Biomaterials and Scaffold Design for 3D Tissue Construction

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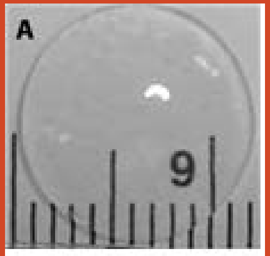

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Current Life Science Topics

1. Fundamentals in Basic Biochemistry & Cell Biology
2. Introduction to Tissue Engineering & Regenerative Medicine
3. Developmental Tissue Reconstruction
4. Wound Healing & Regeneration
5. Natural Tissue Composition & Cell-ECM Interaction
6. Stem Cells & Cell-Based Therapy
7. Biomaterials
8. *Mid-Term Exam*
9. Mechano-transduction & Bioreactors
10. Discussions on Tissue Reconstruction
11. Regulation & Ethics
12. AI in Current Life Science
13. Machine Learning & Github
14. Deep Neural Network
15. Convolutional Neural Network
16. *Final Exam*

Study Materials

Chapter 30. Principles of RM by A. Atala
Seliktar D 2012 Science
Derby B 2012 Science
Papers from T. Okano group
Tani G 2010 Pediatr Surg Int
2017 Conference Program "Scale-up and Manufacturing of Cell-based Therapies V" ECI (Engineering Conference International), San Diego, USA

demineralized cancellous bone matrix

Ceramics Veneers

Total Knee Replacement Arthroplasty

Biomaterials

[Chapter 30. Principles of RM by A. Atala]

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Categories of Biomaterials

<u>Source</u>

Natural polymer

Synthetic polymer

<u>Degradation behavior</u>

Non-degradable

Bio-degradable

<u>Composition</u>

Organic polymer

Inorganic polymer

Hybrid materials

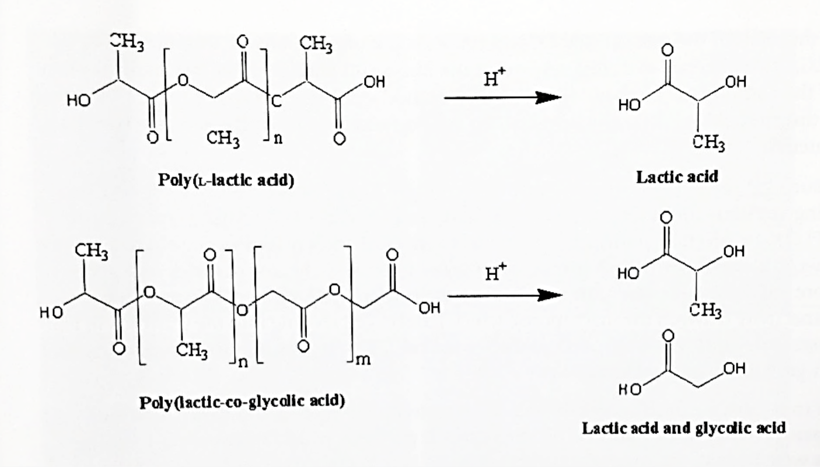
Synthetic and Biological Scaffold Materials in Regenerative Medicine

<table>
<tr><td rowspan="2"></td><td colspan="2" rowspan="2">Synthetic polymers</td><td colspan="2">Biologic materials</td></tr>
<tr><td>Purified ECM components</td><td>Intact tissue-derived ECM</td></tr>
<tr><td>Example(s)</td><td><u>Non-degradable</u>:
Polypropylene (PP)
Polyethylene (PE)
Polytetrafluoroethylene (PTFE)
others</td><td><u>Degradable</u>:
Poly(ethylene glycol) (PEG)
Poly-L-Lactide (PLA)
Polycaprolactone(PCL)
Poly(Lactic-co-glycolic acid) (PLGA)
Others</td><td>Collagen
Chitosan
Hyaluronan
Alginate
Agarose
Others</td><td>Urinary bladder matrix (UBM)
Small intestinal submucosa (SIS)
Dermis
Pericardium
Fascia lata
Cell culture-derived
Others</td></tr>
<tr><td>Advantage(s)</td><td colspan="2">• Predictable mechanical and physical properties
• Well characterized biochemistry
• Accessibility to controlled chemical modifications and various processing
• Techniques to meet site-specific requirements</td><td>• Predictable mechanical, physical, and biologic properties
• Well characterized biochemistry
• Tailored towards multiple endpoints (i.e. biomechanics, biologics)</td><td>• Great mechanical and physical heterogenicity</td></tr>
<tr><td>Disadvantage(s)</td><td colspan="2">Undesired immunogenic effect(s), often tailored towards single endpoint (i.e. biomechanics)</td><td>• Limited accessibility to structural modification, which may also lead to undesirable physiological effects (non-degradable vs degradable scaffolds)</td><td>• Heterogenic mechanical, physical, and biologic properties between and within preparations
• Limited accessibility to structural modification, which may also lead to undesirable physiological effects (non-degradable vs degradable scaffolds)</td></tr>
</table>

Biodegradable synthetic polymers

Aliphatic polyesters

- Surgical sutures
- Bone fixing screws
- Bulk erosion process
- Poly(α -hydroxyesters):
    - Poly(L-Lactic acid), PLA
    - Poly(lactic co-glycolic acid), PLGA
    - Poly-caprolacton, PCL



Polyanhydrides

- Surface-erosion process: particularly useful for sustained drug delivery systems
- Hydrophilic sebacic acid (SA)
- Hydrophobic p-carboxyphenoxyproane (CPP)

Poly(amino acids), peptides

Non-degradable Synthetic Polymers

Polymers w/ a C-C backbone

- Polyethylene & derivatives
  - Polyethylene, polypropylene, polystyrene
  - Catheters, highly durable hip prosthesis, syringe bodies, non-degradable drug delivery device
- Poly(tetrafluoroethylene) PTFE
  - Well known as Teflon (DuPont)
  - Porous PTFE fiber meshes (Goretex)
  - Popular synthetic vascular graft material

Poly(meth)acrylates & polyacrylamides

- Poly(meth)acrylate hydrogels
  - Ocular application (contact lenses)
  - Drug delivery systems
  - Poly(methyl methacrylate, poly(2-hydroxyethyl methacrylate), poly(N-isopropylacrylamide)

- Polyether
  - Polyethylene glycol (PEG)
  - Degradable or non-degradable polymers for drug delivery
    - Gene delivery, tissue engineering scaffold, medical devices, implants
- Polysiloxanes
  - Silicon-oxygen backbone
  - PDMS (poly(dimethylsiloxane))
    - Silicon surface inhibit blood from clogging
    - Used for the fabrication of silicon-coated needles, syringes, and other blood-collecting instruments
    - Prosthetic silicon implants
- Other non-degradable polymers
  - Poly(ethylene terephthalate), PET
  - Polyurethanes, PU

Natural-based polymers

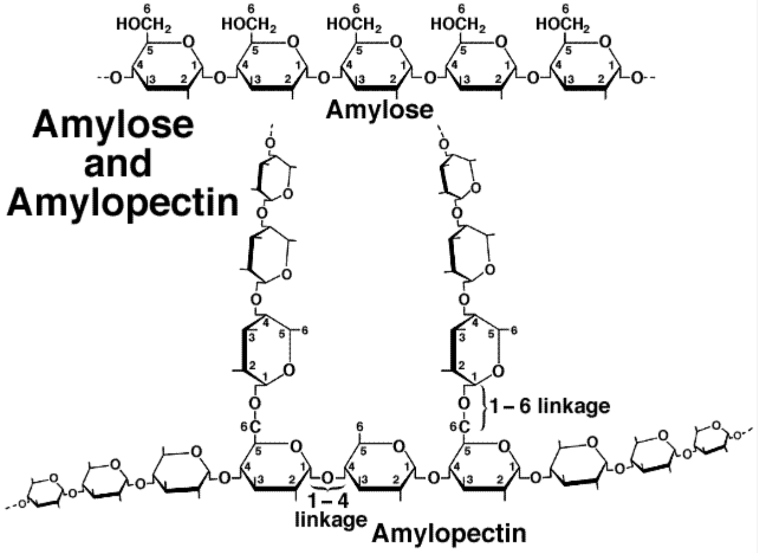
Starch
Chitosan
Alginate
Agarose
Polyhydroxyalkanoates
Silk fibroin

- Collagen
- Chitosan
- Hyaluronan
- Intact ECM
- Others

Starch

Two types of α-glucan polymers

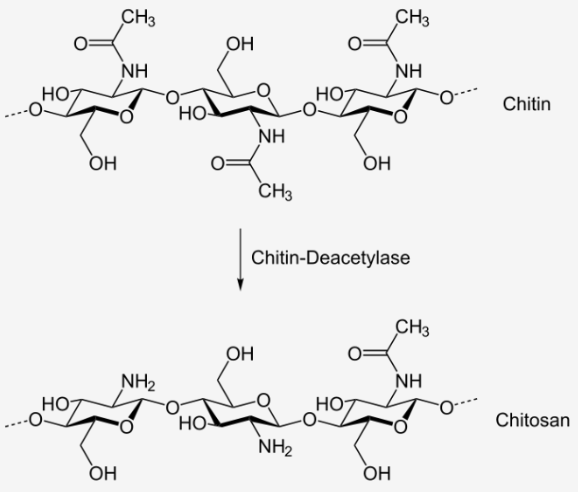
- Thermoplastic behavior
- Amylose
  - Minor constituent
  - Consist mainly of α(1-4) linked D-glucopyranosyl units
- Amylopectin
  - Major component
  - Branched polysaccharide composed of hundreds of short (1-4)-α-glucan chains interlinked by (1-6)-α-linkages



Chitosan

Chitin

- homopolymer of β(1-4)-linked N-acetyl-D-glucosamine residues

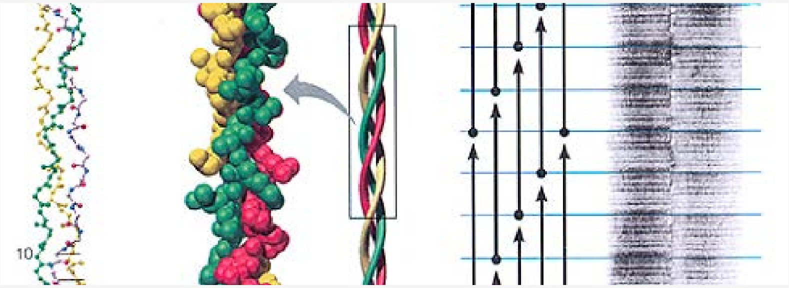


Chitosan

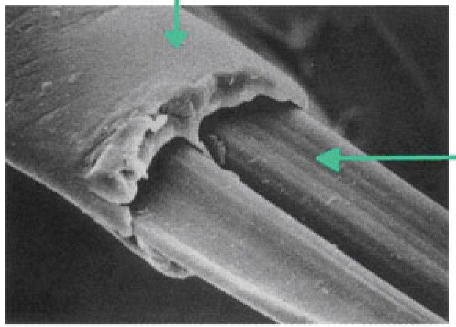
- obtained from the alkaline deacetylation of the biopolymer chitin
- linear polysaccharide consisting of N-glucosamine
- Degree of deacetylation (DD)
  - Ratio of Glucosamine/N-acetylglucosamine
  - Usually can vary depending on the source
  - Chitosan is insoluble in aqueous solutions above pH7. But, in dilute acids, the free amino groups are protonated and the molecule becomes fully soluble below around pH5.
    ($-NH_2$ -> $-NH_3^+$)
- Chitosan support the adhesion and proliferation of osteoblasts

Collagen

The most abundant structural protein
Gives mechanical stability, strength, and toughness to a range of tissues
The stiffness is improved by the inclusion of minerals in cases such as bone and dentin



