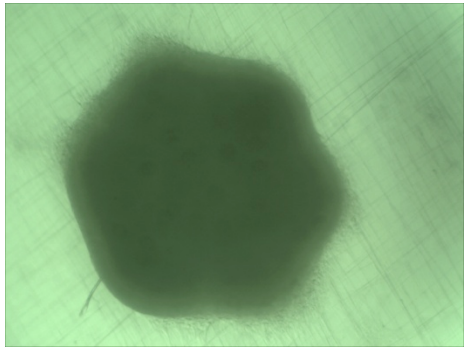
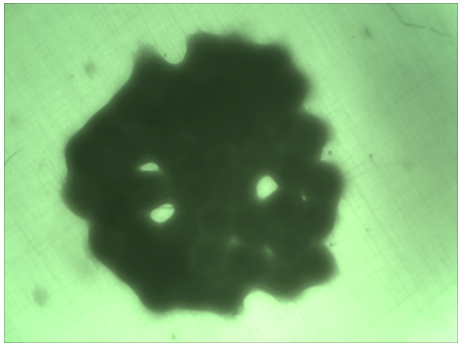
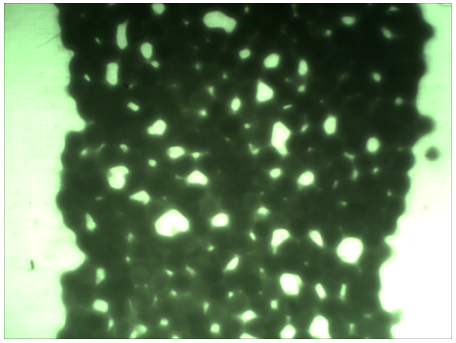
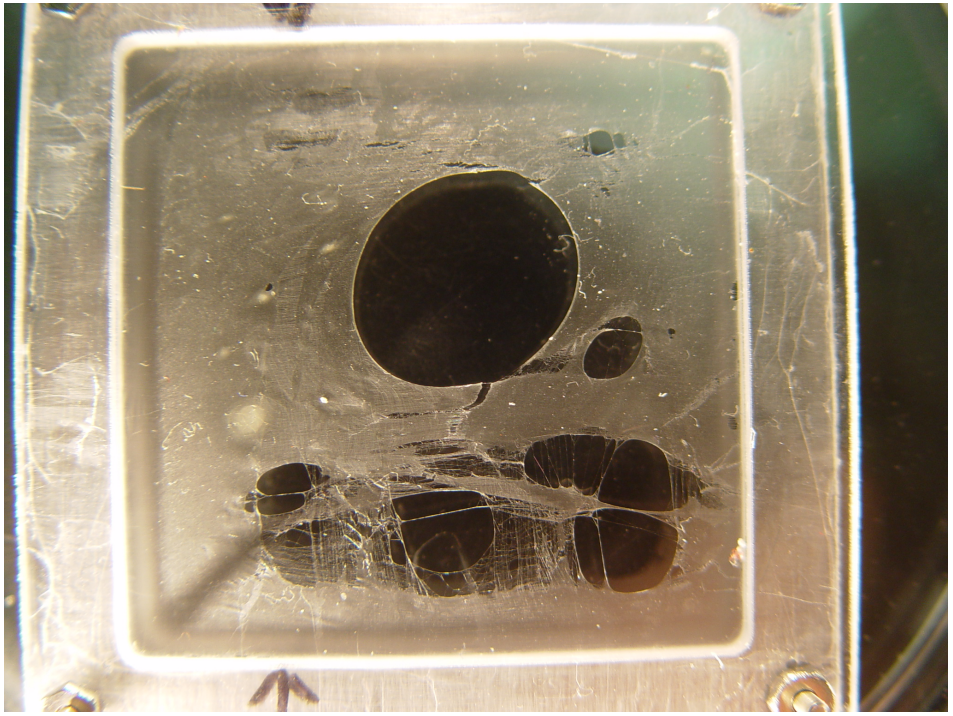
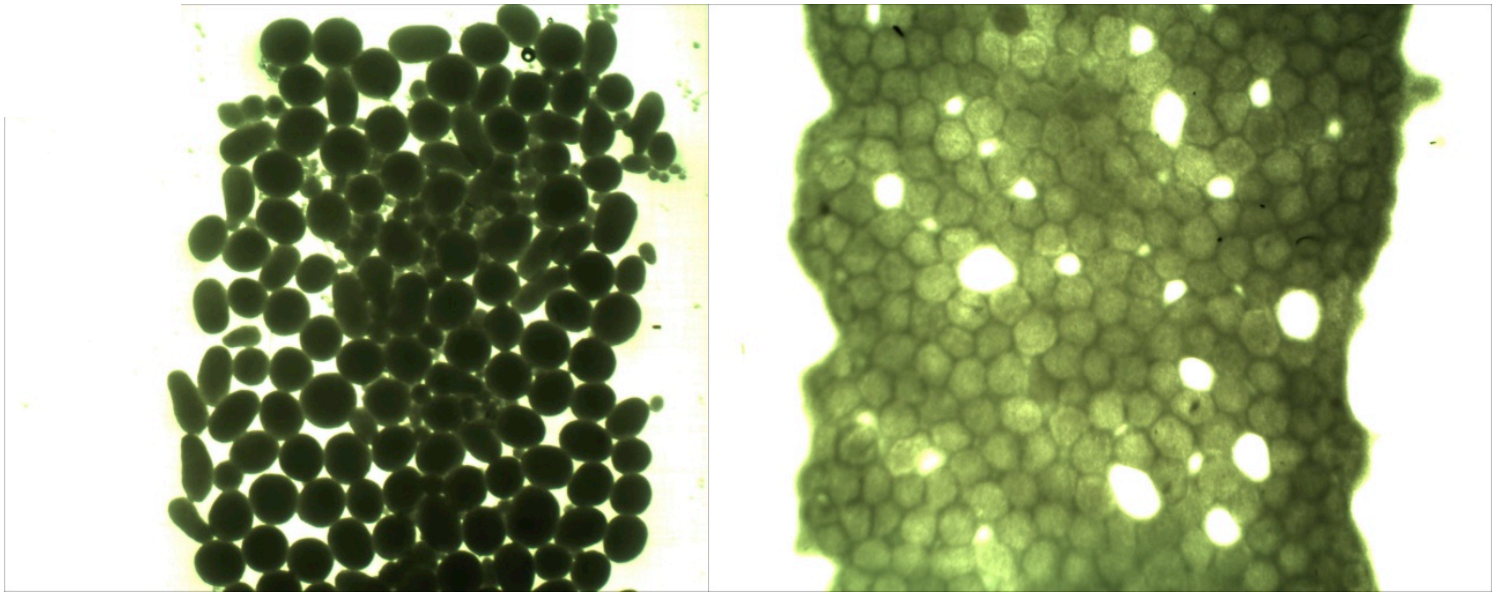
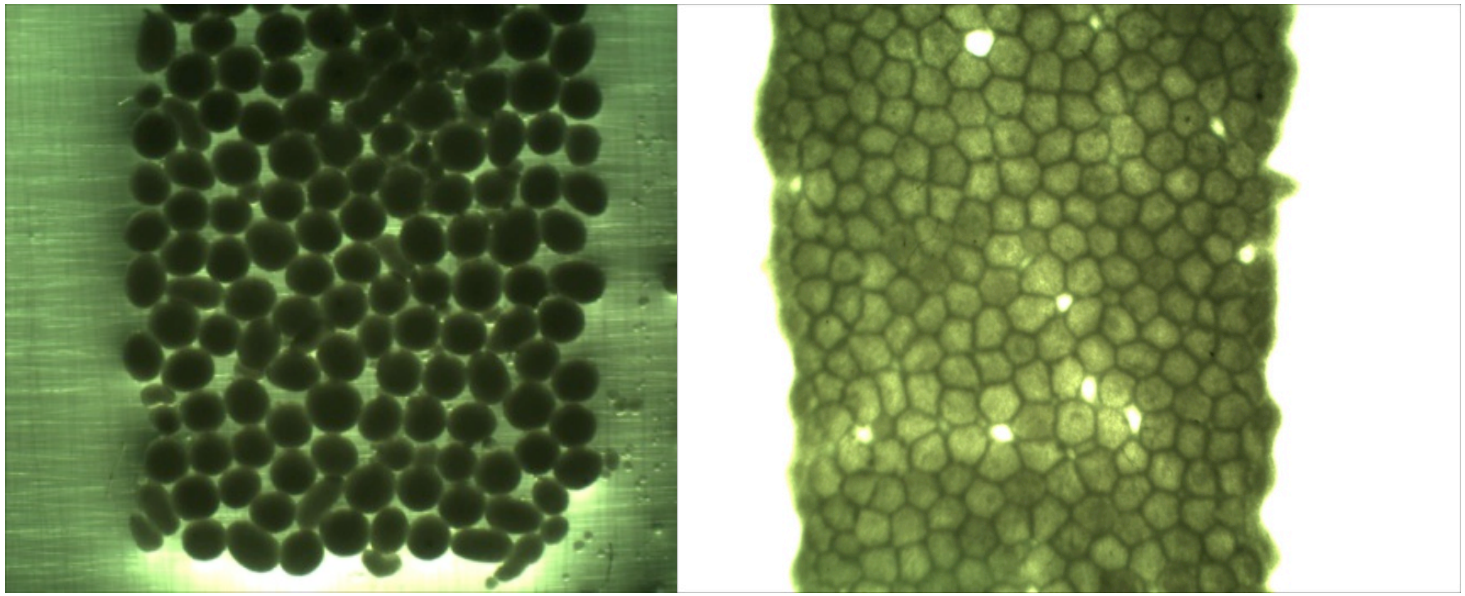
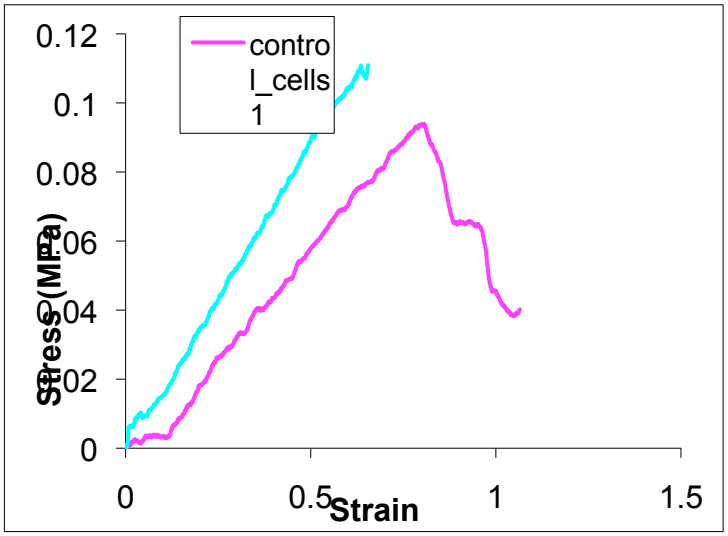
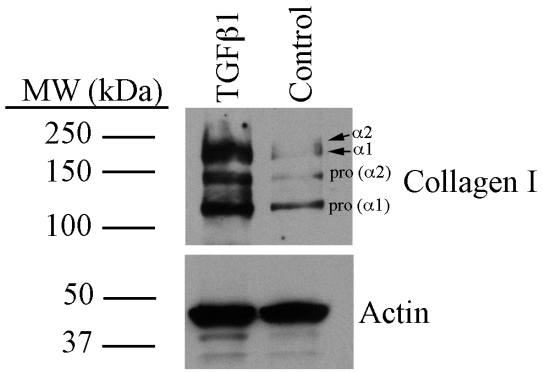






