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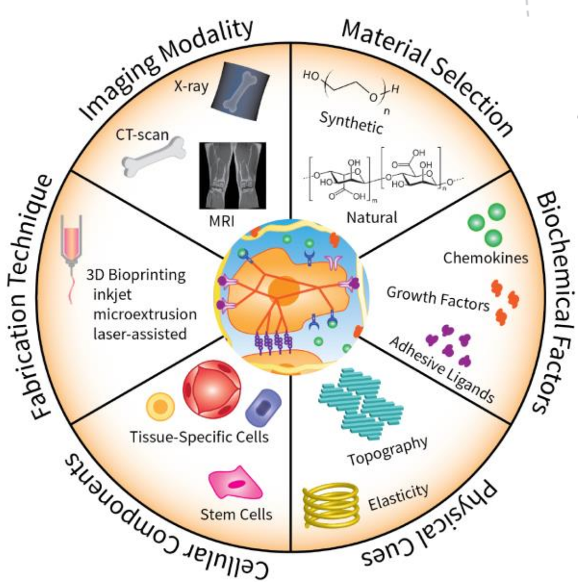
Hydrogel structuring

Mol3014 Øyvind Halaas, IKM

2 Bio-inspired 3D microenvironments: a new dimension in tissue engineering
Chelsea M Magin1,7, Daniel L Alge2,3,7 and Kristi S Anseth4,5,6
Biomedical Materials, Volume 11, 2



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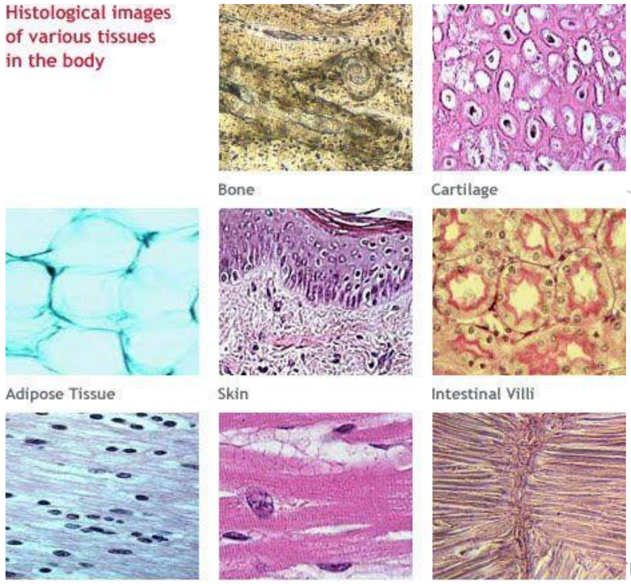
Histological images of various tissues in the body



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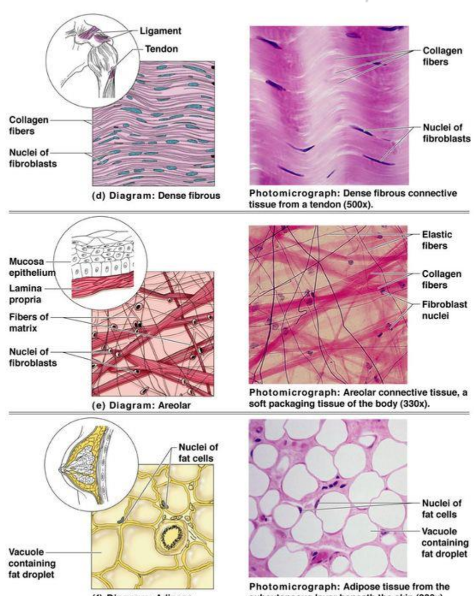
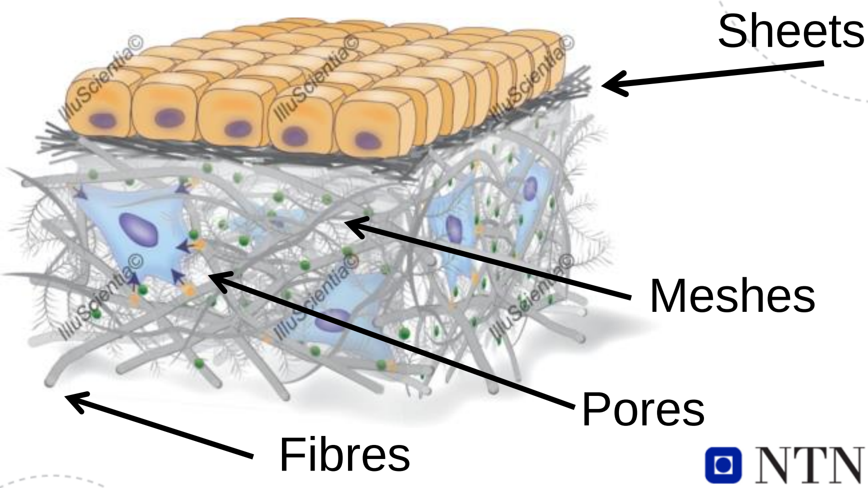
All tissues contain different nano and microstructures