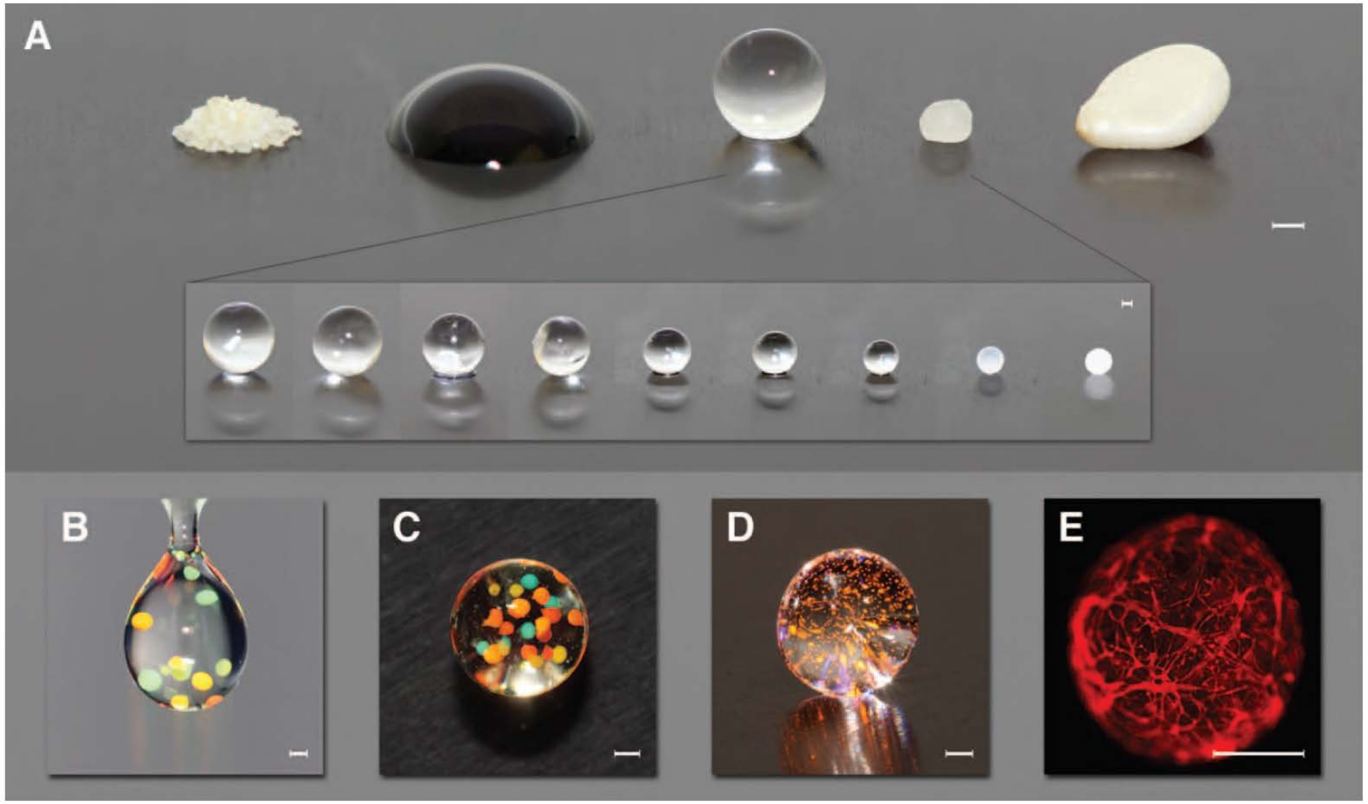
Silk fibroin



Natural fibrous proteins
Composed of fibroin & sericin

- Fibroin
    - the major component, filament core protein
    - Hydrophobic glycoprotein consists of heavy and light chain polypeptides of ~350 and ~25 kDa, respectively, linked by disulfide bond
    - Unique mechanical property
    - Versatility in its processing
    - Biocompatibility
    - Low inflammatory response
- Sericin
    - A water-soluble glue-like protein that bind the fibroin fibers together
    - Immunological reactions to silk have been largely attributable to the sericin proteins
    - Can be removed by boiling in alkaline solution

Hydrogel

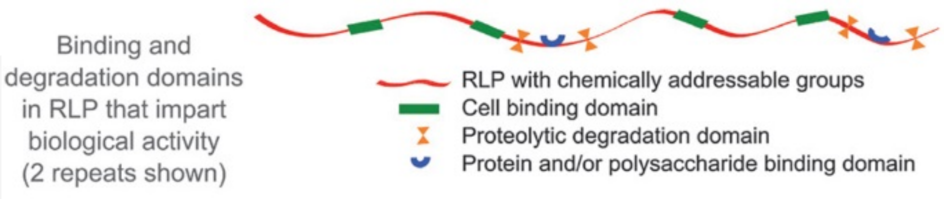


[Seliktar D 2012 Science]

Hydrogel

3D networks composed of cross-linked hydrophilic polymer chains
Can absorb up to thousands of times their dry weight in water
Examples:

- **Soft contact lenses** poly(hydroxyethylmetharylic)acid [poly(HEMA)]
- **Biological adhesive** reconstituted fibrin or albumin
- **Wound dressings** alginate polysaccharide
- **Fillers** hyaluronic acid (HA)



G{GG[GDQK
(GGRPSDSFGAPGGGNGGRPSDSFGAPGGGNGGRPSDSFGAPGGGNGGK)$_2$
GGGRGDSPAEDL]$_2$-
GPQGIWGQGGRGG- CKAAKRPKAAKDKQTKGEDLGDPMASMTGGQQM}$_n$G

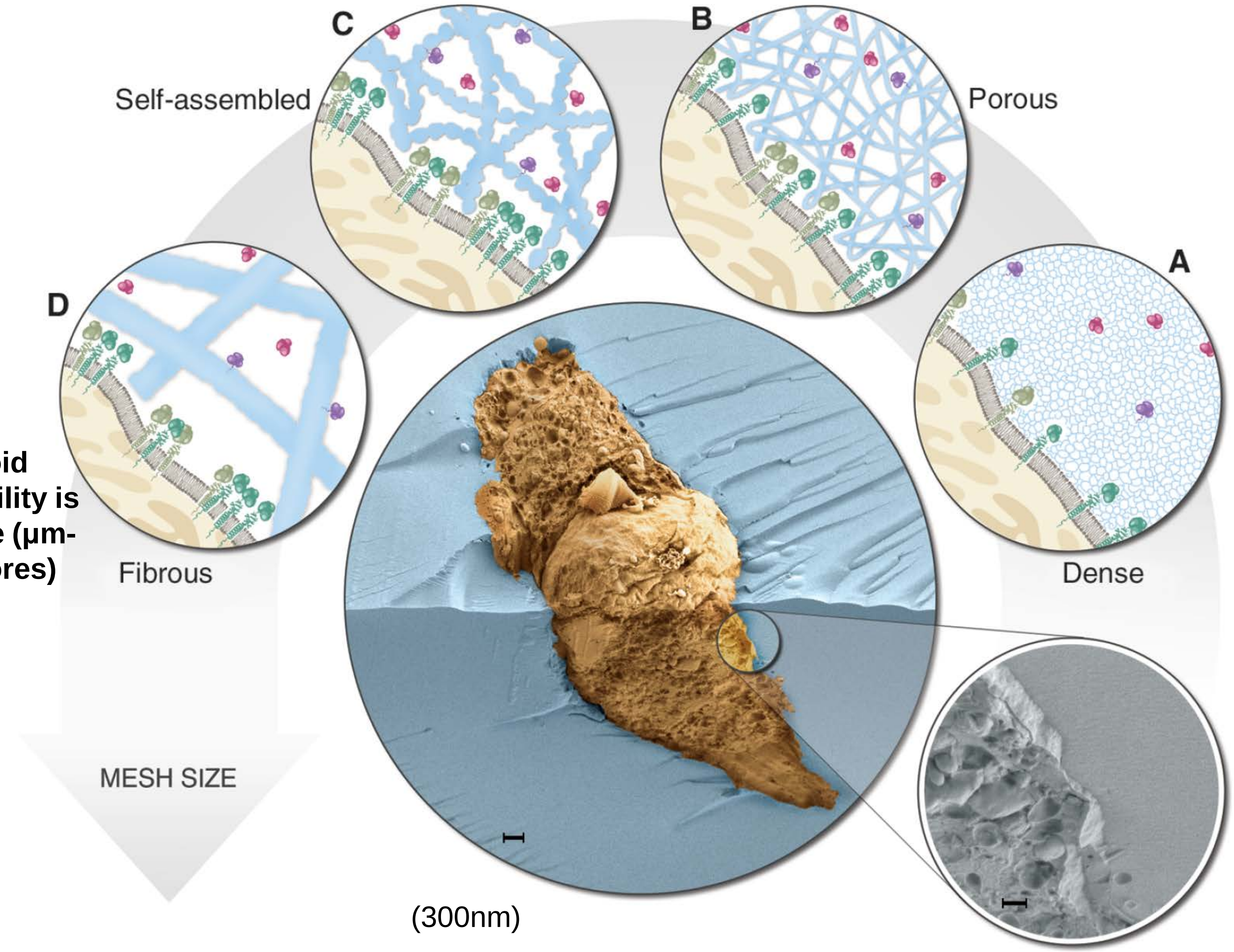
Designing complex hydrogel systems

- Major breakthroughs in synthetic polymer chemistry
- 3D molecular patterning techniques
- Biomimetic rational design approaches

Designing hydrogel molecular building blocks

- Cell-compatible hydrogel: control specific molecular interactions at the cell-material interface (cell adhesion, receptor-ligand complex)
- Designing the molecular structure of a hydrogel to control these spatial and temporal events enables their use of for guiding cell response in vivo and in vitro
  - Tirrell et al.- hydrogels that undergo reversible physical cross-linking based on shifts in temperature or pH
  - Kopecek et al.- stimuli-responsive hydrogels based on grafts of engineered coiled-coil or beta-sheet protein domains with a synthetic polymer backbone
  - Hubbell et al.- the proteolytically responsive synthetic hydrogels
  - Others - combined a variety of synthetic polymers and protease substrates – almost any synthetic hydrophilic polymer milieu can be designed to facilitate controlled cellular degradation and invasion
  - Biomimetic features – cell-adhesive integrin-binding domains, controlled release affinity binding domains, and transglutaminase cross-linking domains

Cell membrane – hydrogel interface



Amoeboid cell motility is possible (µm-sized pores)

(300nm)

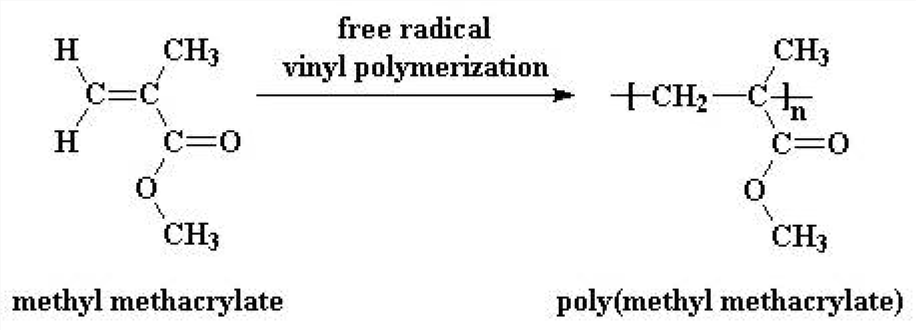
Conjugation in semi-synthetic hydrogel

$$\text{-CHO} + \text{H}_3\text{N-} \rightarrow \text{-CH=N-}$$

Schiff-base reaction

$$\text{-SH} + \text{HS-} \rightarrow \text{-S-S-}$$

Disulfide bonding



Free-radical initiated copolymerization

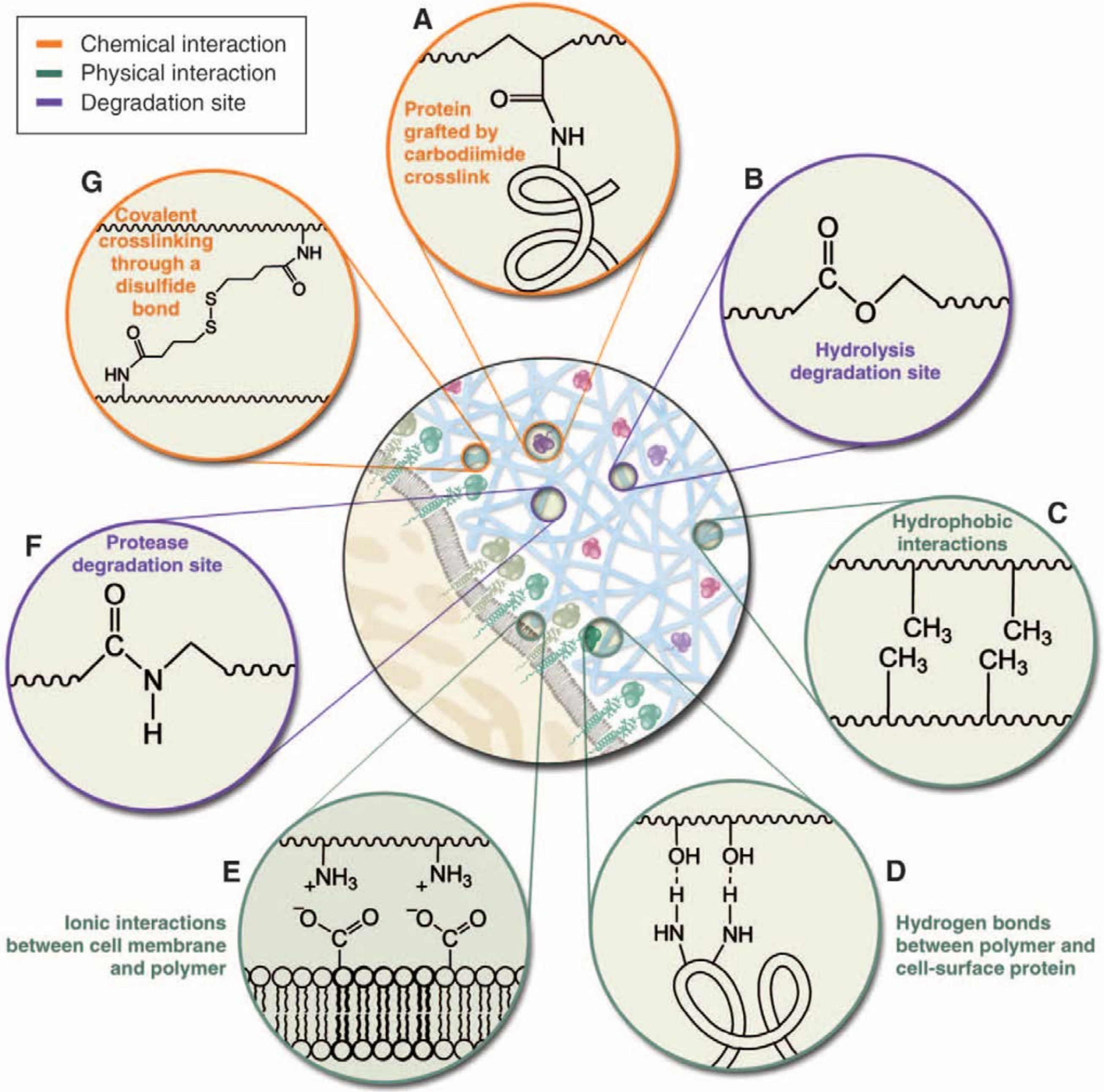
Semi-synthetic approach

- Covalent conjugation of hydrophilic polymers with reconstituted ECM proteins
- Relatively easy to manufacture
- Can be reproduced in large quantities
- Provide a more reliable material as compared with natural ECM hydrogels