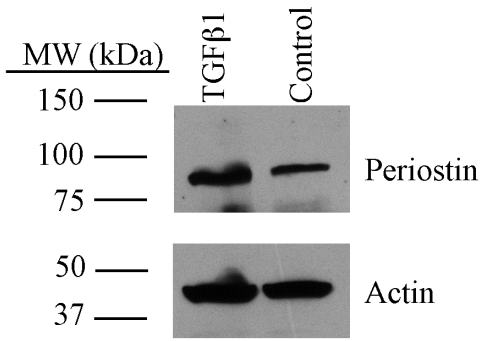




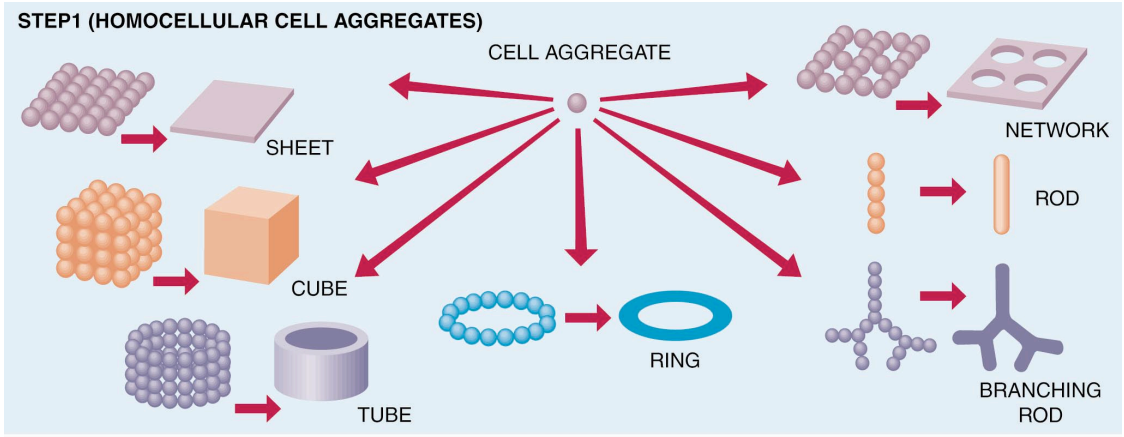
Periostin as a maturogen



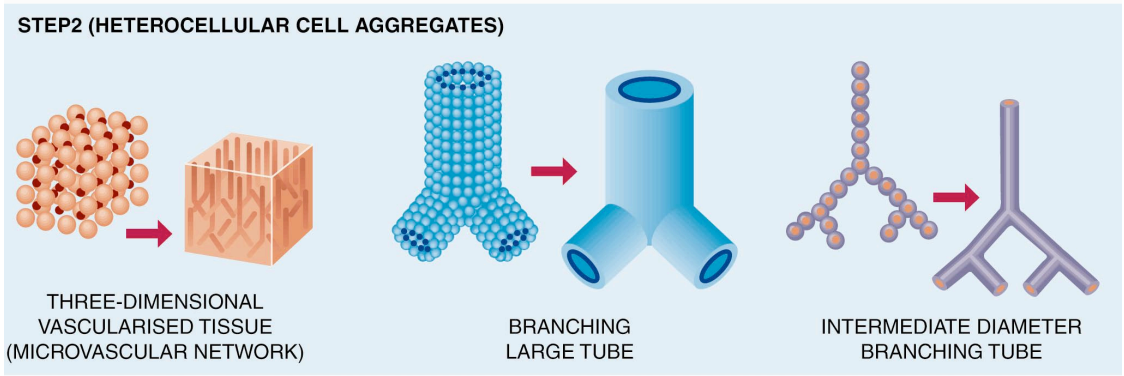




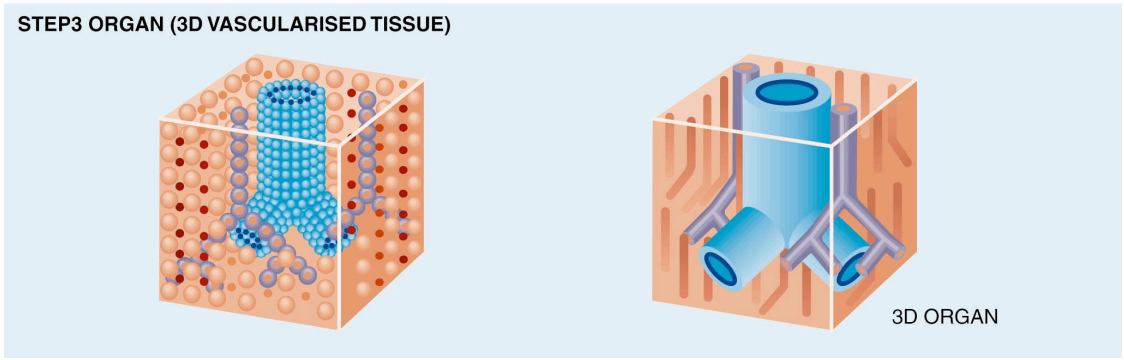
Road Map & Timeline for Organ Printing



2003

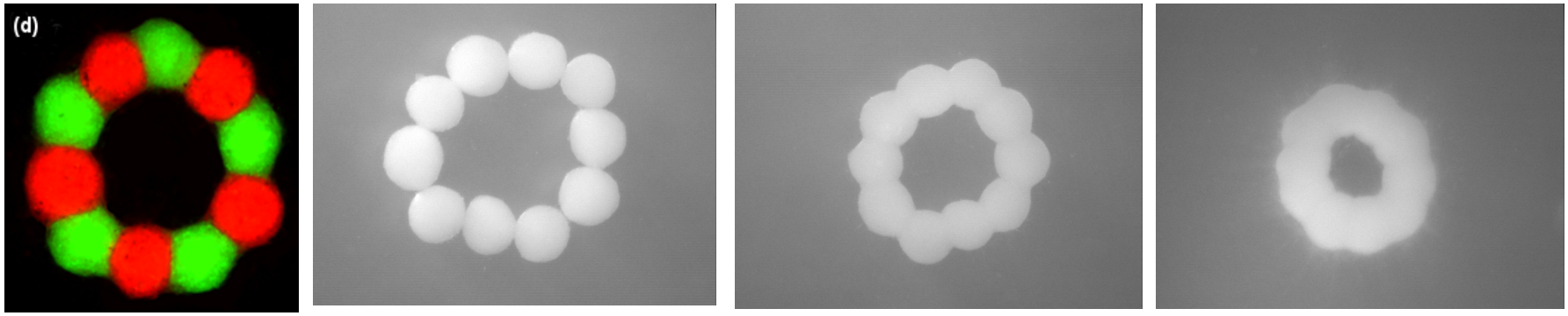


2009

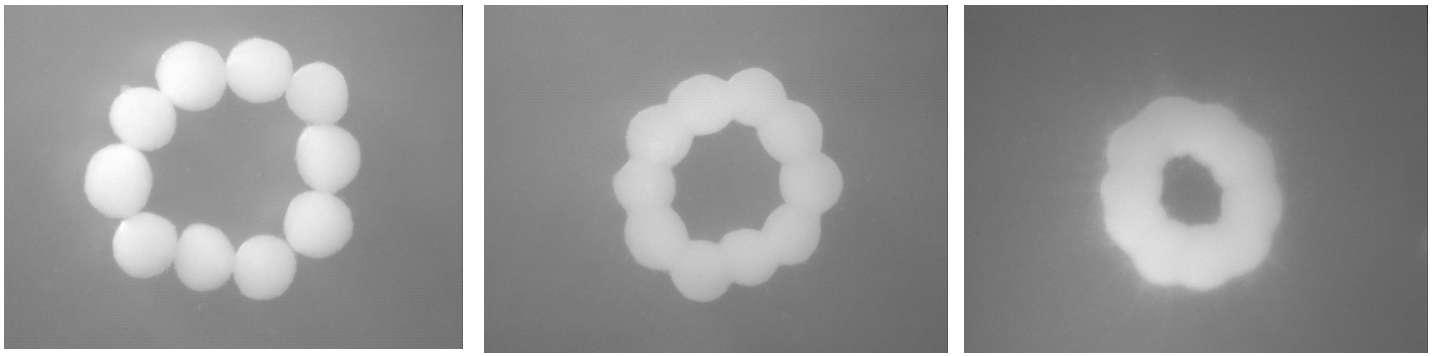


2020?