5

Basic structures



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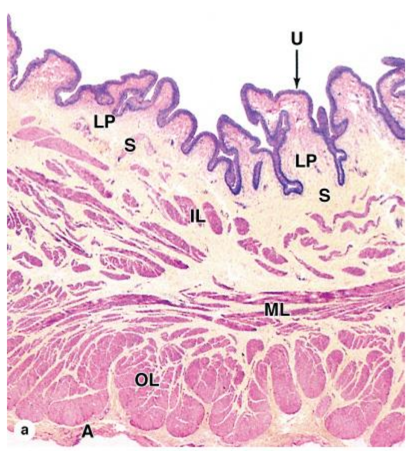
Sheets form membranes that can fold into tubes, invaginations, ducts and globules. These structures compartmentalize tissues, increase surface and is necessary for organ function



Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition:* http://www.accessmedicine.com

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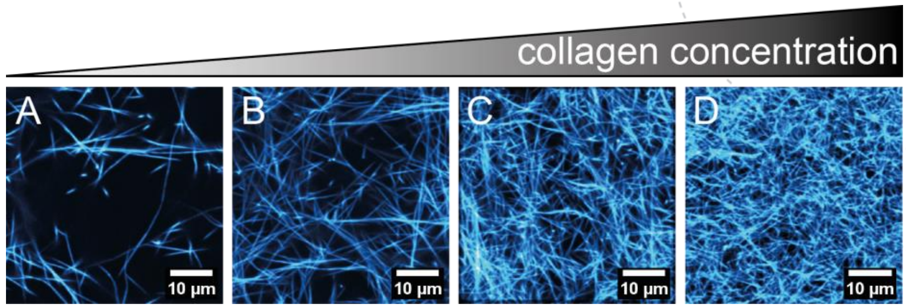
Fibres



Fibres give tensile and elastic strength and gives orientation and crosslinking in meshes and membranes

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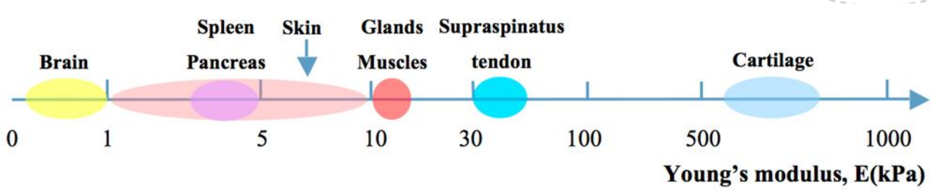
Different tissues have different mechanics (elasticity, deformability)



Biomaterial focus for macrostructures

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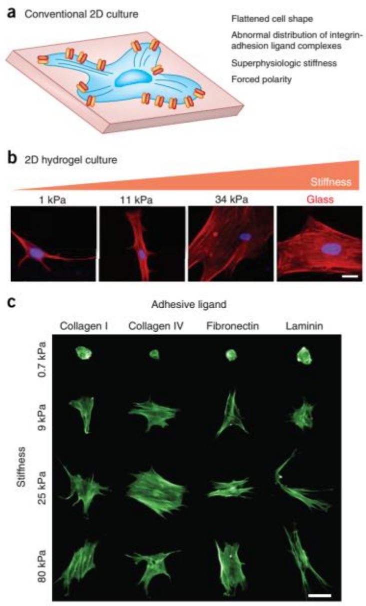

Stiffness determines cell fate

Figure 1 | Cell culture atop 2D hydrogels. (**a**) Conventional 2D culture on superphysiologically stiff plastic or glass substrates leads to cells displaying aberrant phenotypes. (**b**) Culturing cells on 2D hydrogel films has some of the same disadvantages as conventional methods but permits user-defined control of the substrate stiffness and adhesive ligand presentation. Human mesenchymal stem cells (MSCs) cultured on increasingly stiff 2D substrates display increasing spread area. Left to right: 1 kPa polyacrylamide (PA), 11 kPa PA, 34 kPa PA, and glass (~GPa). Scale bar, 10 μm. Images modified from ref. 65 with permission. (**c**) Substrate stiffness (*y* axis) and adhesive ligand type (*x* axis) combine to regulate MSC morphology. Human MSCs spread more with increasing stiffness, but cells on laminin-coated hydrogels are smaller than those on other ECM protein coatings. Images modified from ref. 64 with permission. Scale bar, 50 μm.

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Introducing mechanical properties (stretching, twisting, folding)

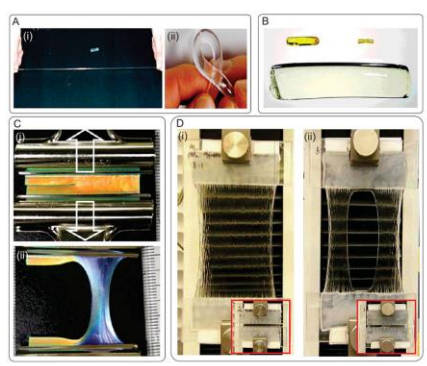

Figure 2.
Examples of composite elastomers. A) N-isopropylacrylamide/clay nanocomposite hydrogel; i) with high level of elongation and ii) torsion. Reproduced with permission.[95] Copyright 2002, John Wiley & Sons, Inc. B) Volume change of a superabsorbent polyrotaxane gel swelled to 45 times the initial weight; before volume change, in dried state, and in swollen state (up to 400% of its dry weight). Reproduced with permission.[97] Copyright 2001, John Wiley & Sons, Inc. C) Crack resistance of a PDGI/PAAm gel; (i) hydrogel with an initial sharp crack along the longitudinal direction, (ii) the hydrogel was stretched perpendicular to the crack direction up to a strain of 3. Reproduced with permission.[99] Copyright 2011, American Chemical Society. D) Highly stretchable alginate/acrylamide gel; i) the gel was glued to two rigid clamps and stretched up to 21 times its initial length, ii) a notch was cut into the gel before stretching to 17 times its initial length. Reproduced with permission.[100] Copyright 2012, Nature Publishing Group.