Hydrogel structure and properties

Design criteria should be focused on…

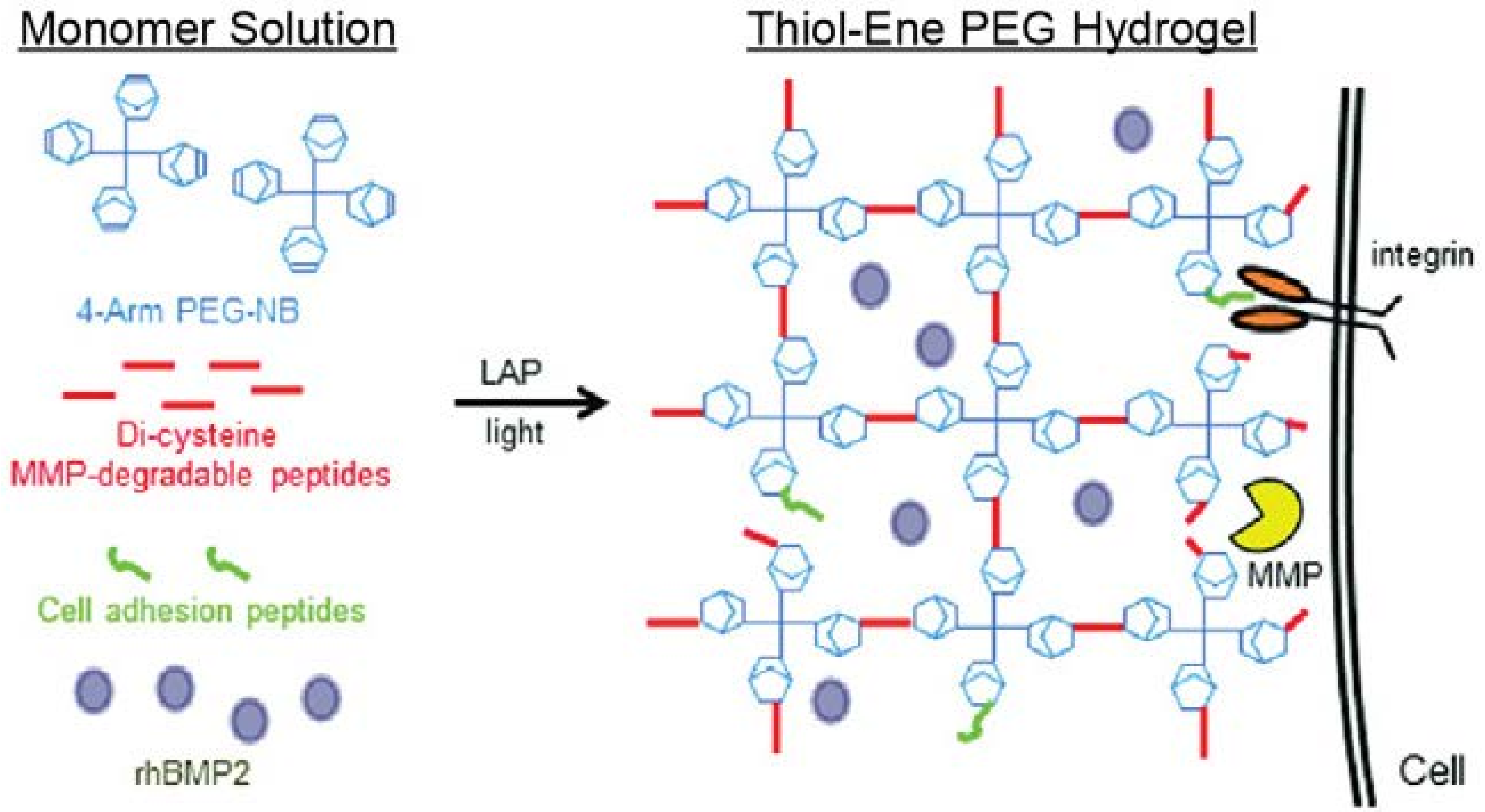
- Transport properties (controlled release)
  - Hydrophobic/hydrophilic nature of hydrogel
  - Hydrogel′s porosity (mesh size)
  - Hydrogel structure and mesh size – engineered for the size of protein
- Tissue interactions (bioactivity)
  - GF bioavailability regulated by nonspecific associations between GF and ECM
  - Covalent GF immobilization
- Chemical stability (degradability)
  - Essential for biomedical applications that require controlled resorption
  - Engineering the spatiotemporal aspects of this presents a challenge
- Bio-adhesion
  - Important property that allows cells and tissue to adhere to hydrogels has enabled their use as tissue adhesives in surgical repair (most of hydrogels are not adhesive)
  - Hydrogel modification using catechol moiety, Fibrin glue
- Mechanical properties
  - Convey physical cues to cells



Chemical interaction
Physical interaction
Degradation site
A
Protein grafted by carbodiimide crosslink
O
NH
B
O
C
O
Hydrolysis degradation site
C
Hydrophobic interactions
CH3
CH3
CH3
CH3
D
OH
OH
H
H
HN
NH
Hydrogen bonds between polymer and cell-surface protein
E
+NH3
+NH3
-O
C
O
Ionic interactions between cell membrane and polymer
F
Protease degradation site
O
C
N
H
G
Covalent crosslinking through a disulfide bond
NH
O
S
S
O
HN

Kristi Anseth (U. Colorado)
“Design of novel materials to regulate stem and progenitor cell expansion and differentitation”

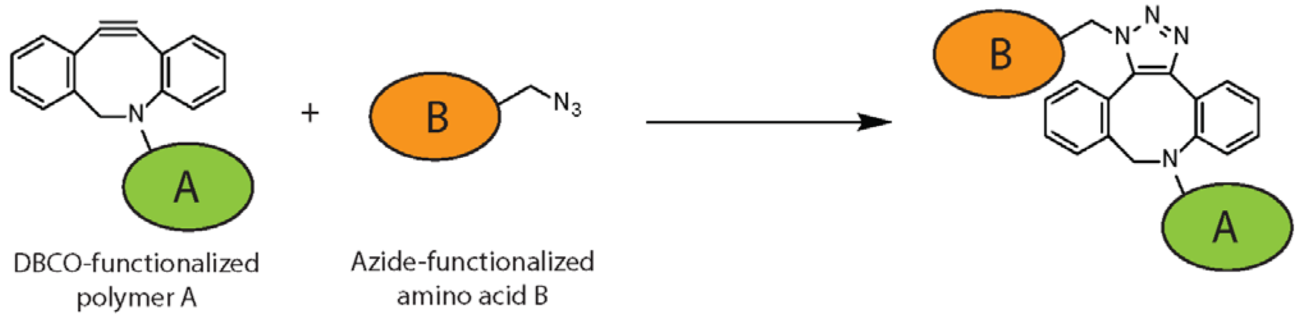
PEG network with poly-acrylamide kinetic chains



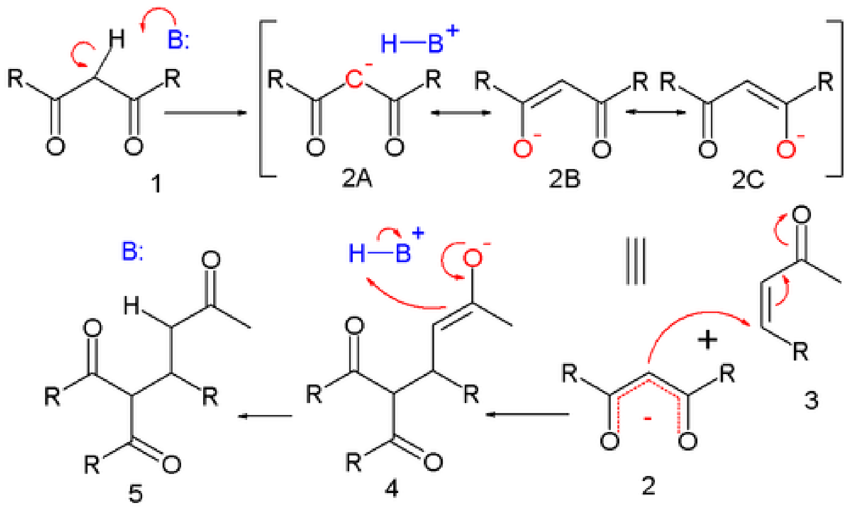
Kristi Anseth (U. Colorado)
Bio-click reactions

- Ca-catalyzed azide-alkyne
- SPAAC
- IED DAC
- Michael addition
- Photo-initiated thiol-ene coupling

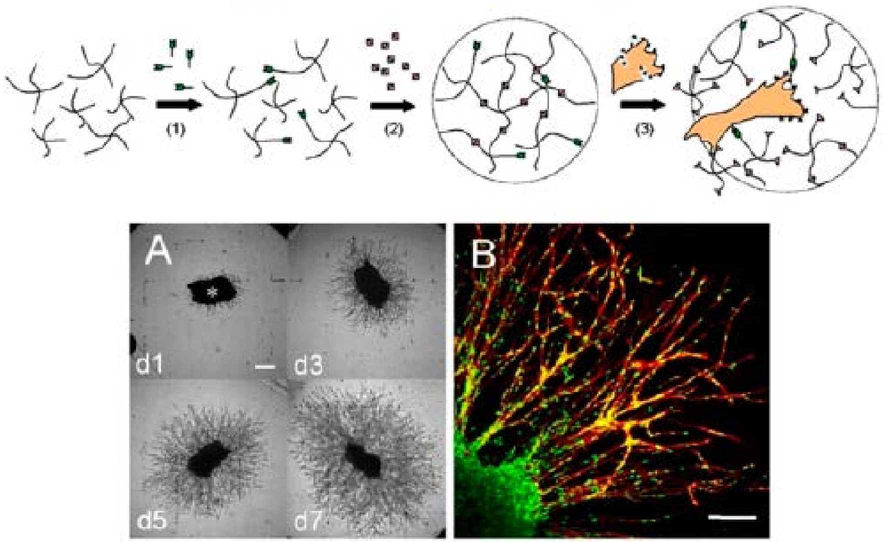
Strain-promoted alkyne-azide cycloadditions (SPAAC)