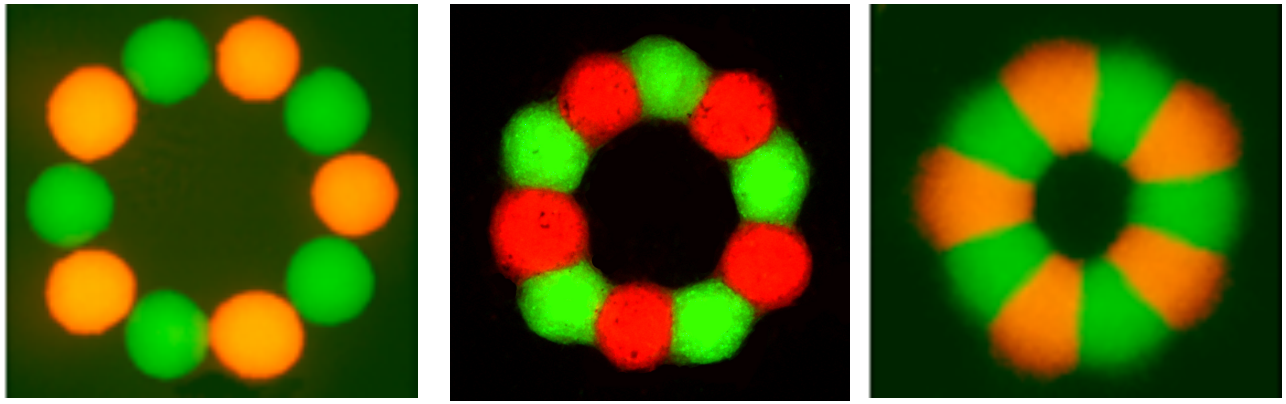
Bioprinting of Vascular Tree



Virtual and Physical Prototyping, 2009



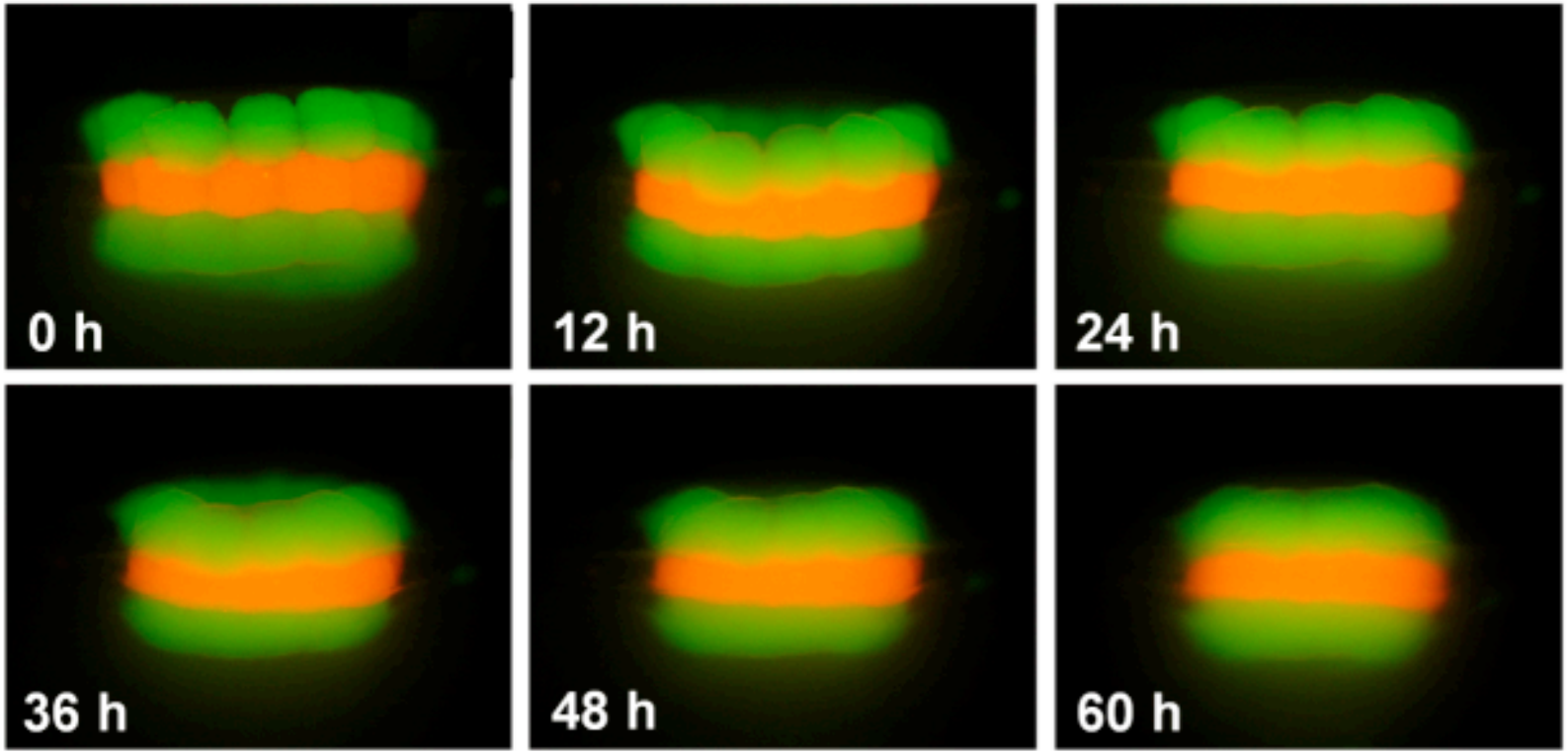
Tissue compaction during fusion of vascular tissue spheroids