11

Hydrogels

- tunable physical, chemical, and biological properties
- high biocompatibility
- versatility in fabrication
- similarity to native ECM

- regenerative medicine
- drug/gene delivery
- stem cell and cancer research
- cell therapy

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Hydrogel raw materials

Natural	**Synthetic**
collagen	poly(ethylene glycol) (PEG)
chitosan	poly(vinylalcohol)(PVA)
hyaluronic acid (HA)	poly(2-hydroxyethyl methacrylate) (PHEMA)
alginate	polyacrylamide (PAM)
gelatin	
elastin	
chondroitin sulfate	
heparin	

The optimal materials would be hybrid composites

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13

Table 1 | Representative hydrogels that can be used for cell culture studies

Material	Example vendors	Notable material features
Natural materials		
Collagen	BD BioSciences, Advanced BioMatrix (PureCol, FibriCol), Vitrogen, Flexcell (Thermacol, Collagel)	Typically sourced from rat tail tendon or bovine skin and tendon Usually purchased in pepsin- or acid-solubilized form and stored at low pH and temperature Enzymatically degradable Exhibits structural and mechanical properties reminiscent of native tissues Presents native cell adhesion ligands
Fibrin	Baxter (Tisseel, Artiss), Johnson & Johnson (Evicel), Sigma	Typically sourced from human plasma Enzymatically degradable Provides good substrate for studying wound-healing phenomena *in vitro* Low mechanics limit utility
Alginate	NovaMatrix-3D, PRONOVA (FMC BioPolymer)	Derived from brown algae Must be modified with adhesive ligands for cell attachment Ionic crosslinking with divalent cations enables easy cell encapsulation and recovery Additional covalent crosslinking often needed for strength
Synthetic materials		
Polyacrylamide (PA)	Sigma	Wide range tuning of substrate mechanics Probably the most standardized material as far as protocols for making hydrogels and using for culture Suitable for 2D cell culture only
Polyethylene glycol (PEG)	QGel Inc. (QGel), Sigma, Cellendes (3-D Life Dextran-PEG or PVA-PEG), BioTime Inc. (PEGgel)	'Blank slate' synthetic material enables a wealth of user modifications Premodified versions and various molecular weights are readily available Can be engineered to present different adhesive ligands and to degrade via passive, proteolytic, or user-directed modes
Hybrid materials		
Hyaluronic acid (HA)	Lifecore (Corgel BioHydrogel), BioTime Inc. (HyStem), BRTI Life Sciences (Cell-Mate3D)	Usually produced via bacterial fermentation, but can also be sourced from animal products Wide variety and high degree of potential chemical modification enables considerable tunability Interacts with cell receptors but must be modified with adhesive ligands to permit cell attachment
Polypeptides	Corning (PuraMatrix), PepGel LLC (PGmatrix), Sigma (HydroMatrix)	Typically formed by self-assembly Useful in soft-tissue applications and in conjunction with other materials Protein engineering enables great design flexibility

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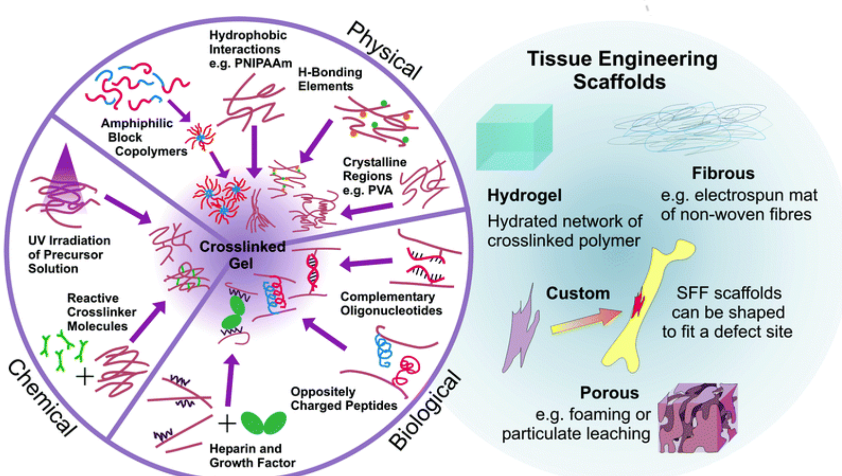
14