Michael addition



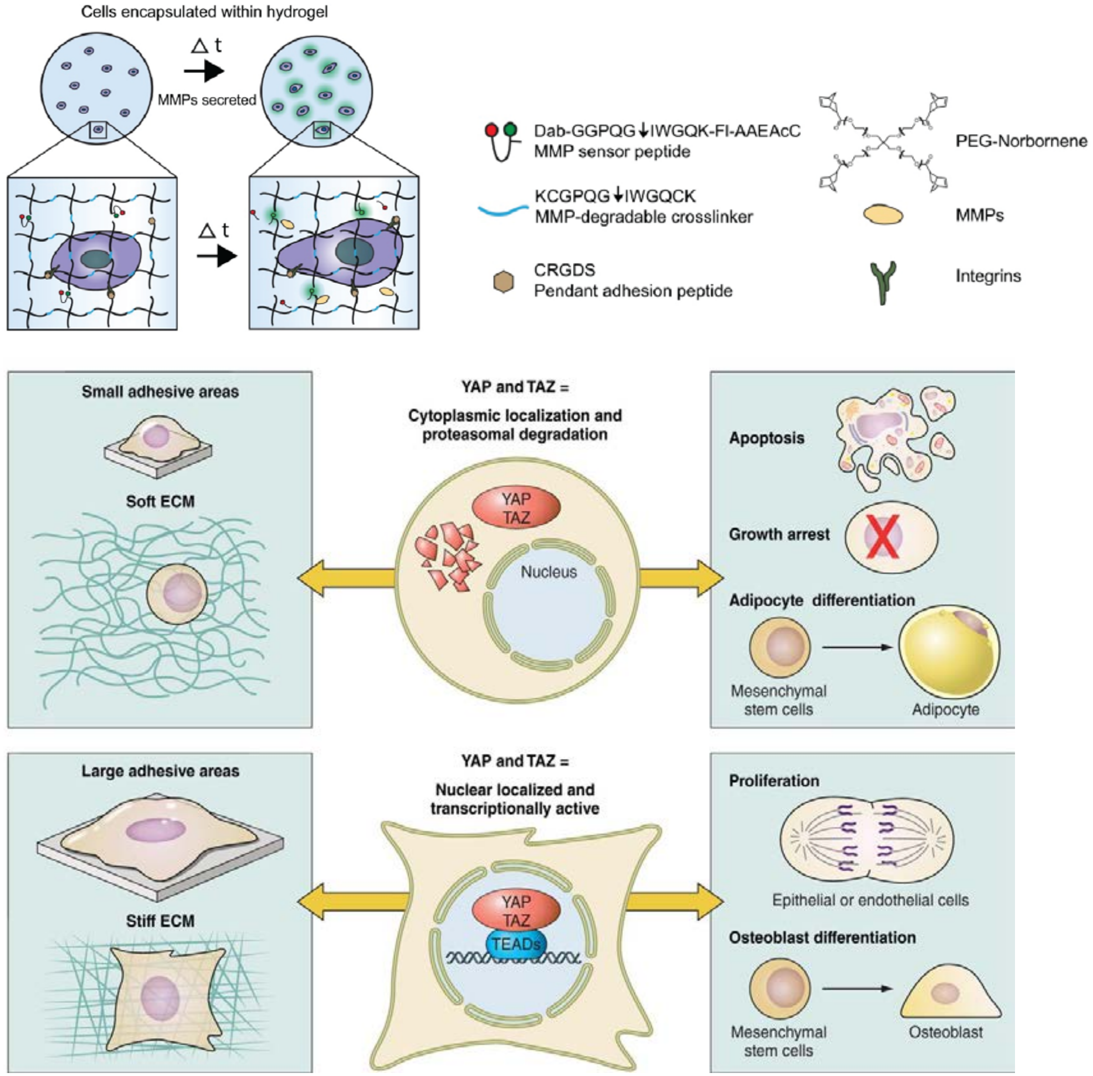
Degradable Hydrogels



* Introduction of photo-degradable linker

- Cell culture on soft hydrogel showed more engraftment after transplant
- YAP translocated to nucleus in cells cultured in stiff surface

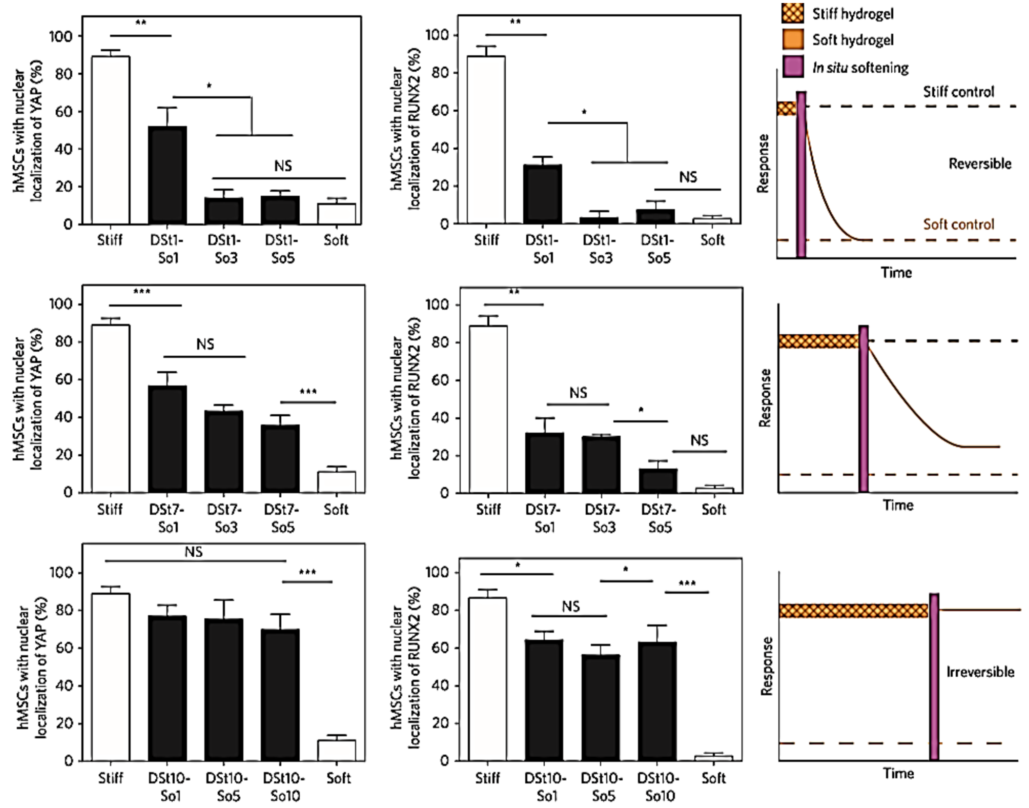
(YAP translocation is dependent on focal adhesion-mediated signaling)



* Mechanical memory and dosing

(by Photo-initiated degradation)

- Engineering organic polymer backbone to induce cleavage by light
- High dose mechanical surface cannot be reverted easily. (High dose = longer time exposure)
- This response also biases differentiation (direct the tendency of differentiation)
- PI3K is activated on stiff substrates compared with Soft substrates



Natural Origin Materials for Bone Tissue Engineering

Features for Ideal Scaffold in Tissue Engineering

- **Biocompatibility**
- **Porosity**
- **Biodegradability**
- **Cell-friendly environment.**

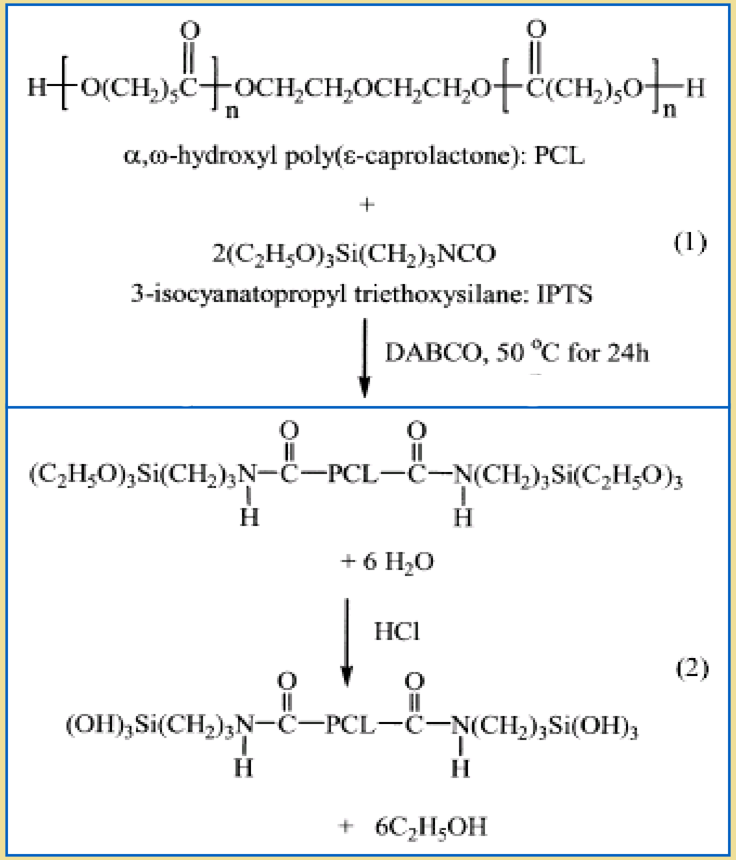
Bone biomaterials

Osteo-conductivity
Osteo-inductivity

-Needs mineralization starting point
(inorganic component)

Osteo-conductive vs. Osteo-inductive Materials

Polycaprolacton-based bone scaffold



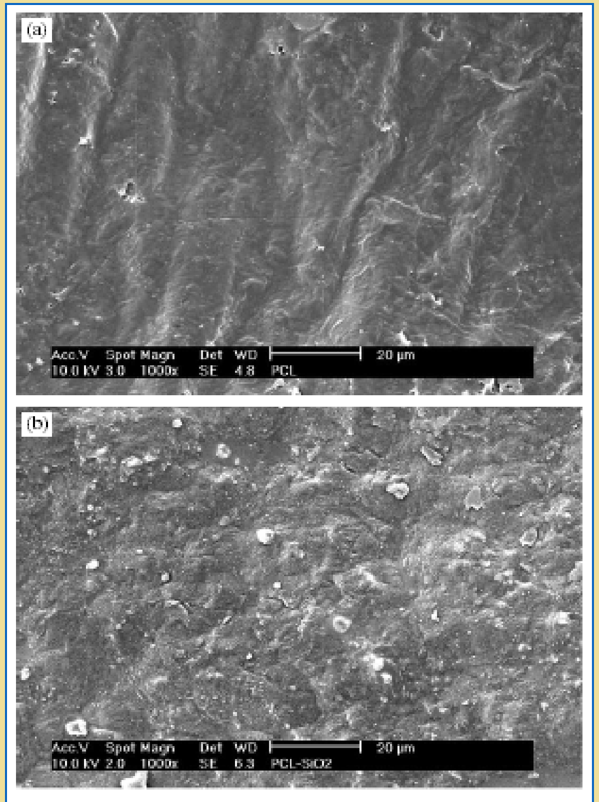


Fig. 2. SEM photographs of as-prepared specimens (a) P and (b) S.

S. H. Rhee *et al.*, *Biomaterials* 2002, 4915

Simulated Body Fluid (SBF)

	Plasma	SBF
Na^{+}	142.0	142.0
K^{+}	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^{-}	103.0	147.8
MCO^{3-}	27.0	4.2
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.2-7.4	7.4

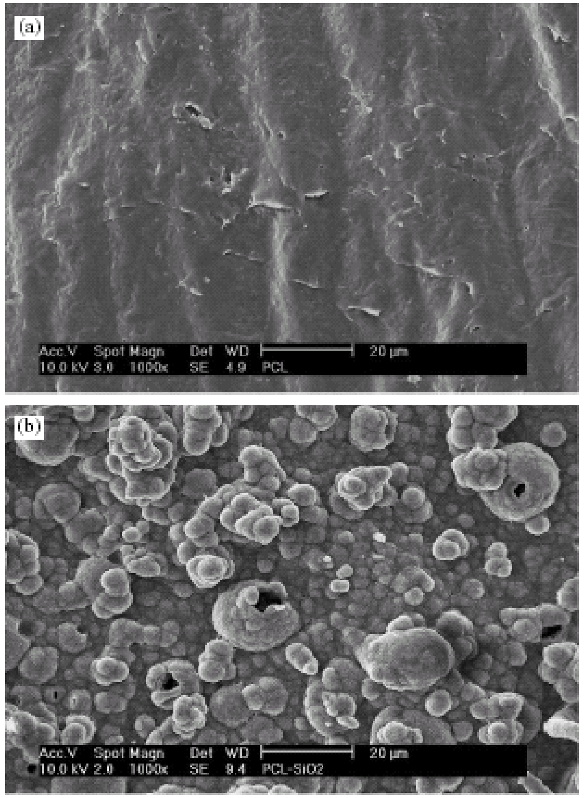


Fig. 5. SEM photographs of specimens (a) P and (b) S after soaking in the SBF for 1 week at 36.5°C.

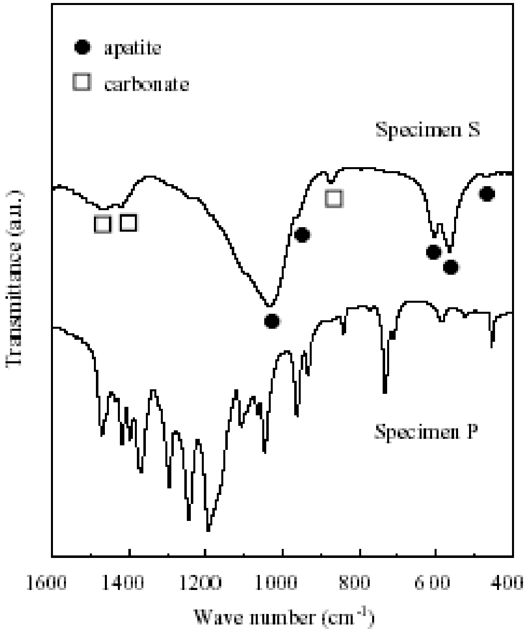


Fig. 6. FT-IR spectra of specimens P and S after soaking in the SBF for 1 week at 36.5°C.