Tissue fusion without cell mixing

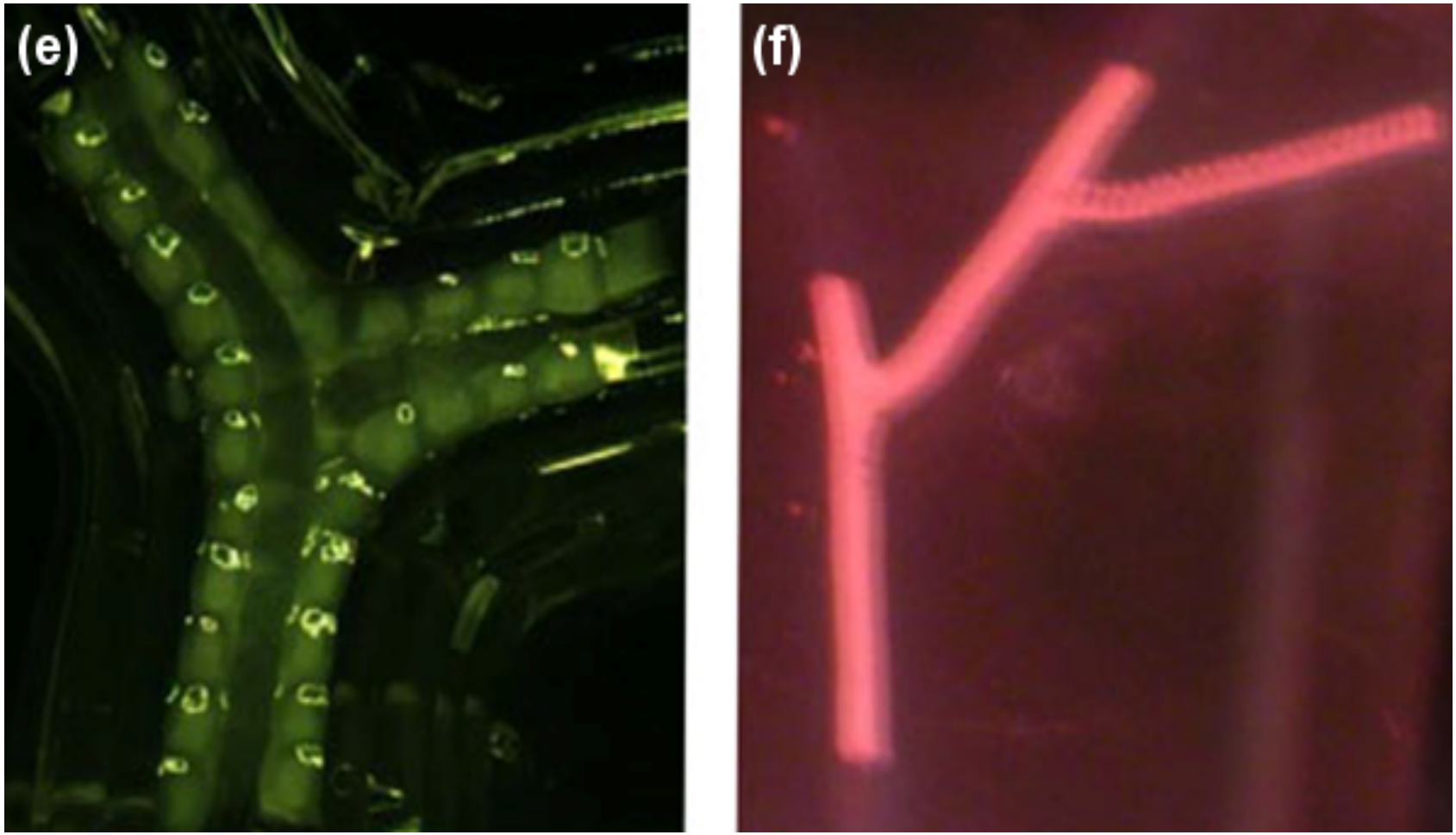
Virtual and Physical Prototyping, 2009

Bioengineering Vascular Tube Using Self-assembling Tissue Spheriods

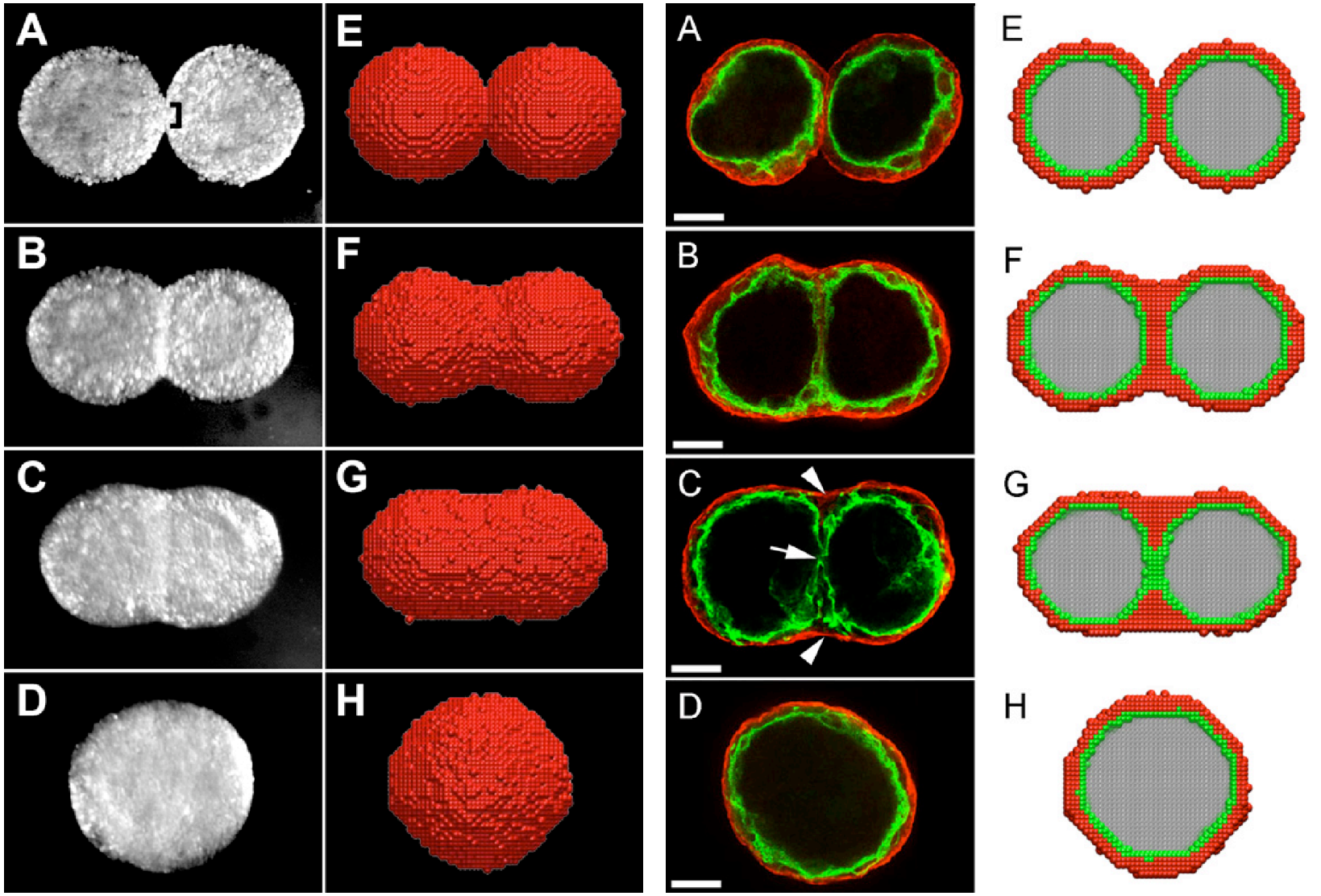


Tissue Engineering, 2008

Bioprinting of Vascular Tree



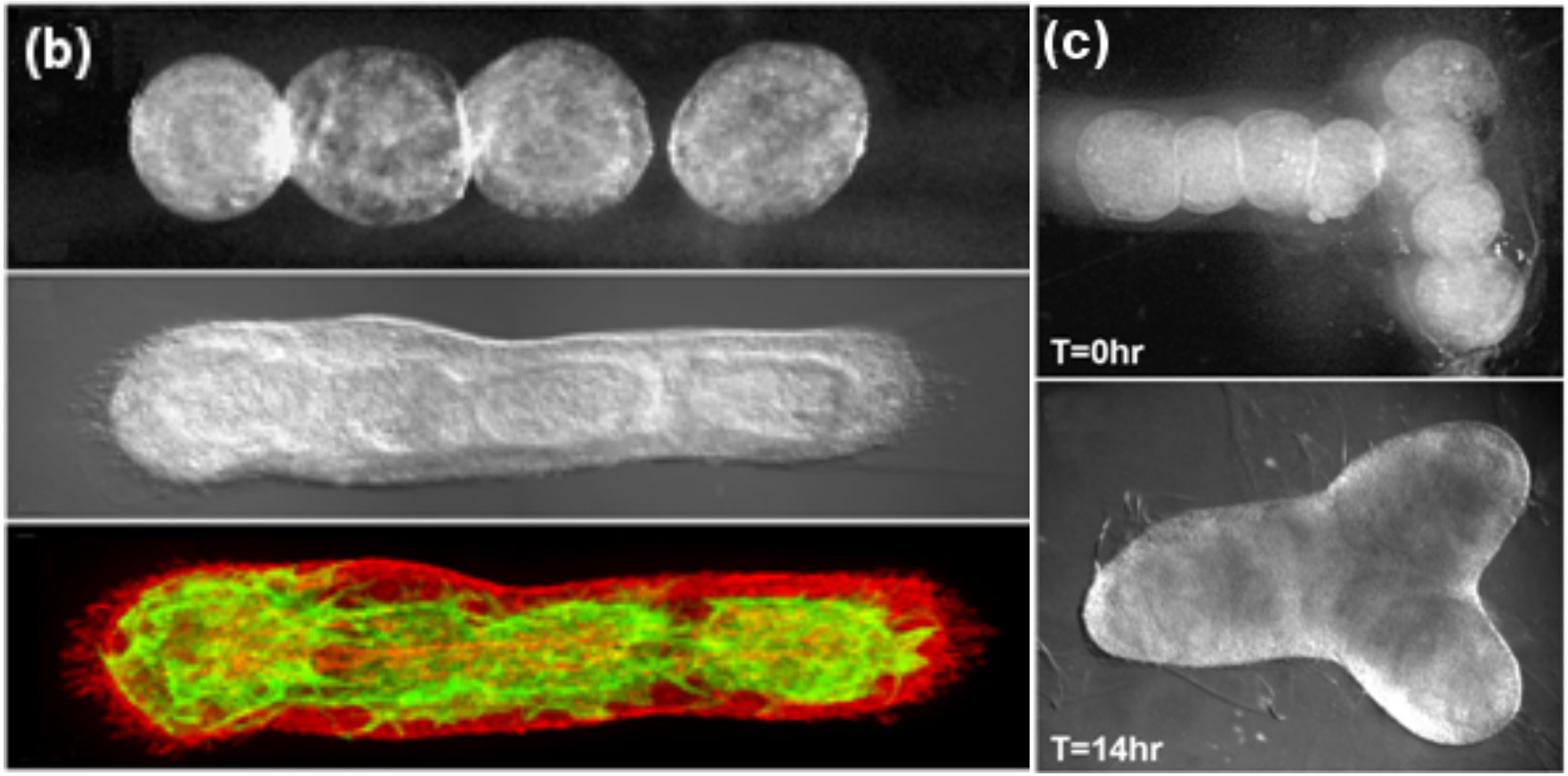
‘Tissue Engineering’ (2008), featured in ‘Nature News’



Dev. Dynamics (submittted)