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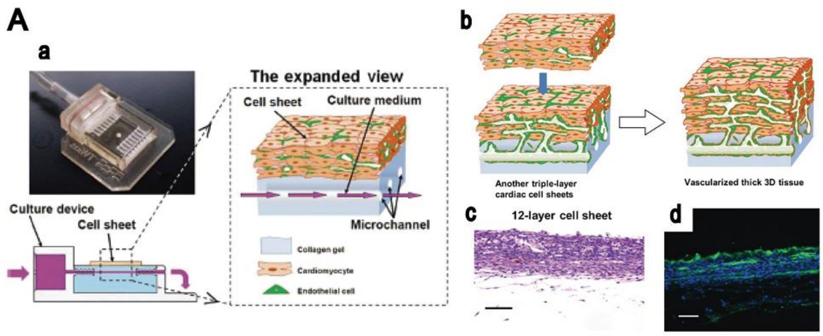
Larger constructs needs vascularization
A solution is microfluidics



Underlaid perifusion with
several added sheets of cells

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Structuring options

- Micromoulding
- Photocrosslinking
- 3D printing
  - Extrusion (continous)
  - Ink-jet (droplets)
- Extrusion of fibers
- Self assembly

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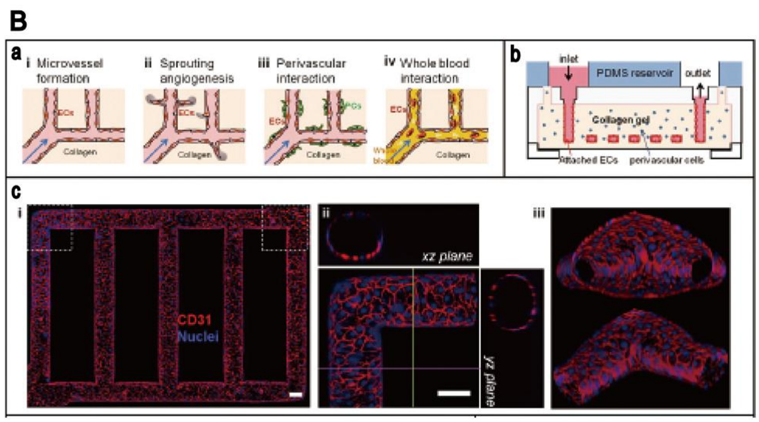
Micromoulding (previous lecture)



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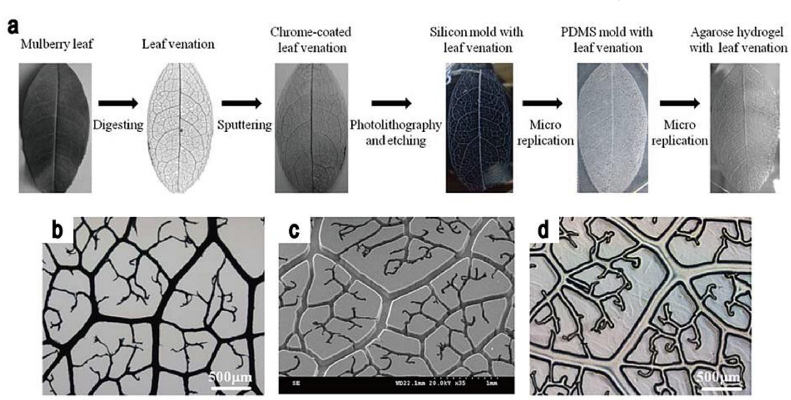
Using natural structures as micromoulding templates for vascularization



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Photocrosslinking chemistry

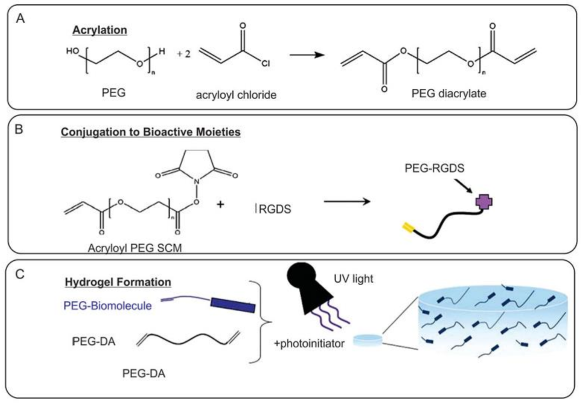


Figure 3.
Schematic for generation of photocrosslinkable hydrogels. A) Modification of PEG polymer with photocrosslinkable acrylate groups. B) Conjugation of biological molecules to photocrosslinkable PEG polymer precursor. C) Formation of hydrogel upon exposure to UV light. Reproduced with permission.[112] Copyright 2011, Wiley Periodical Inc.

20

Designer-crosslinking using click chemistry

Click chemistry is highly specific for the conjugation-pairs

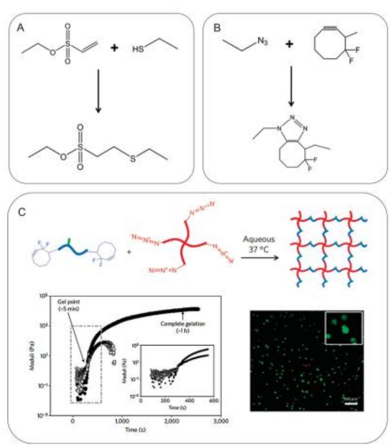


Figure 4.
Biocompatible click-based hydrogels. A) A Michael-type addition between thiol and vinyl sulfone. B) A "copper-free" click chemistry between azide and difluorinated cyclooctyne. Both reactions occurred under physiological conditions. C) Cell-encapsulating hydrogel was fabricated by copper-free click chemistry between 4-arm PEG-tetrazide and bis(difluorocyclooctyne)-polypeptide. Reproduced with permission.[166] Copyright 2009, Nature Publishing Group.