S. H. Rhee *et al.*, *Biomaterials* 2002, 4915

Natural-based ceramics

Calcium phosphate – HAp

Silicates

Calcium Phosphate

Hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$

- Comprise bone substitute

$CaCO_3$ (calcium carbonate)**: calcium precursor for obtaining different ceramics**

- Species possessing $CaCO_3$ skeleton: corals, sponges, mollusk shells, fish bones
- Shells
  - Multi-layered microstructures consist of calcium carbonate
  - Organic component 1-5 wt%
- Nacre
  - 95-98wt% inorganic
  - 2-5 wt% organic matter
- Sponges
  - Hierarchical organization
  - Fibrous skeletons
  - Mineralized spicules
  - Contain amorphous silica or calcium carbonate
  - Coral skeletal carbonate: Substitute materials for orthopedics and dentistry

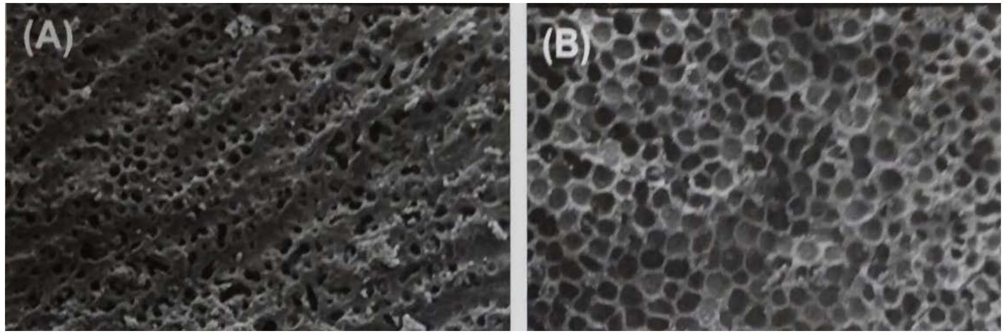
$10CaCO_3 + 6(NH_4)2HPO_4 + 2H_2O$ ->

$Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)2CO_3 + 4H_3CO_3$

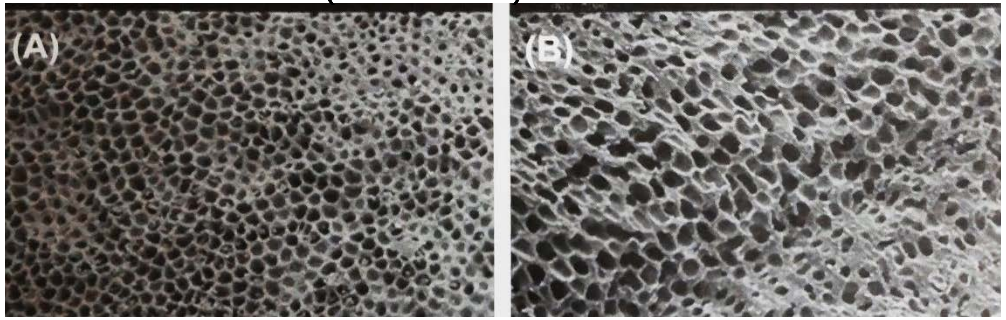
SEM micrograph of Red algae *Lithothamnion glaciale*

Porosity, pore size, pore interconnectivity
-> key issues in bone tissue regeneration

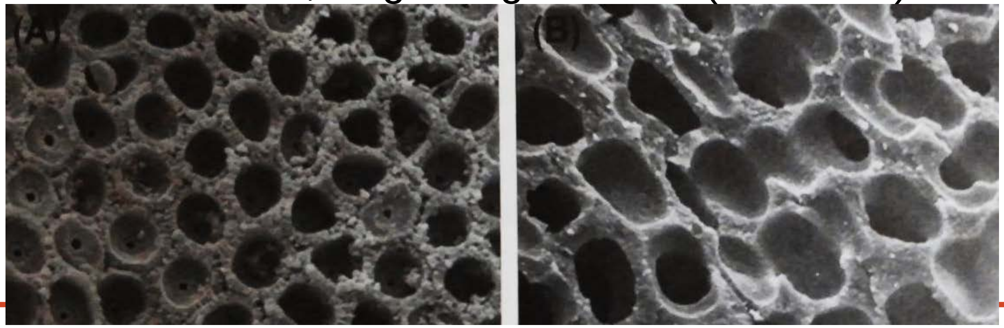
No treatment



Heat treatment (400°C/3h)



Heat treatment, High magnification (400°C/3h)



- Marine-derived calcium carbonate skeletons are unsuitable for most applications due to its fast dissolution rate & poor stability
- Converting the hard calcium carbonate skeleton of mineralized algae into calcium phosphates without destroying its native architecture by combined treatment (thermal & chemical)

Silicates

Sponges & diatom
Sponges contain amorphous silica spicules
Diatom can accumulate silica at intracellular concentrations up to 250 times higher than that in the surrounding media

- Centric diatoms: radially symmetrical
- Pennate diatoms: elongated & bilaterally symmetrical

Silicate glass can be converted to HAp by soaking the substrates in a solution of K_2HPO_2 with a pH value of 9.0 at 37^oC
The silica/collagen hybrid materials supported the adhesion, proliferation, and osteogenic differentiation of human mesenchymal stem cells

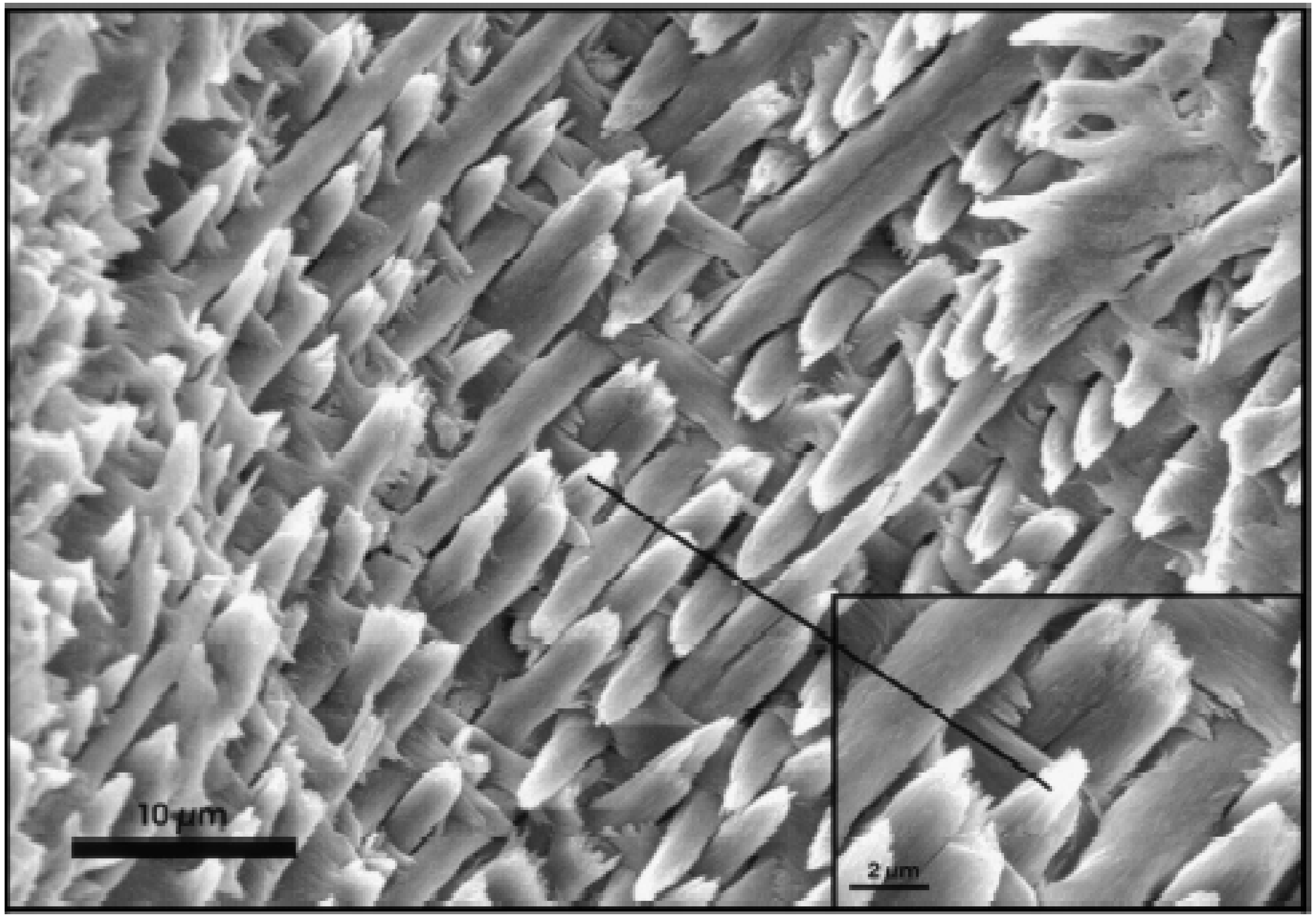


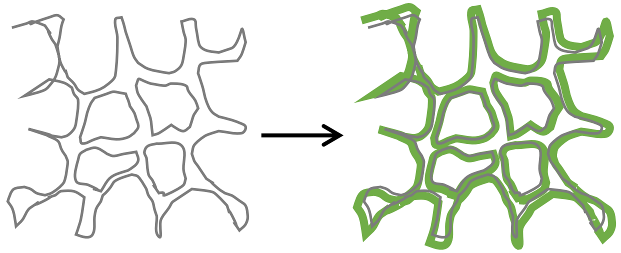
FIG. 1. The organization of dental enamel. Scanning electron micrograph of the surface of an acid-etched ground section of mature mouse incisal dental enamel. Ordered arrays of enamel prisms are each constructed of parallel bundles of carbonated hydroxyapatite enamel crystallites. Each prism structure is considered to arise as the product of a single secretory ameloblast cell of the inner enamel epithelium. (Inset) An enlargement of the center section of the images as indicated. [Electron micrograph courtesy of Dr. W. Luo.]

Fincham A.G. *et al.*, *J Structureal Biology.* 1999, 126:270

Demerit of synthetic bone graft materials

Type of Hybrid Material

ECM on Ceramic



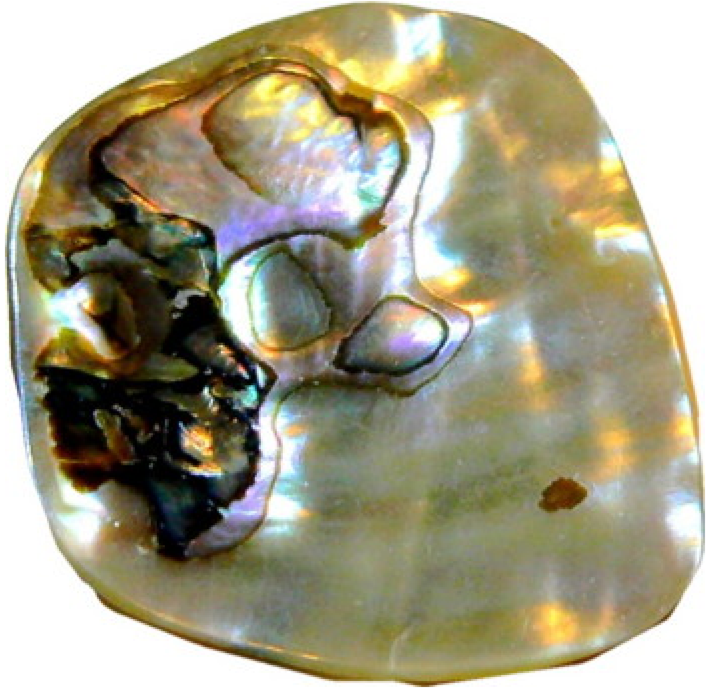
Mechanical property
depends on **ceramic**

Ceramic on organic polymer

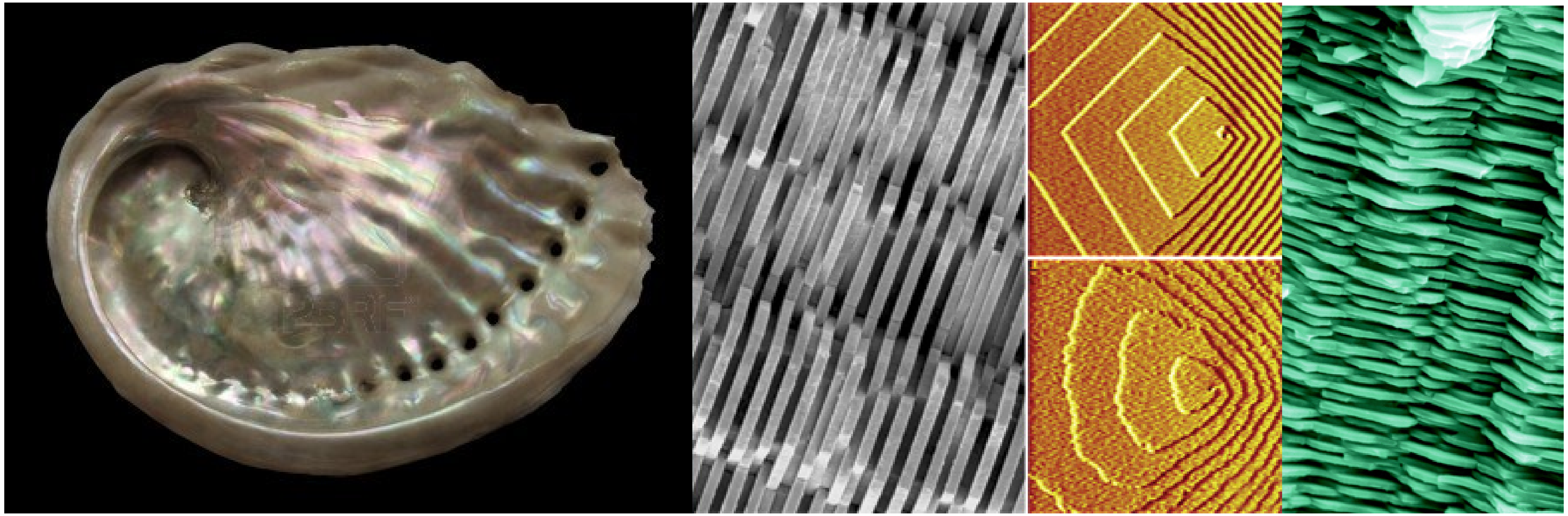
Mechanical property
depends on **organic polymer**