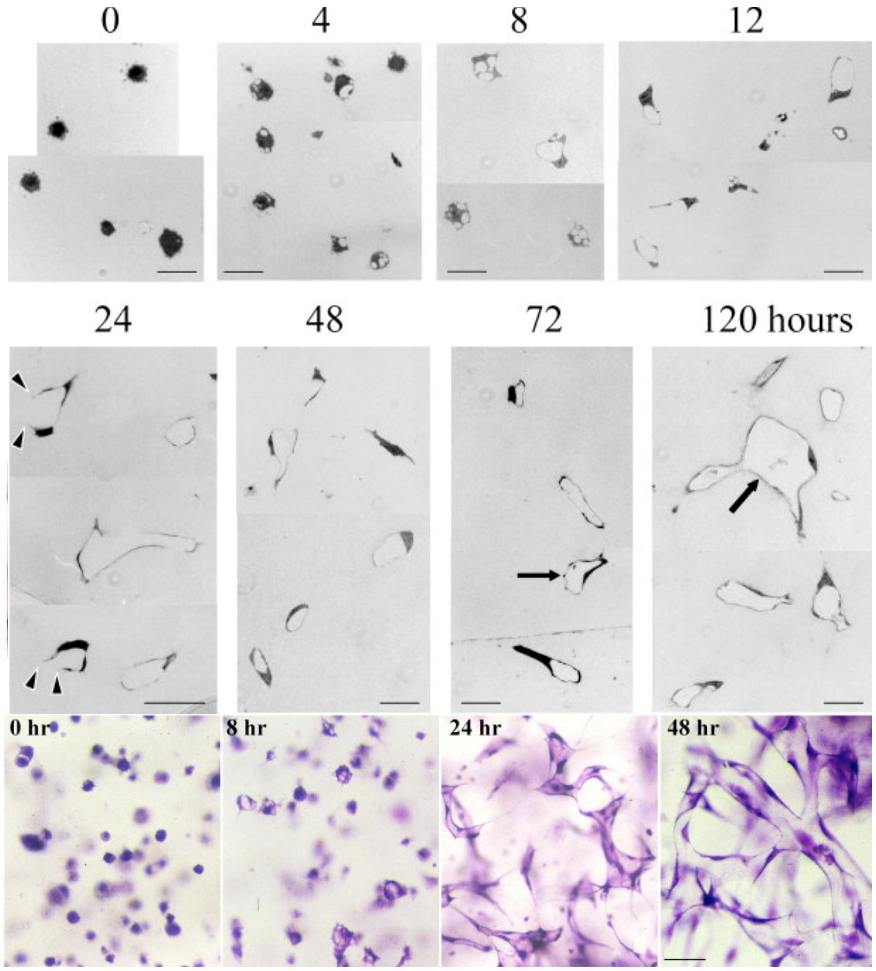
Vascular Tissue Spheroids Fusion

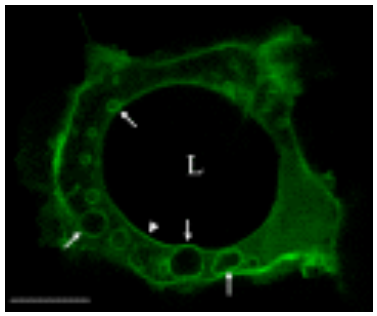
(Linear and Branched Segments of Vascular Tree)

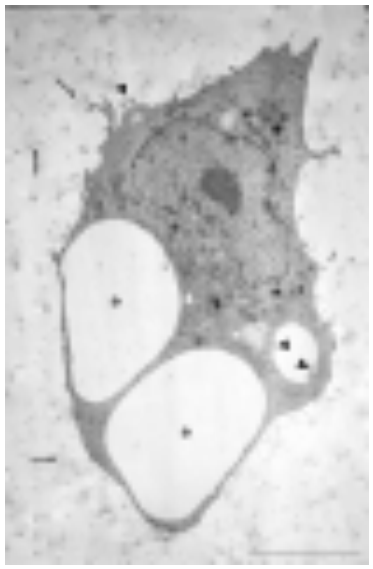


Biomaterials, 2009

Mechanism of Lumen Formation

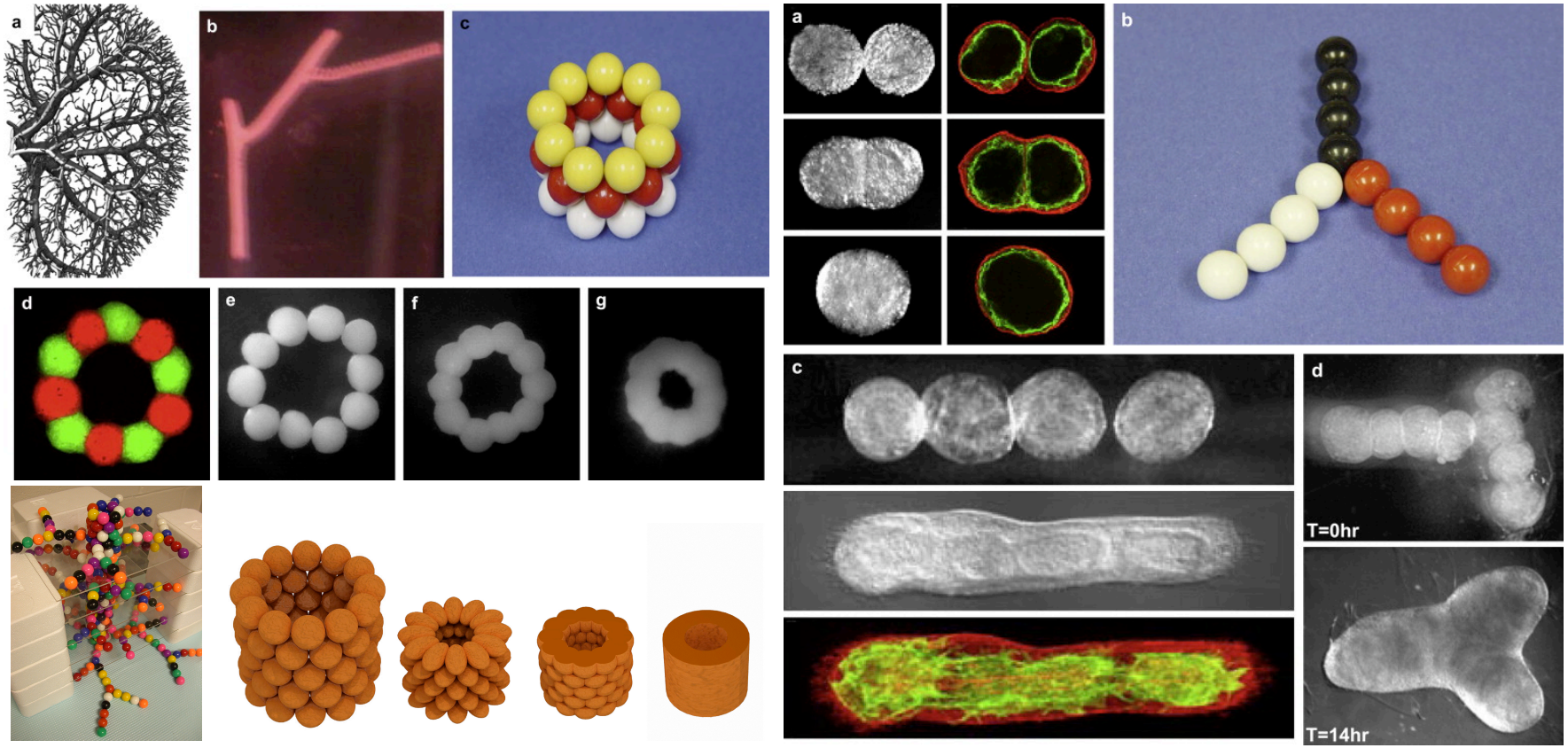






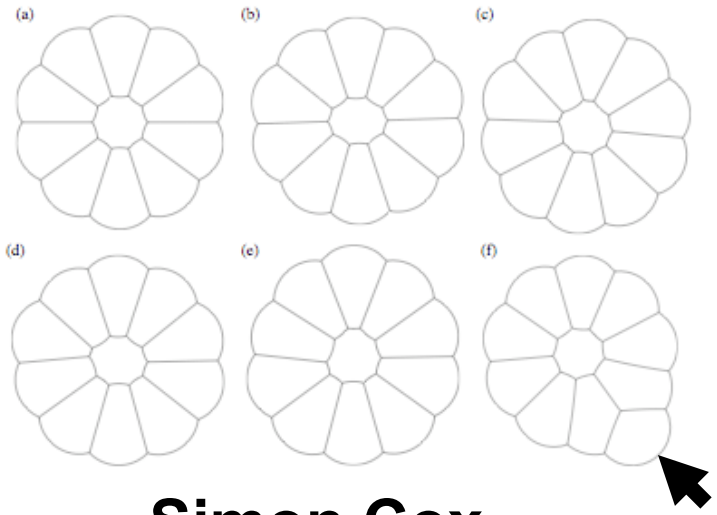
Kamei M, Saunders WB, Bayless KJ, Dye L, Davis GE, Weinstein BM. Endothelial tubes assemble from intracellular vacuoles in vivo. Nature. 2006 Jul 27;442(7101):453-6