21

Photopatterning of 3D hydrogels

Photocrosslinking of 3D pattern (compare to 2D biopatterning in previous lecture)

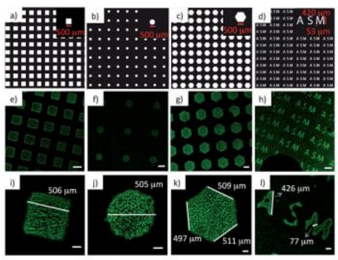
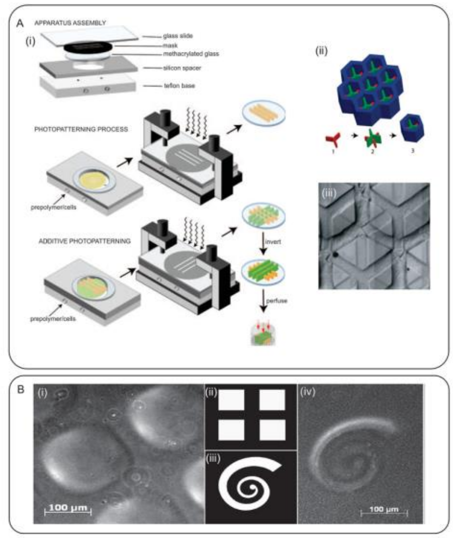


Figure 3. Light-triggered gel patterning: a–d) mask layouts. Inset: a single pore of each mask. e–h) CLSFM of gel patterns formed after irradiation through the mask. Scale bar: 500 µm. i–l) shape and size of the pattern features. Scale bar: 100 µm. General conditions: ([1]/[2]/[PAH] = 16:96:1 at millimolar concentrations).



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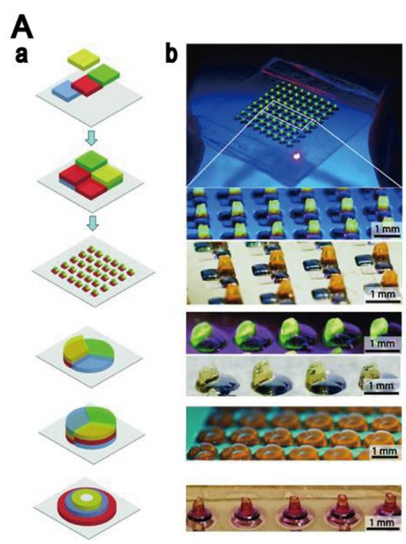
Multilayered 3D specified photolithography makes it possible to create smaller structures with complexity



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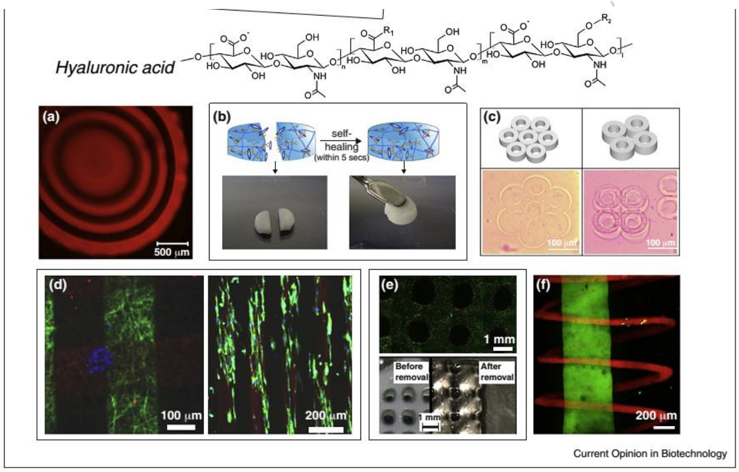
3D photo-crosslinking and patterning of HA, a natural ECM component, modified with UV-sensitive moelcules



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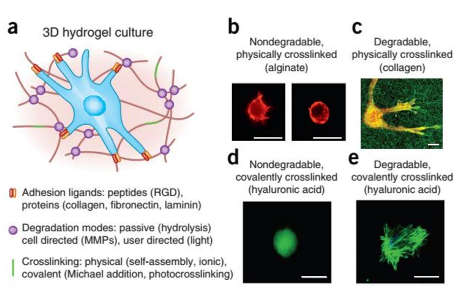
Degradability in cross-linked networks increase cell perfomance and function



A fully crosslinked hydrogel is static, therefore it is necessary to incorporate tissue remodelling moieties

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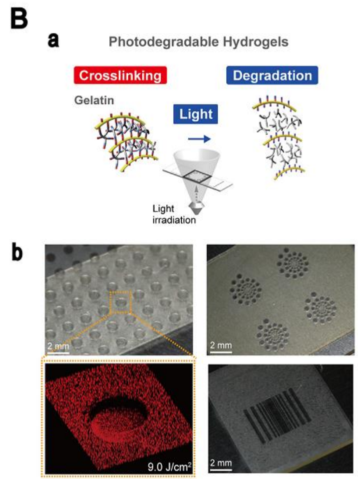
Reverse thinking