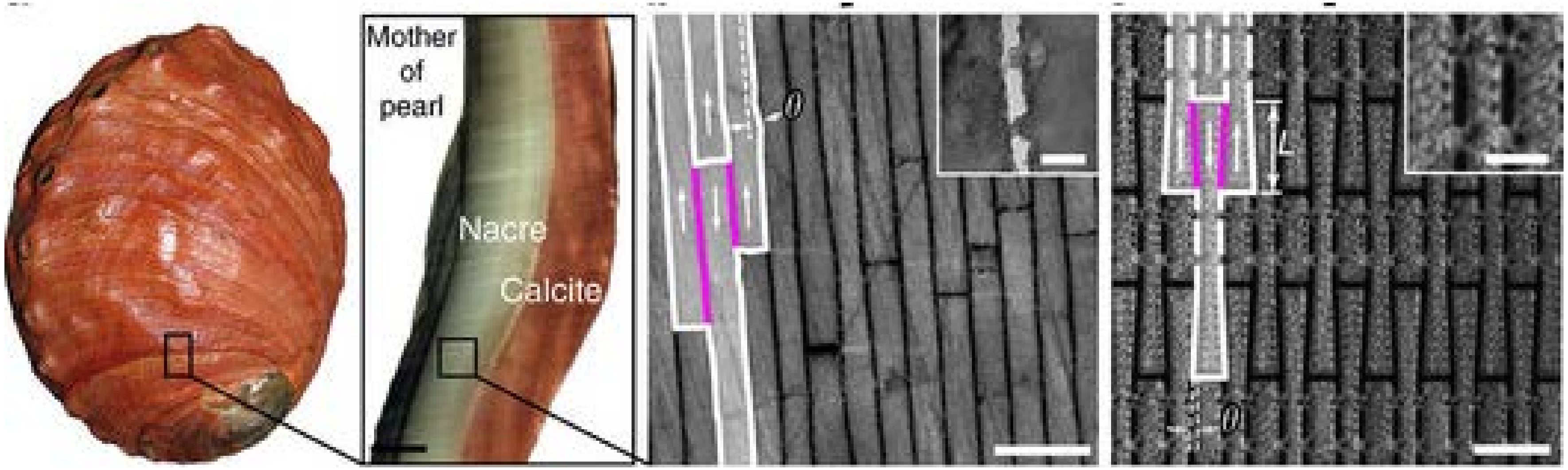
Inorganic material
Organic material

Nacre

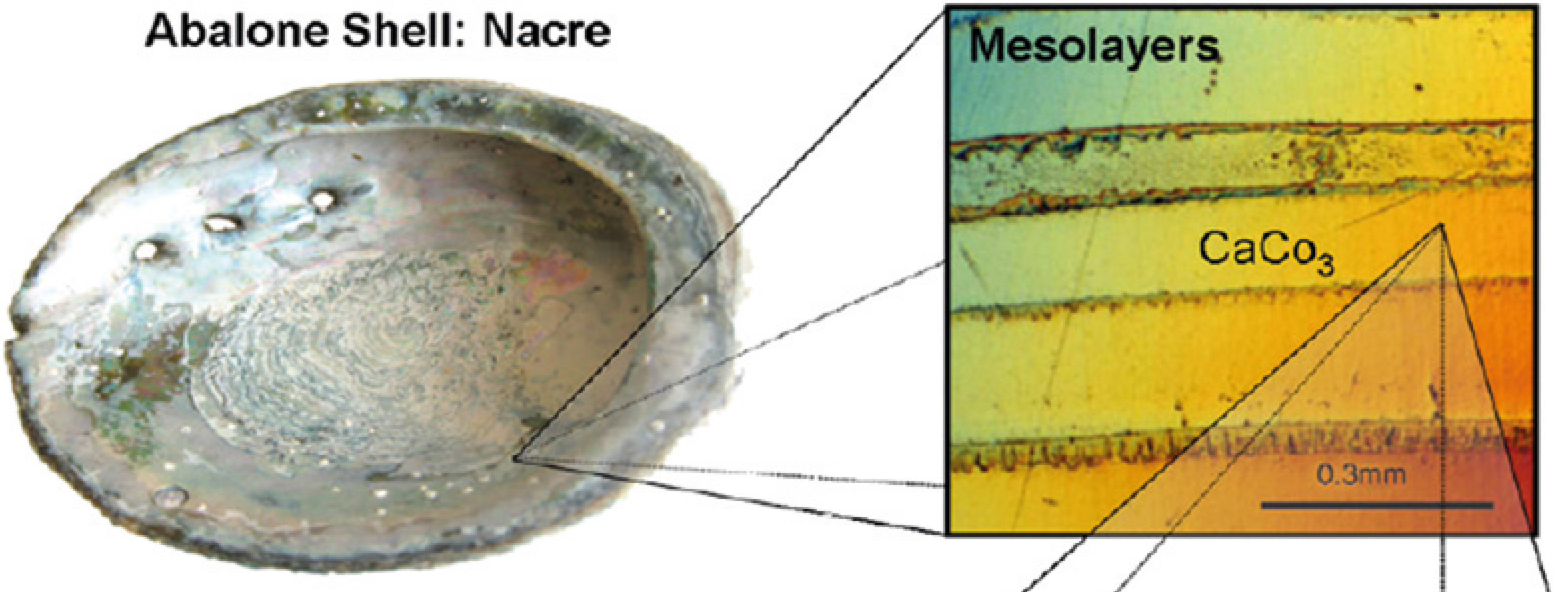


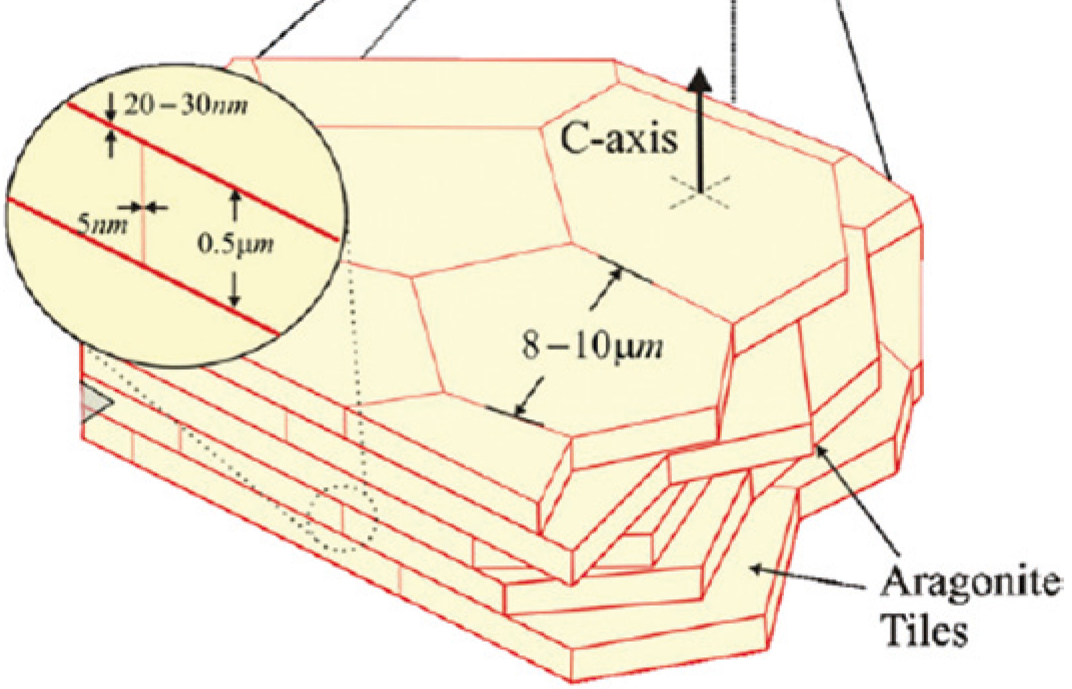
Nacre

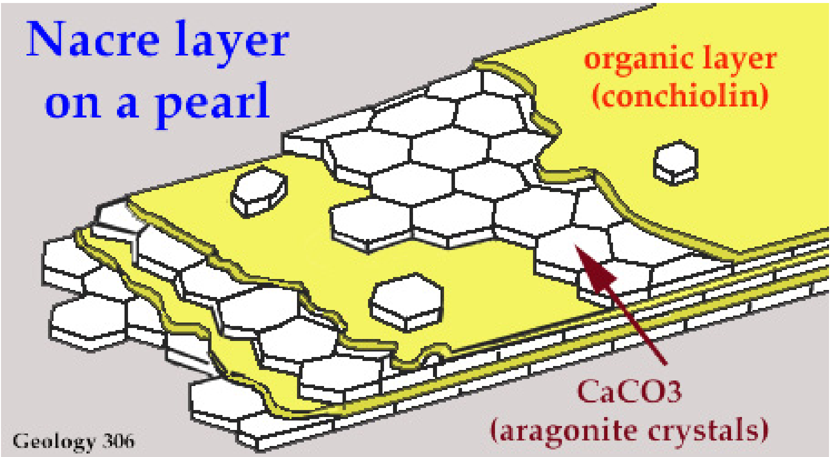




Abalone Shell: Nacre







One-dimensional tissue engineering

- Bone marrow transplantation
  - One fluid is replaced by another
  - Due to its simplicity, it is the most routine demonstration of stem cell-based therapy to date

Two-dimensional tissue engineering

- Reconstruction of skin and corneal surfaces

Three-dimensional tissue engineering

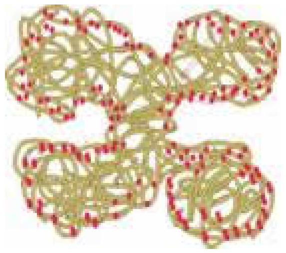
- Reconstruction of bones and teeth
  - More difficult in terms of maintaining the appropriate spatial relationships between different cell types, and importantly, in establishing appropriate vascularization.

3D Structure Matters!!!

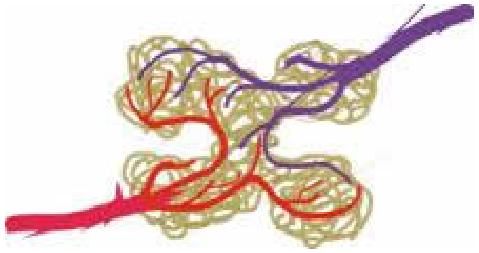
Realization of…

- 3D contour
    - Patient-customized structure with precision
- Vascularity
    - Viability of tissue/organ
- Complexity of stroma composition
    - Functionality of tissue/organ
- Cellularity
    - Distribution of cells in the scaffold