Tissue engineered branched segments of vascular tree

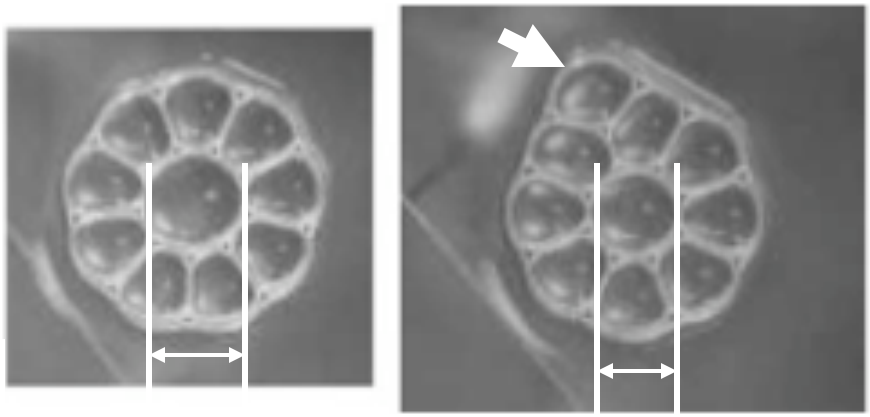


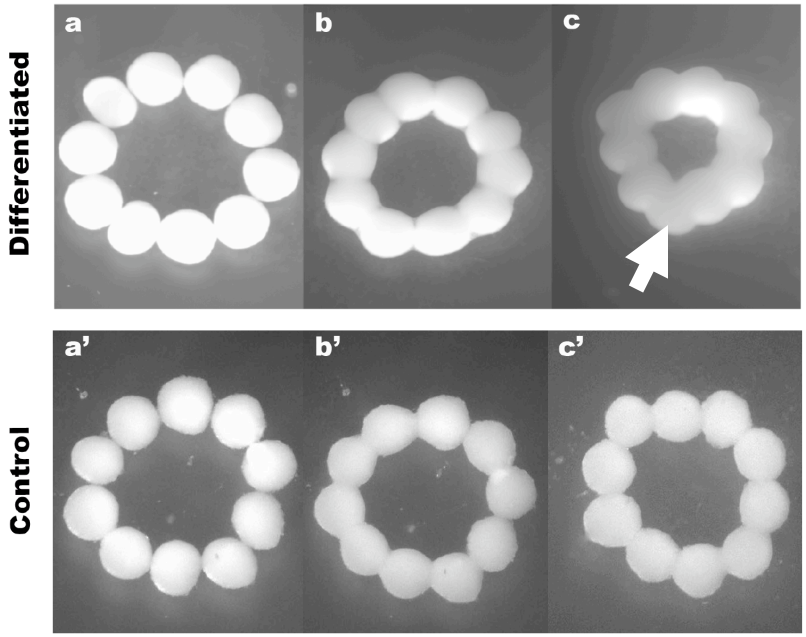
Regenerative Medicine, 2008 & Biomaterials, 2009

Buckling instability & tissue spheroid ejection



Simon Cox



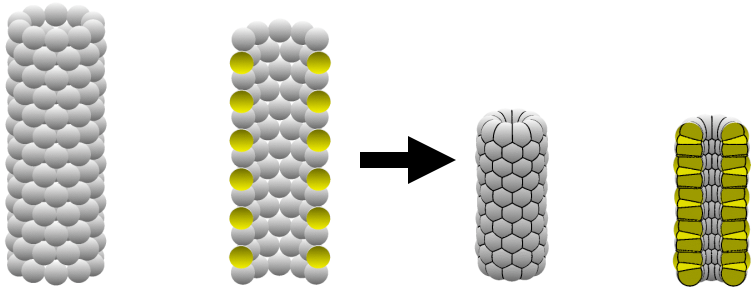


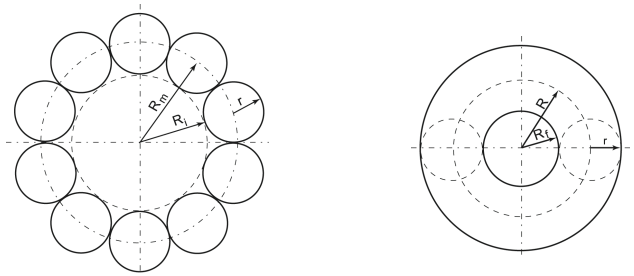
Rick Visconti

In collaboration with Rick Visconti, Simon Cox (UK) & Denis Weaire (IR)