Photocleavable crosslinking for post-gellation structuring



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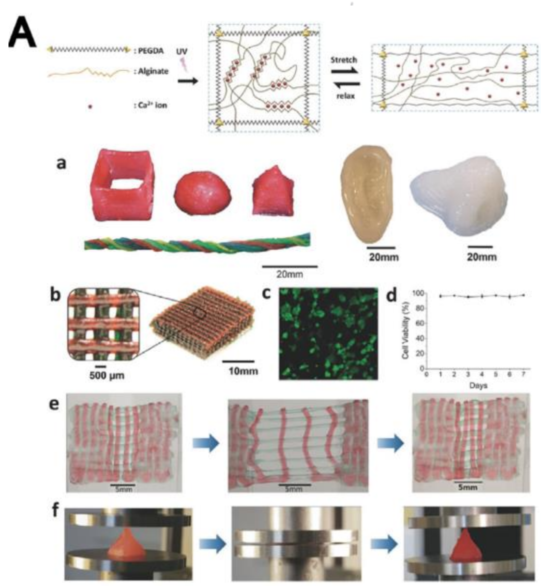
3D printing

Extrusion and photocrosslinking of materials



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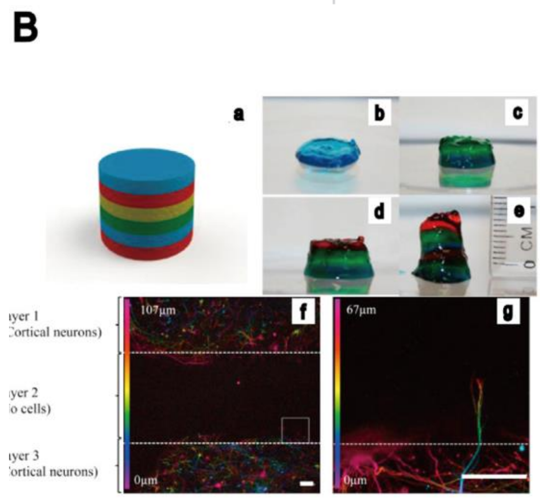
Extrusion-based 3D printed layered neural hierarchy



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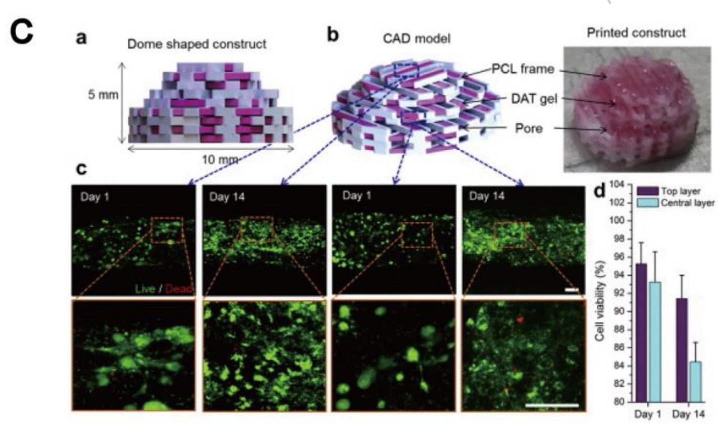
Two-component 3D extrusion printing



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Process of 3D printing perfused vascular channels

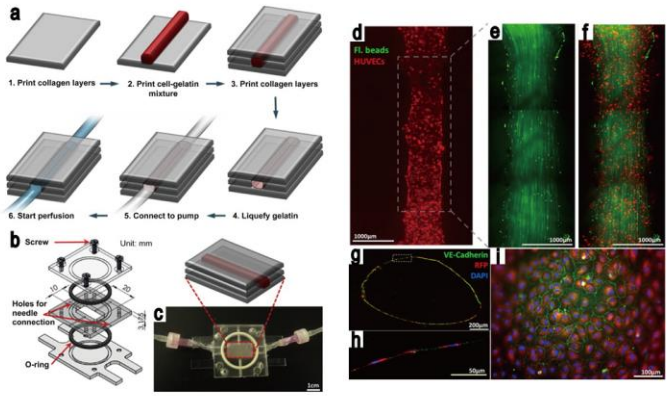

Fig. 7. The fabrication of perfused functional vascular channels, using 3D bioprinting technology. (a) The schematics of the vascular channel construction procedure using cell gelatin mixture. (b–c) Custom-designed flow chamber. (d) Fluorescent images of printed vascular channel with perfusion, after five days of culture. (e–f) The visualization of fluorescent bead motion with flow. (g–i) Vascular channel images, following five days of cell culture, with flow. Blue: DAPI nuclei staining; Red: RFP-transfected HUVECs; Green: VE-cadherin.
Source: Lee et al. [136], copyright (2014), with permission from Elsevier Ltd.