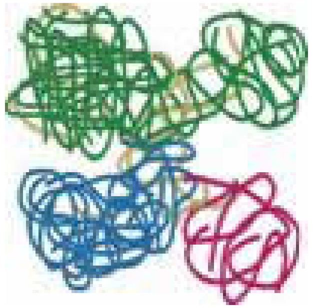
Current Scaffolding Issues



Cell Distribution



Vascularization

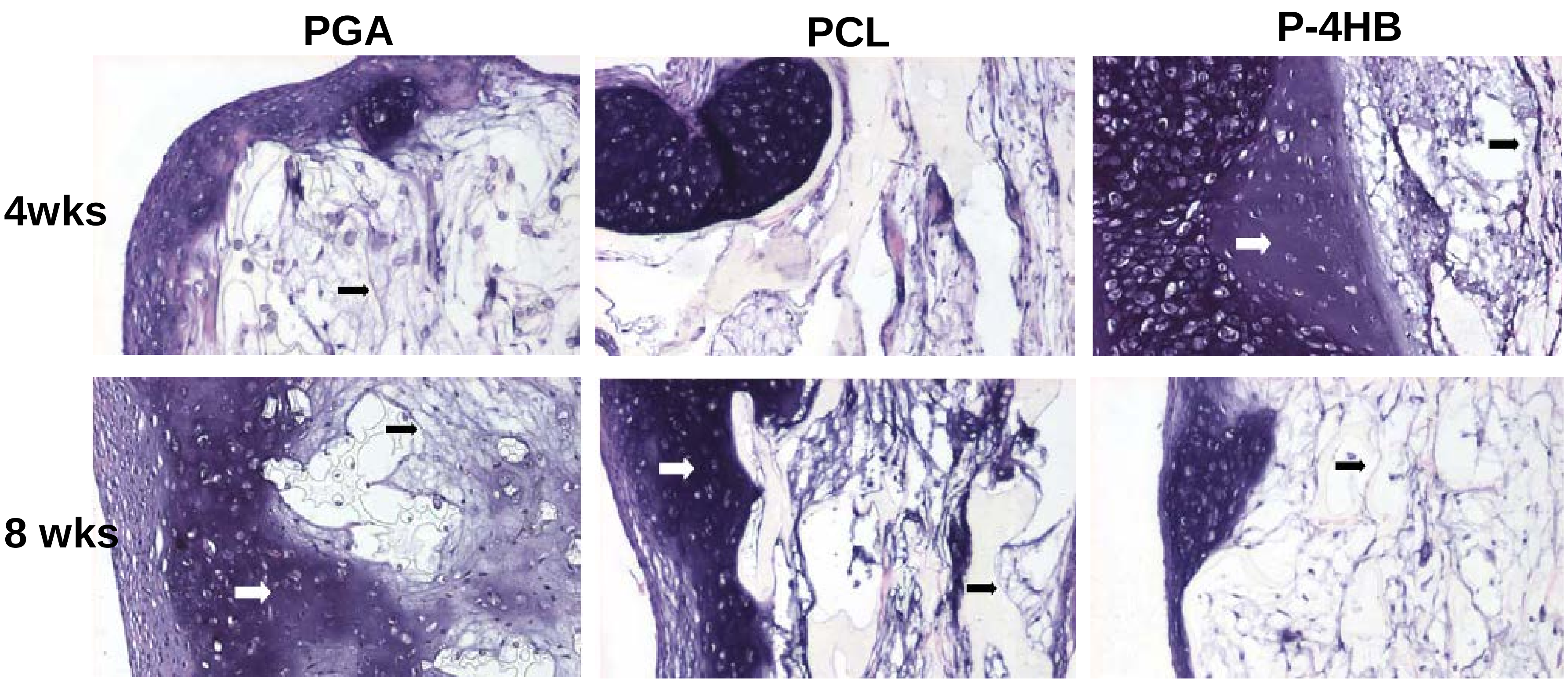


Heterogeneous Composition

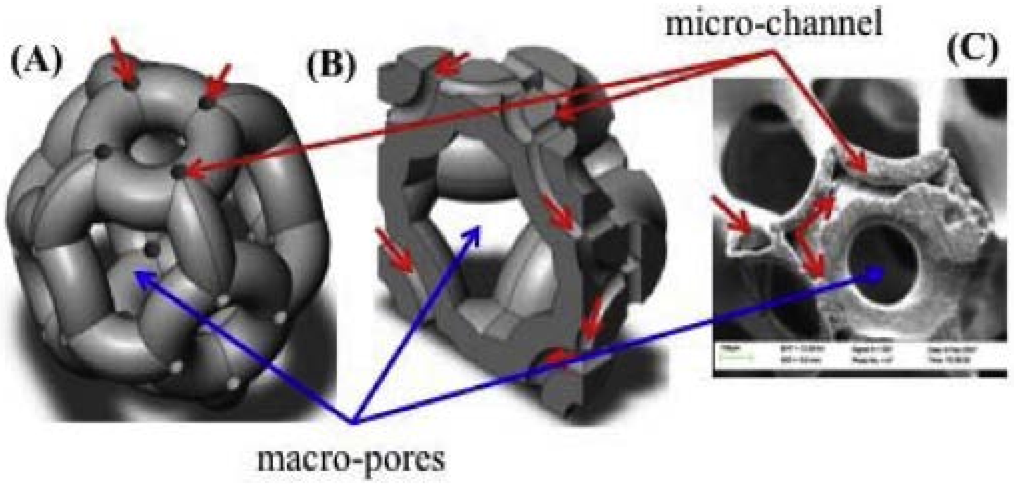
Diffusion limitations of nutrients

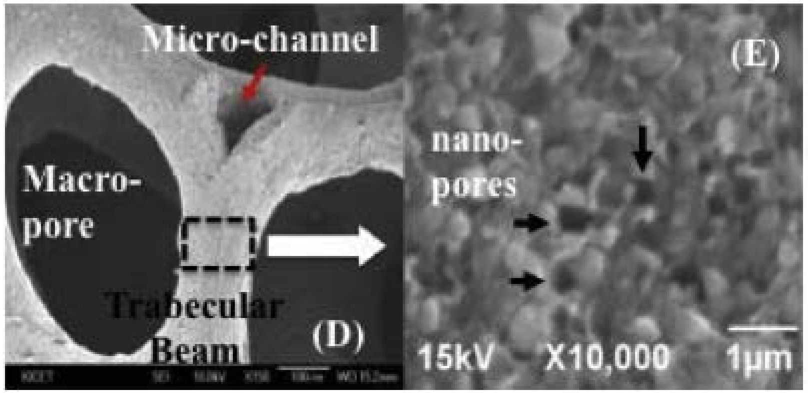
Vascular supply can grow into the scaffold after implantation

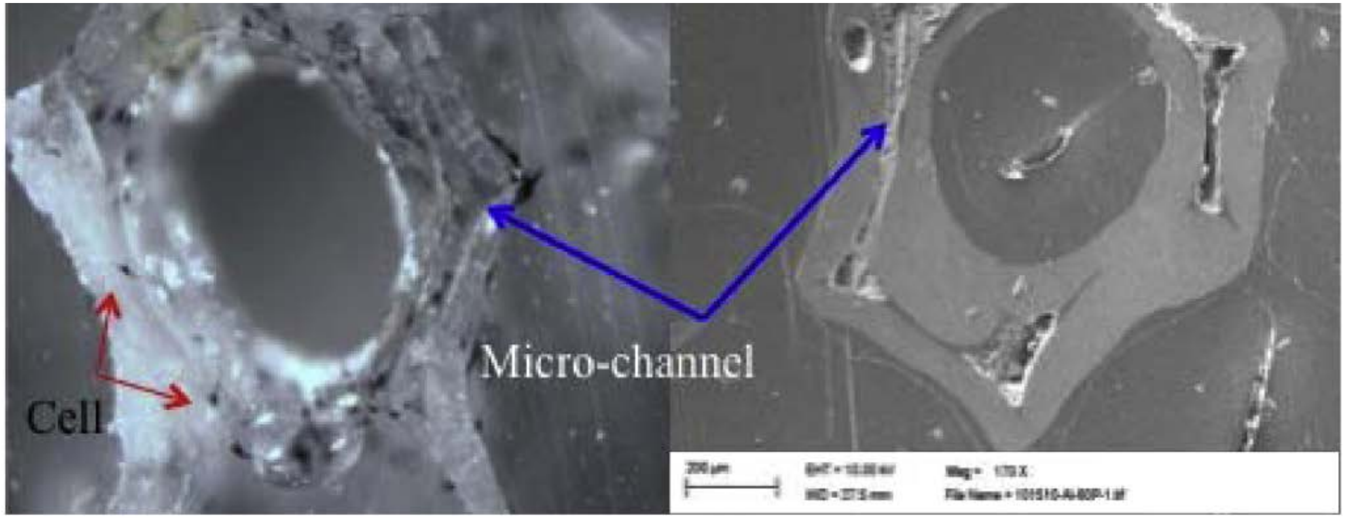
Problem Caused by Insufficient Cell Penetration