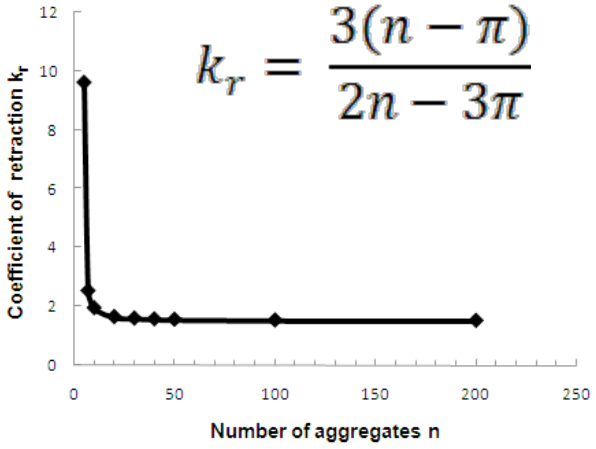
Olympic Waterhouse, Beijing

Bioprinting of Vascular Tree

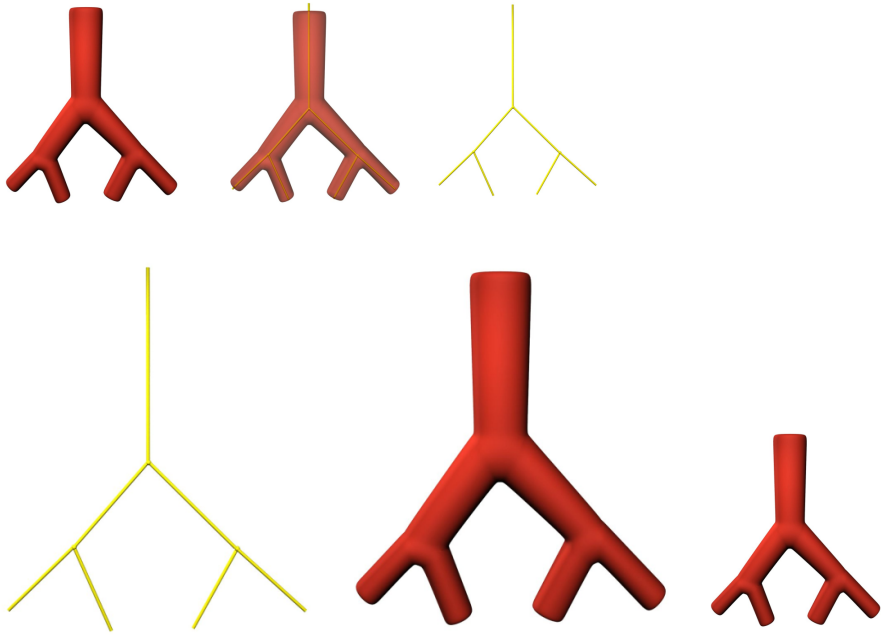




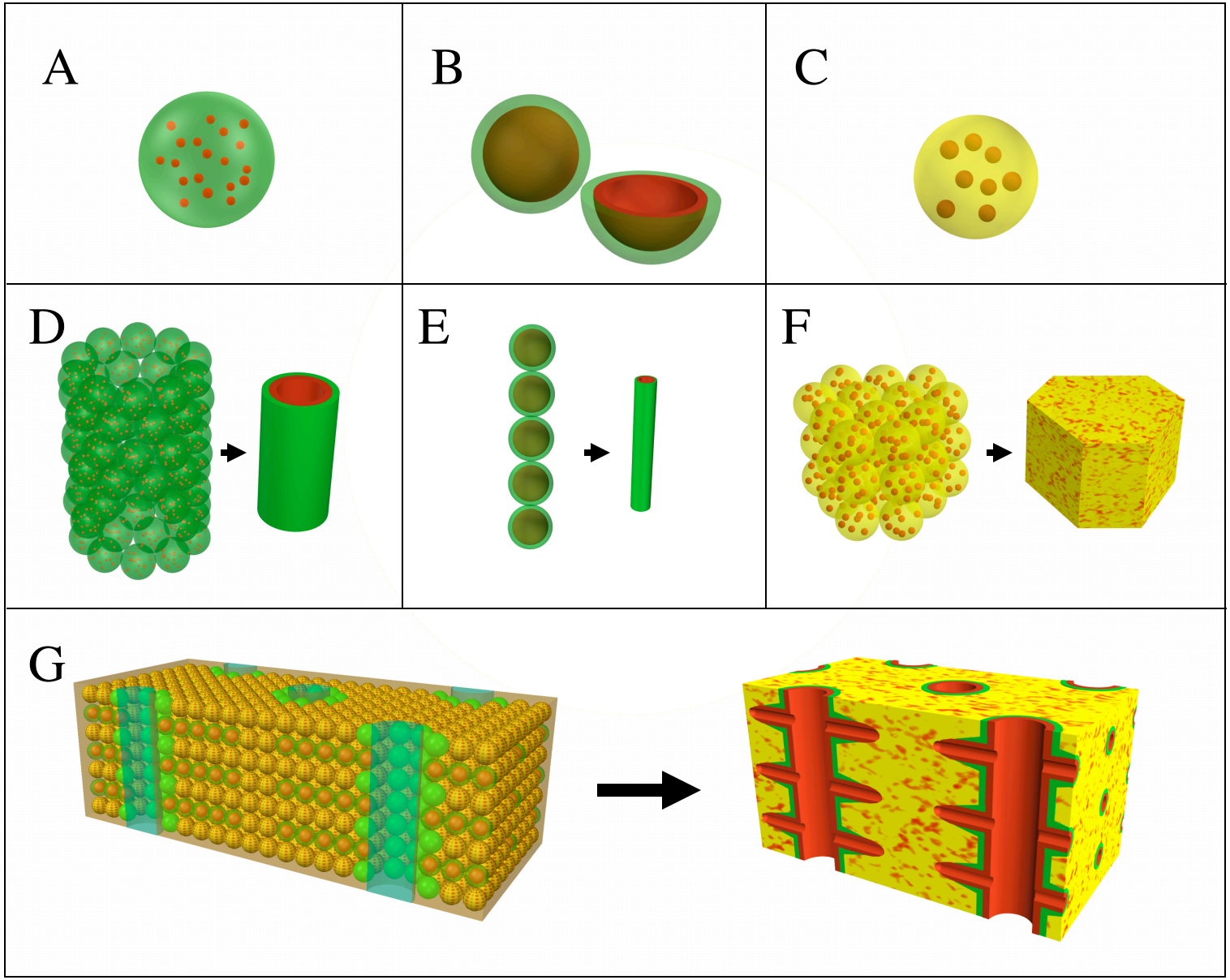
‘Surface Evolver’, Ken Brakke



$$k_r = \frac{3(n-\pi)}{2n-3\pi}$$



Virtual and Physical Prototyping, 2009


A
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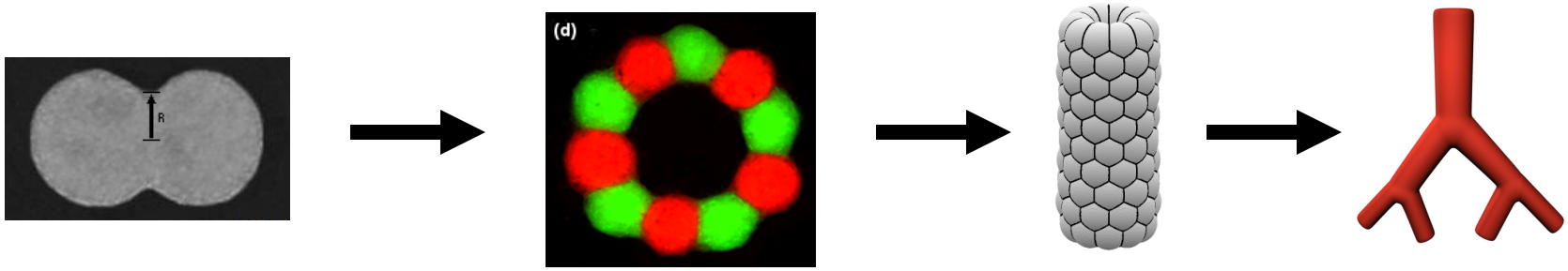
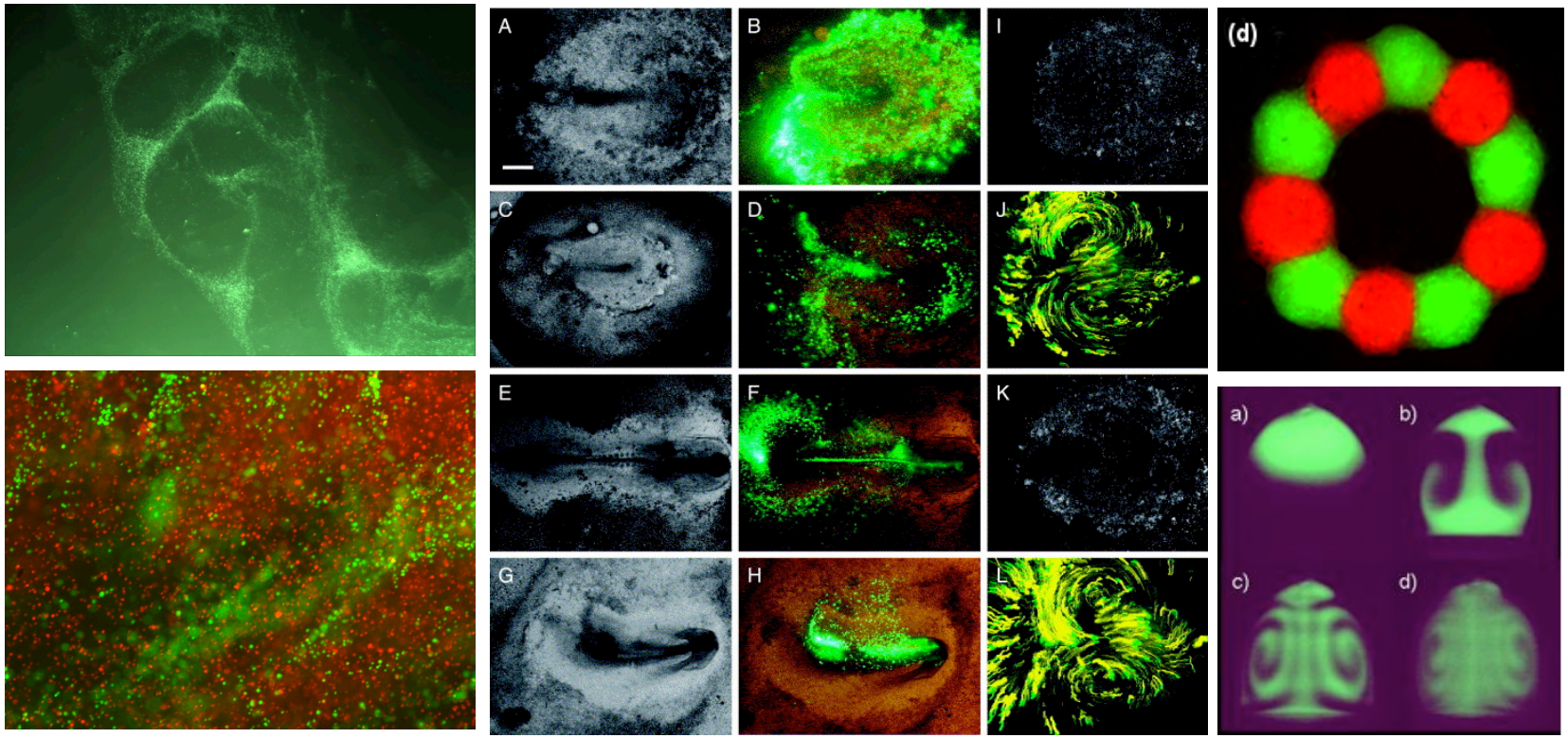
Biofabrication of vascular tree from self-assembled vascular tissue spheroids

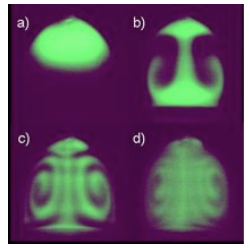




‘I like to compare morphogenesis with hydrodynamics…
There are certain principles of spatial order in organism…
…can be written down as rules and equations…
exactly the same way you can with liquids’

Brian Goodwin (UK) - antidarvinist, structuralist and author of famous book
‘How the Leopard Changed Its Spots: The Evolution of Complexity’

Embryonic Morphogenesis as a Problem of Fluid Mechanics:



I. Histodynamics is similar to hydrodynamics?
II. Morphogenesis is predictable and computable?
V. New research paradigm in Developmental Biology?

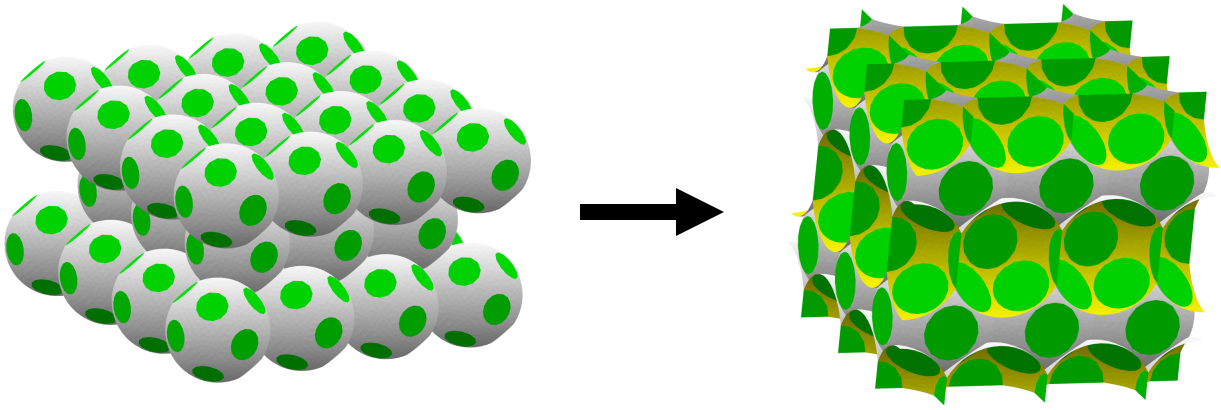
Specific Aims for Possible NSF FIBR grant:

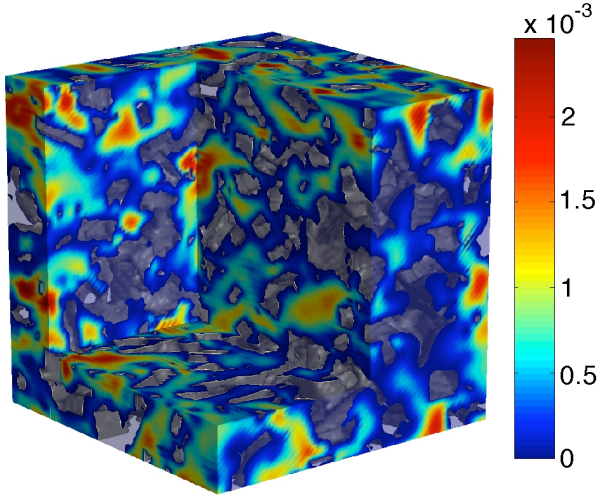
Aim 1. Tracing and visualization of tissue flow & tissue velocity *in vivo*.
Aim 2. Estimation of kinematic viscosity of tissues *in vitro* and *in vivo.*
Aim 3. Estimation of biological determinants of tissue fluidity.
Aim 4. Modeling of tissue flow *in vitro* and mathematical modeling, numeric and computer simulation *in silico.*
Aim 5. Employing of microfluidics and fluid mechanics in injectable tissue engineering and digital bioprinting.