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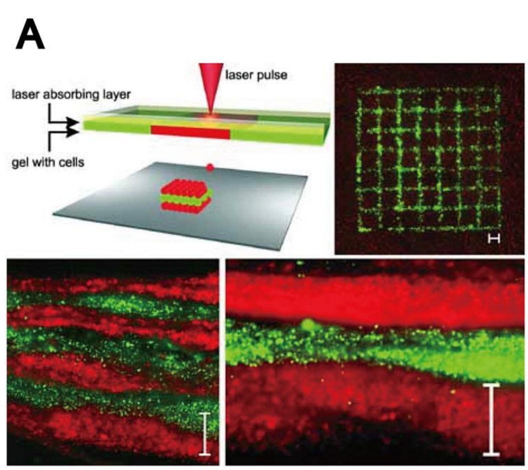
Combine 3D printing with photocrosslinking

Light-assisted bioprinting can create structures not possible using additive manufacturing (extrusion or plotter 3D printing)



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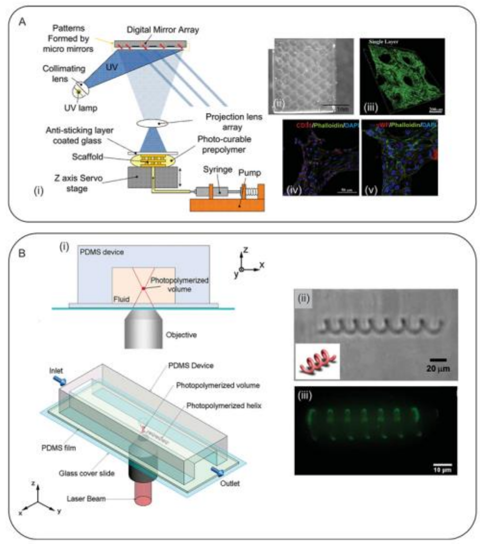
Rapid protoyping and stereolithographic structuring of hydrogels, also within volumes



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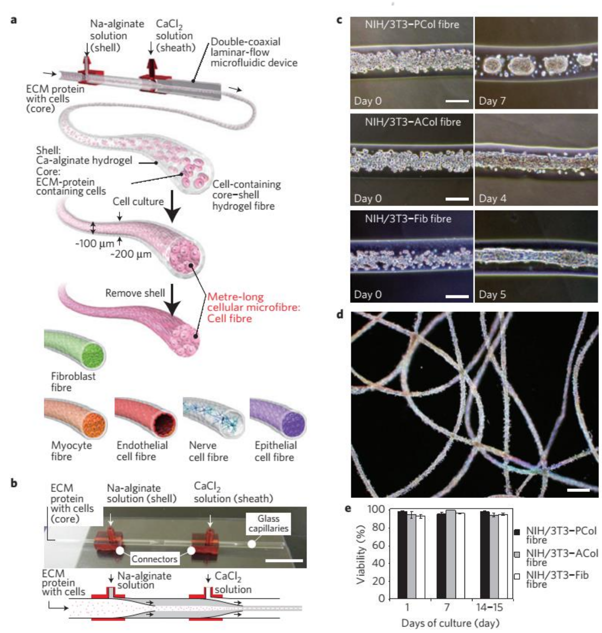
Making fibers by extrusion

DOI: 10.1038/NMAT3606



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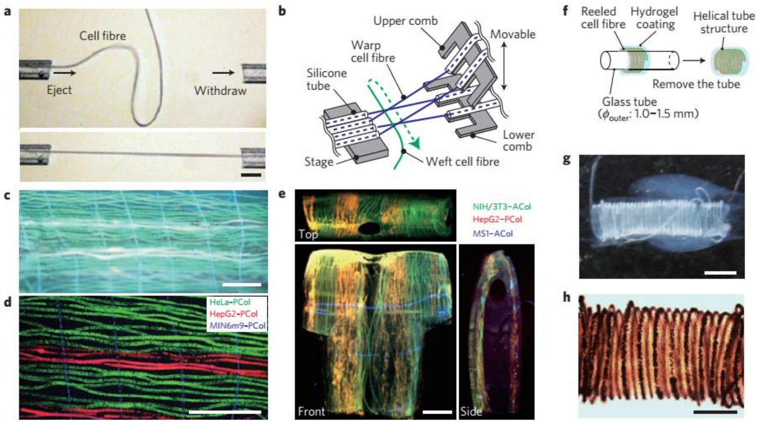
Higher order fibre structuring
weaving, winding, knitting



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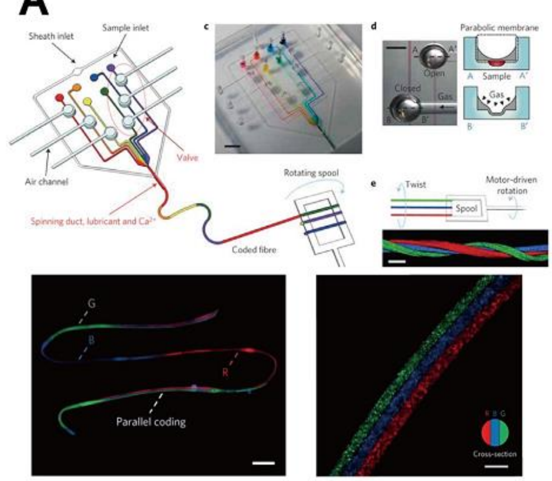
Setup for microifluidic-assisted extrusion printing

A



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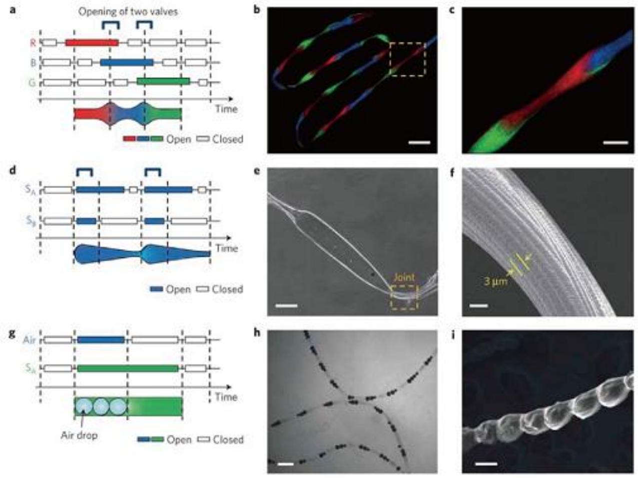
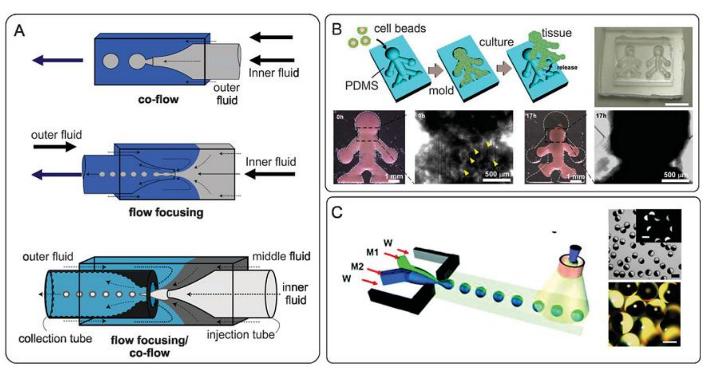
Microfluidic-assisted fiber extrusion for increased complexity



36

Microdroplet hydrogels (previous lecture) can be exploied in bioplotting



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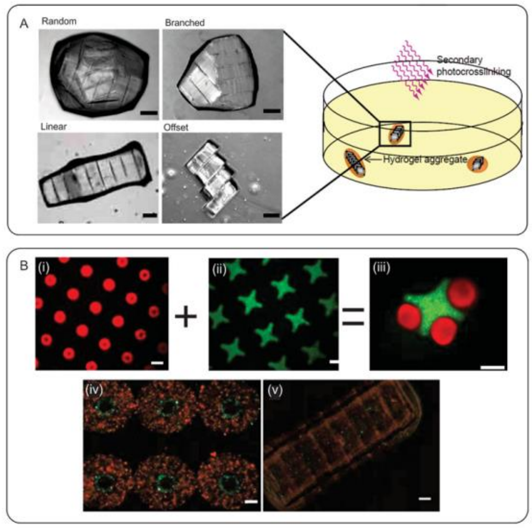
Assembly of microgels



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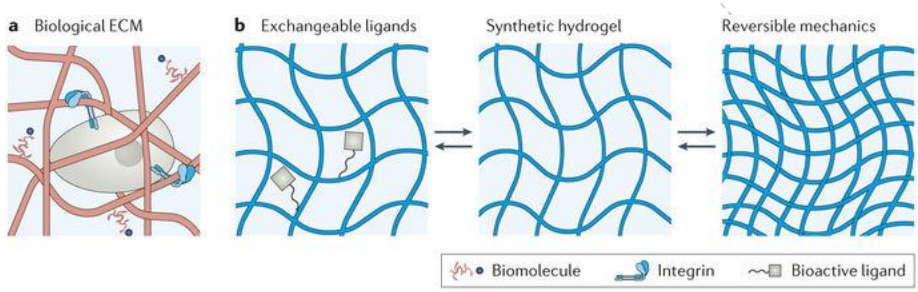
Reversible scaffolds enhance dynamics



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39

Injectable photoreactive hydrogels for clinics

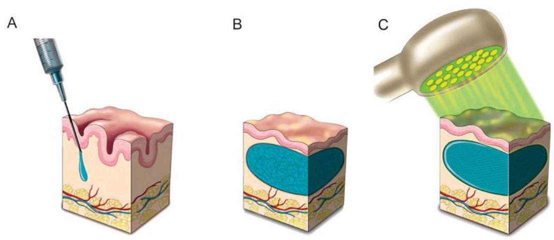

Figure 17.
Transdermal injection of photocrosslinkable PEG/HA hydrogels. A) The composite blend was injected into the dermis, B) the uncrosslinked mixture was massaged into the desirable shape under the skin, C) the material was then crosslinked by using an array of LEDs emitting light, which penetrated up to 4 mm of tissue depth. Reproduced with permission.[424] Copyright 2011, Advancing Science, Serving Society.

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Review

Hydrogel microfabrication technology toward three dimensional tissue engineering

CrossMark

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25th Anniversary Article: Rational Design and Applications of Hydrogels in Regenerative Medicine

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