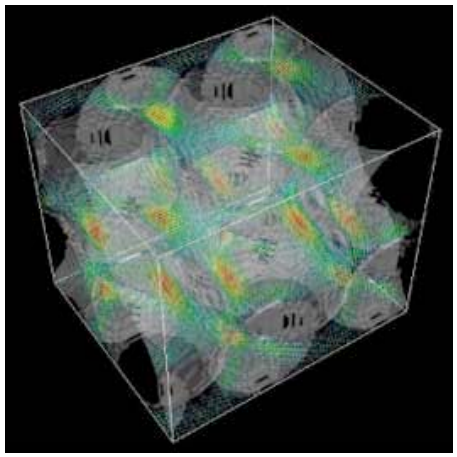
BIG UNANSWERED QUESTIONS:

Is chaotic advection based stirring (not mixing!!!) just a metaphor or it is a real physical mechanism of embryonic morphogenesis?
What is a driving force? Proliferative pressure?

Modeling tissue spheroids fusion using Surface Evolver and superfusion using LBflow

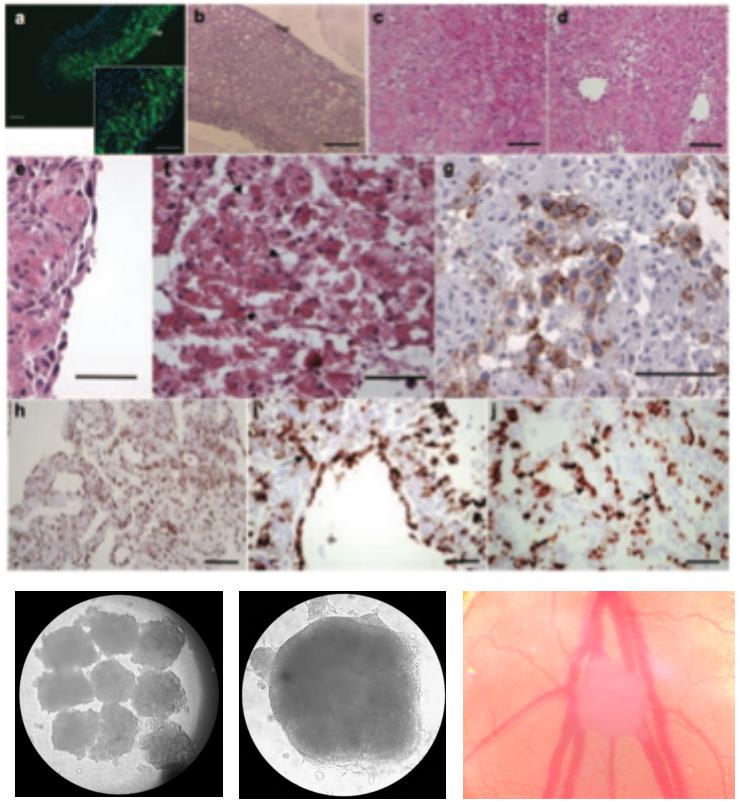


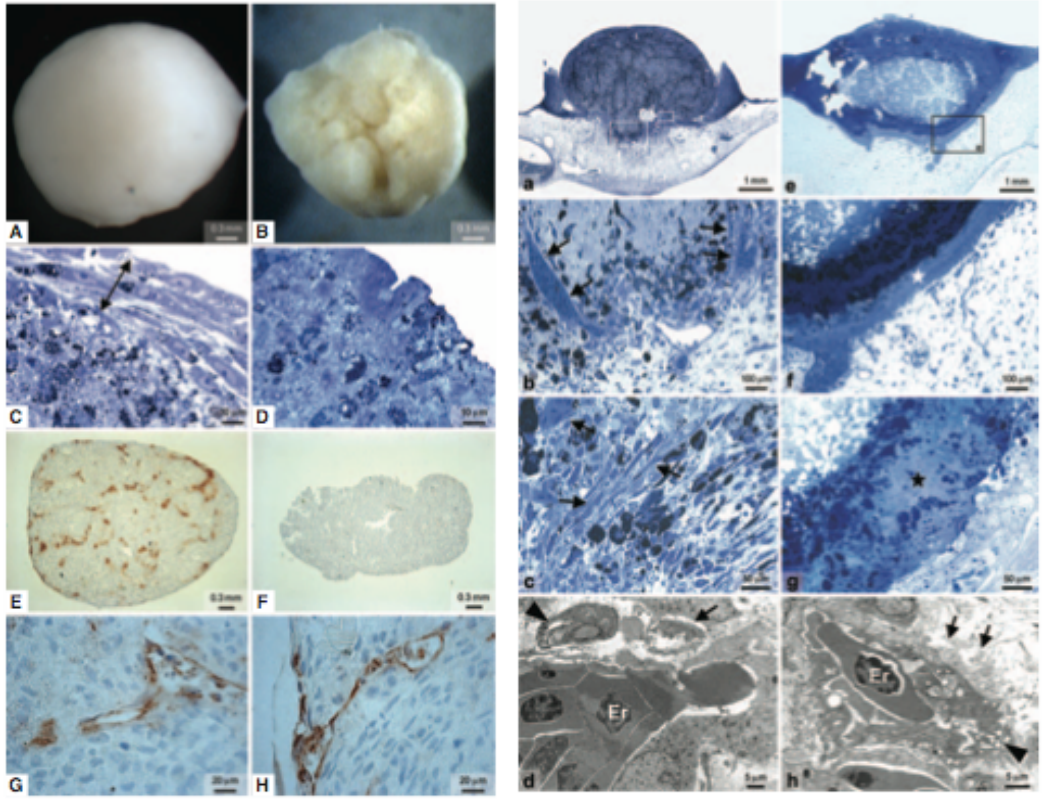




(in collaboration with Ken Brakke & Ed Llewellin, UK)

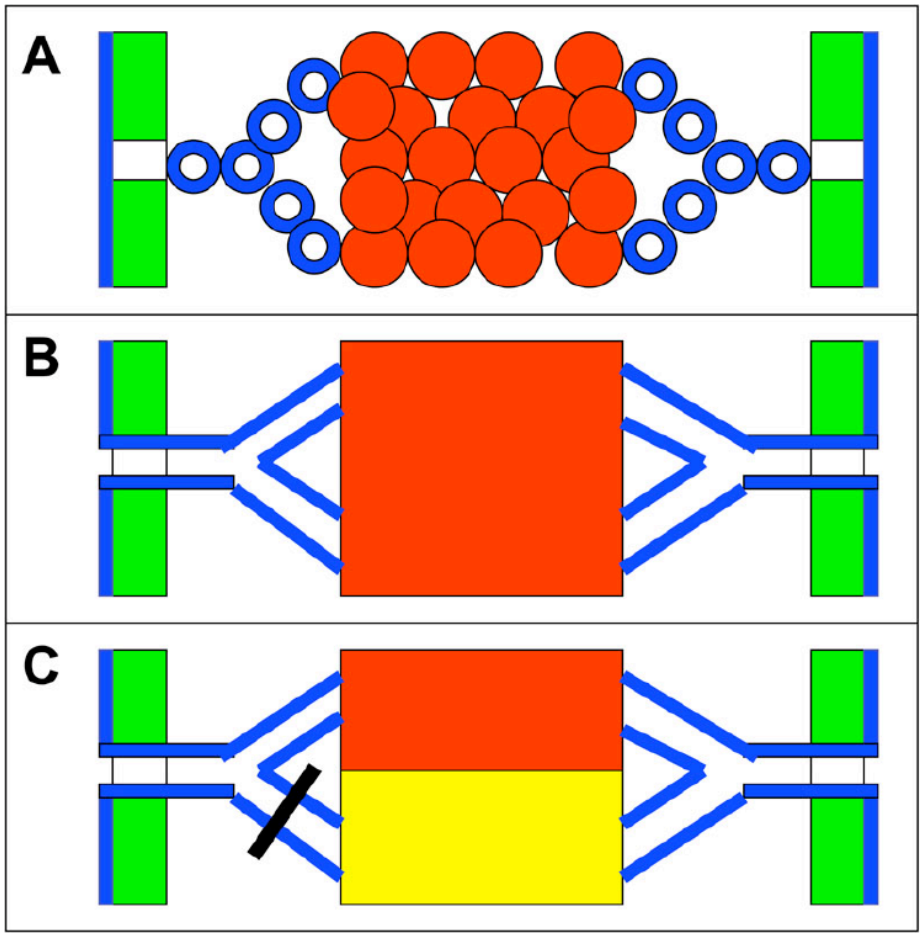
Tissue engineered vascularized myocardium





Tissue Engineering, 2008 & according to Kelm et al. TE

In Vitro 3D MI Model



Biofabrication of nephron