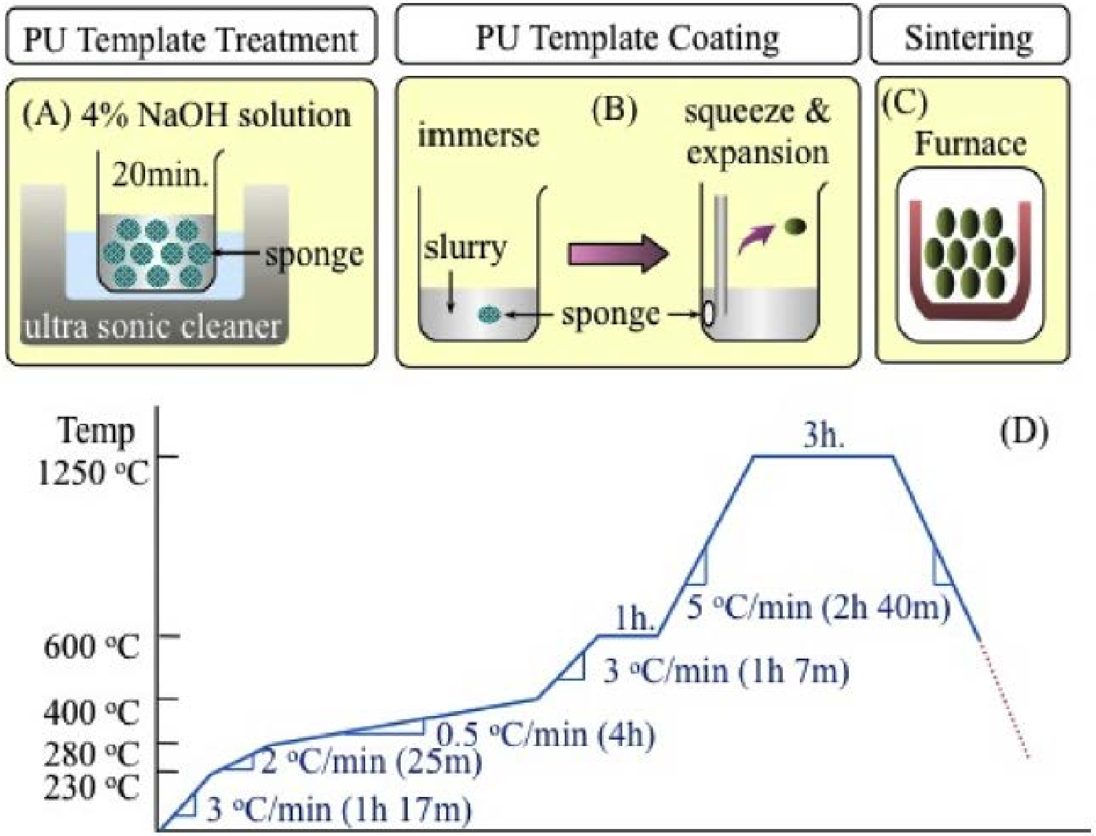


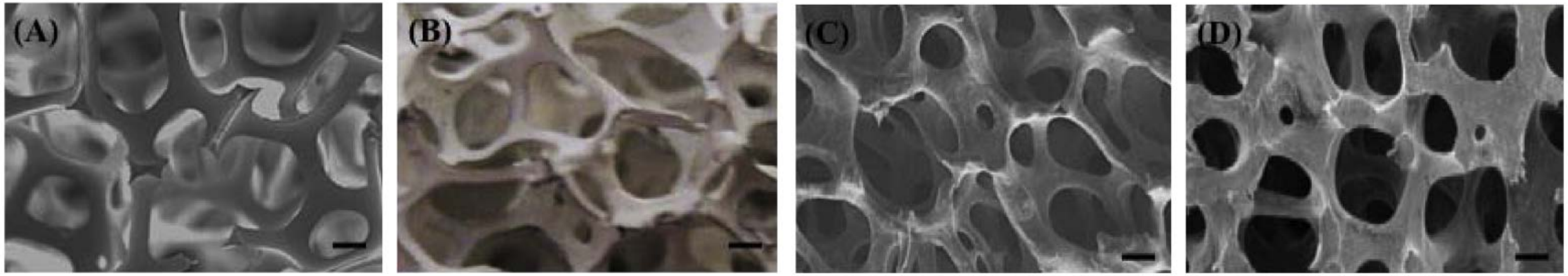
Shieh SJ 2004 Biomaterials

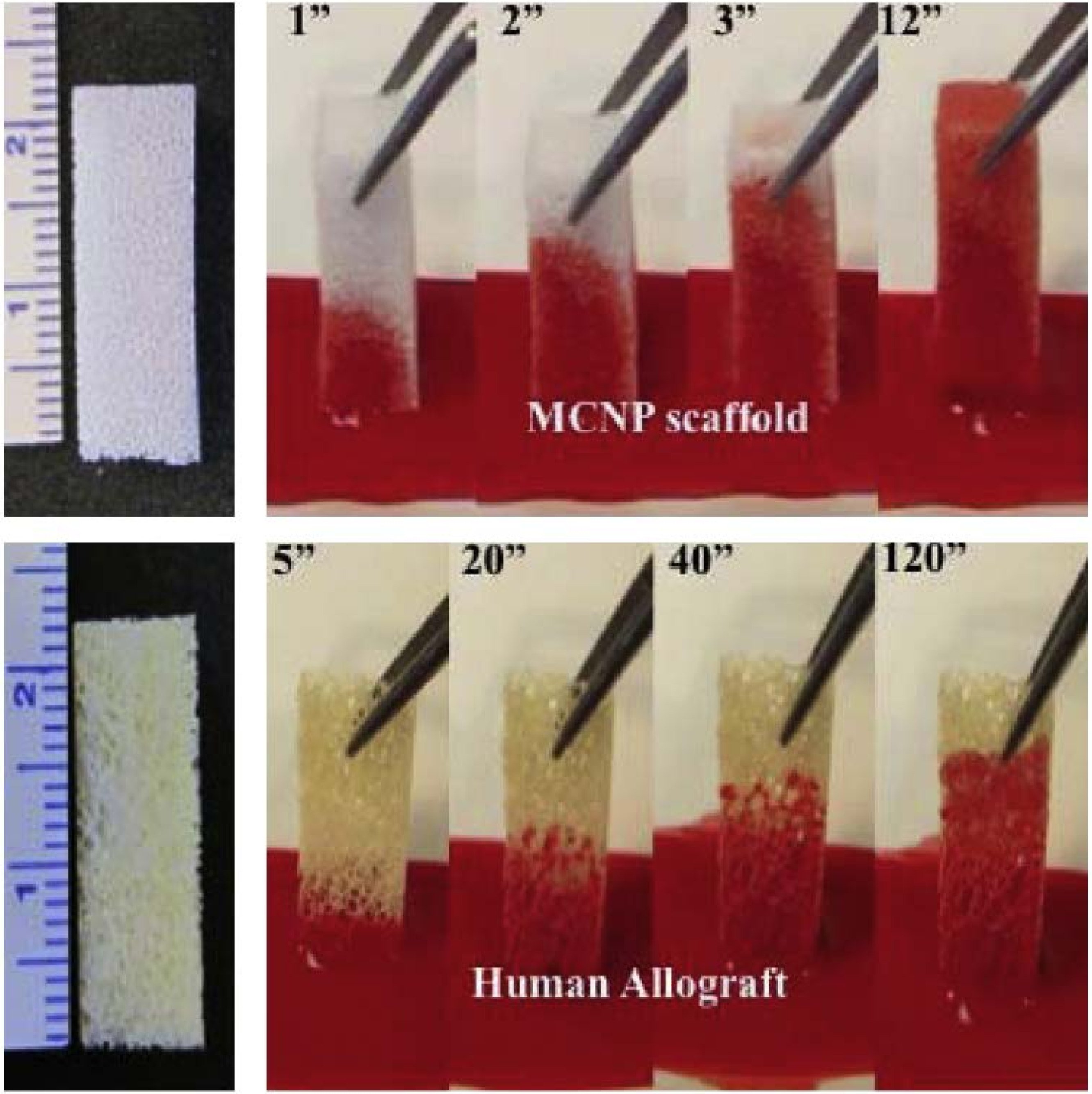

micro-channel
(A)
(B)
(C)
macro-pores

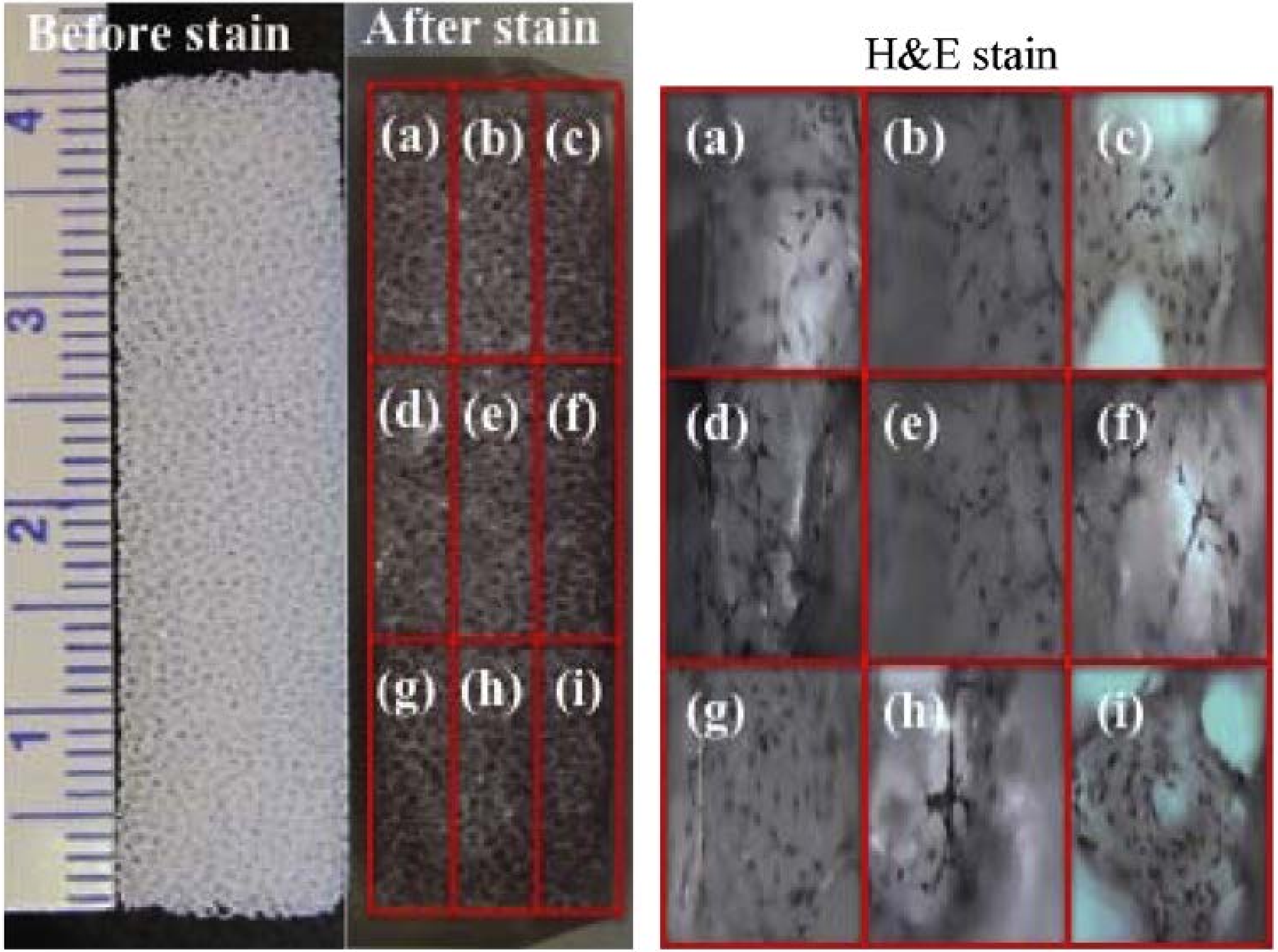

Micro-channel
(E)
Macro-
pore
nano-
pores
Trabecular
Beam
(D)
15kV
X10,000
1μm


Micro-channel
Cell


PU Template Treatment
PU Template Coating
Sintering
(A) 4% NaOH solution
20min.
sponge
ultra sonic cleaner
immerse
(B)
squeeze & expansion
slurry
sponge
(C)
Furnace
Temp
1250 °C
600 °C
400 °C
280 °C
230 °C
(D)
3h.
1h.
5 °C/min (2h 40m)
3 °C/min (1h 7m)
0.5 °C/min (4h)
2 °C/min (25m)
3 °C/min (1h 17m)


(A)
(B)
(C)
(D)


1"
2"
3"
12"
MCNP scaffold
5"
20"
40"
120"
Human Allograft
2
1
2
1


Before stain
After stain
H&E stain
(a) (b) (c)
(d) (e) (f)
(g) (h) (i)
(a) (b) (c)
(d) (e) (f)
(g) (h) (i)
4
3
2
1

Intact ECM

Use of a homogenous scaffold materials: sub-optimal
Intact ECM

- Consist of all the structural and functional molecules secreted by the resident cells
- This diverse collection of molecules is arranged in the unique 3D architecture of the native tissue if processed appropriately
- Manufactured by decellularization

Product	Company	Material	Processing	Form
AlloDerm	Lifecell	Human skin	Natural	Dry sheet
AlloPatch®	Musculoskeletal Transplant Foundation	Human fascia lata	Natural	Dry sheet
Axis™ Dermis	Mentor	Human dermis	Natural	Dry sheet
Bard® Dermal Allograft	Bard	Cadaveric human dermis	Natural	Dry sheet
CuffPatch™	Arthrotek	Porcine small intestinal submucosa (SIS)	Crosslinked	Hydrated sheet
DurADAPT™	Pegasus Biologicals	Horse pericardium	Crosslinked	Dry sheet
Dura-Guard®	Synovis Surgical	Bovine pericardium	Crosslinked	Hydrated sheet
Durasis® SIS	Cook	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Durepair®	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
FasLata®	Bard	Cadaveric fascia lata	Natural	Dry sheet
GraftJacket®	Wright Medical Tech	Human skin	Natural	Dry sheet
Oasis®	Healthpoint	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
OrthADAPT™	Pegasus Biologicals	Horse pericardium	Crosslinked	Dry sheet
Pelvicol®	Bard	Porcine dermis	Crosslinked	Hydrated sheet
Peri-Guard®	Synovis Surgical	Bovine pericardium	Crosslinked	Dry sheet
Permacol™	Tissue Science Laboratories	Porcine skin	Crosslinked	Hydrated sheet
PriMatrix™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Restore™	DePuy	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Stratasis®	Cook	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
SurgiMend™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Surgisis®	Cook	Porcine small intestinal submucosa (SIS)	Natural	Dry sheet
Suspend™	Mentor	Human fascia lata	Natural	Dry sheet
TissueMend®	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Vascu-Guard®	Synovis Surgical	Bovine pericardium	Crosslinked	Dry sheet
Veritas®	Synovis Surgical	Bovine pericardium	Crosslinked	Hydrated sheet
Xelma™	Molnlycke	ECM protein, PGA, water		gel
Xenform™	TEI Biosciences	Fetal bovine skin	Natural	Dry sheet
Zimmer Collagen Patch®	Tissue Science Laboratories	Porcine dermis	Crosslinked	Hydrated sheet

EunAh Lee @ KHU

7/26/2017, 5:18AM

Turning pig organs to human organs

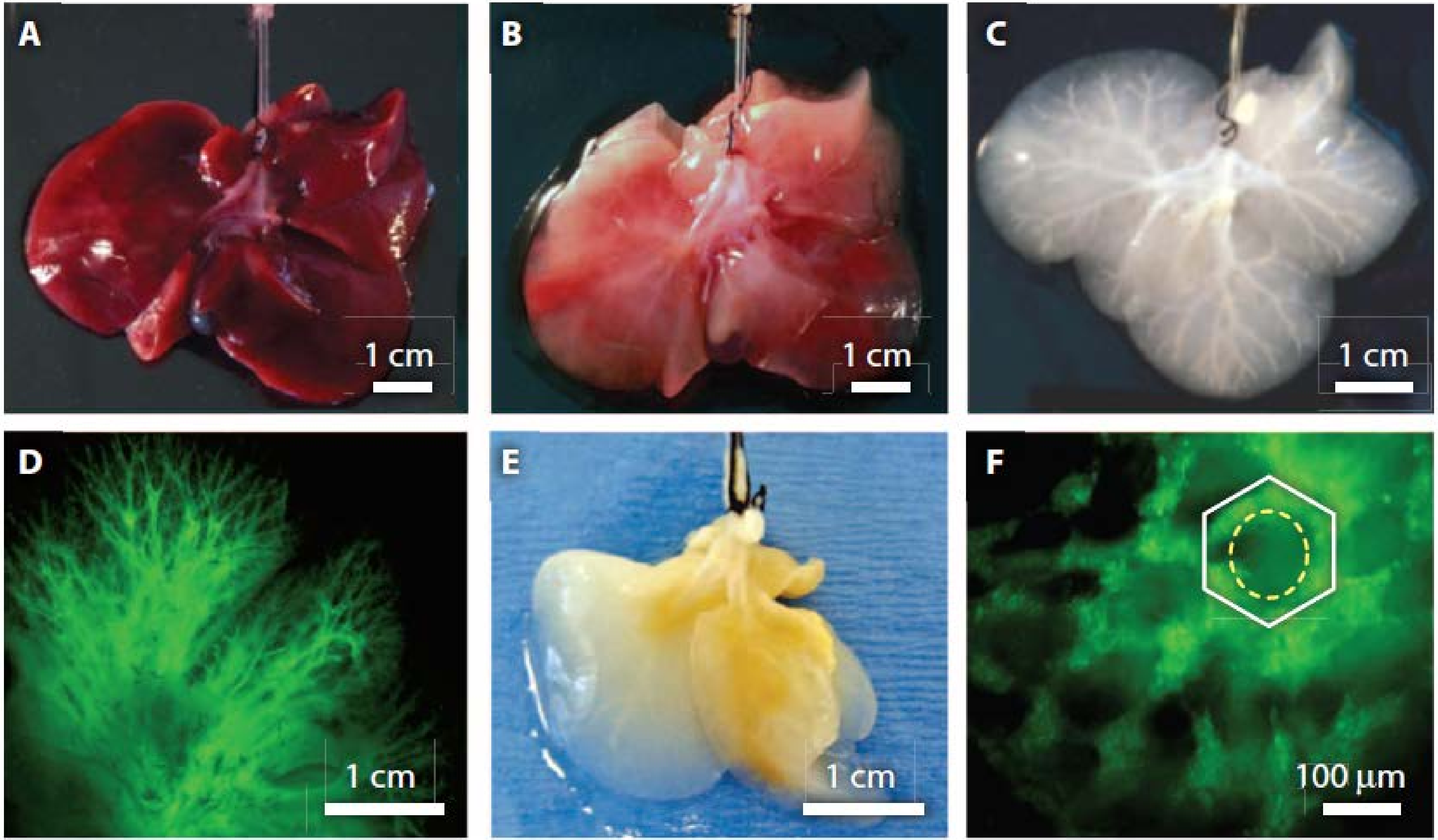
With research stemming from the University of Minnesota, researchers are attempting to repopulate pig organs with human cells.

By SYDNEY BAUM-HAINES

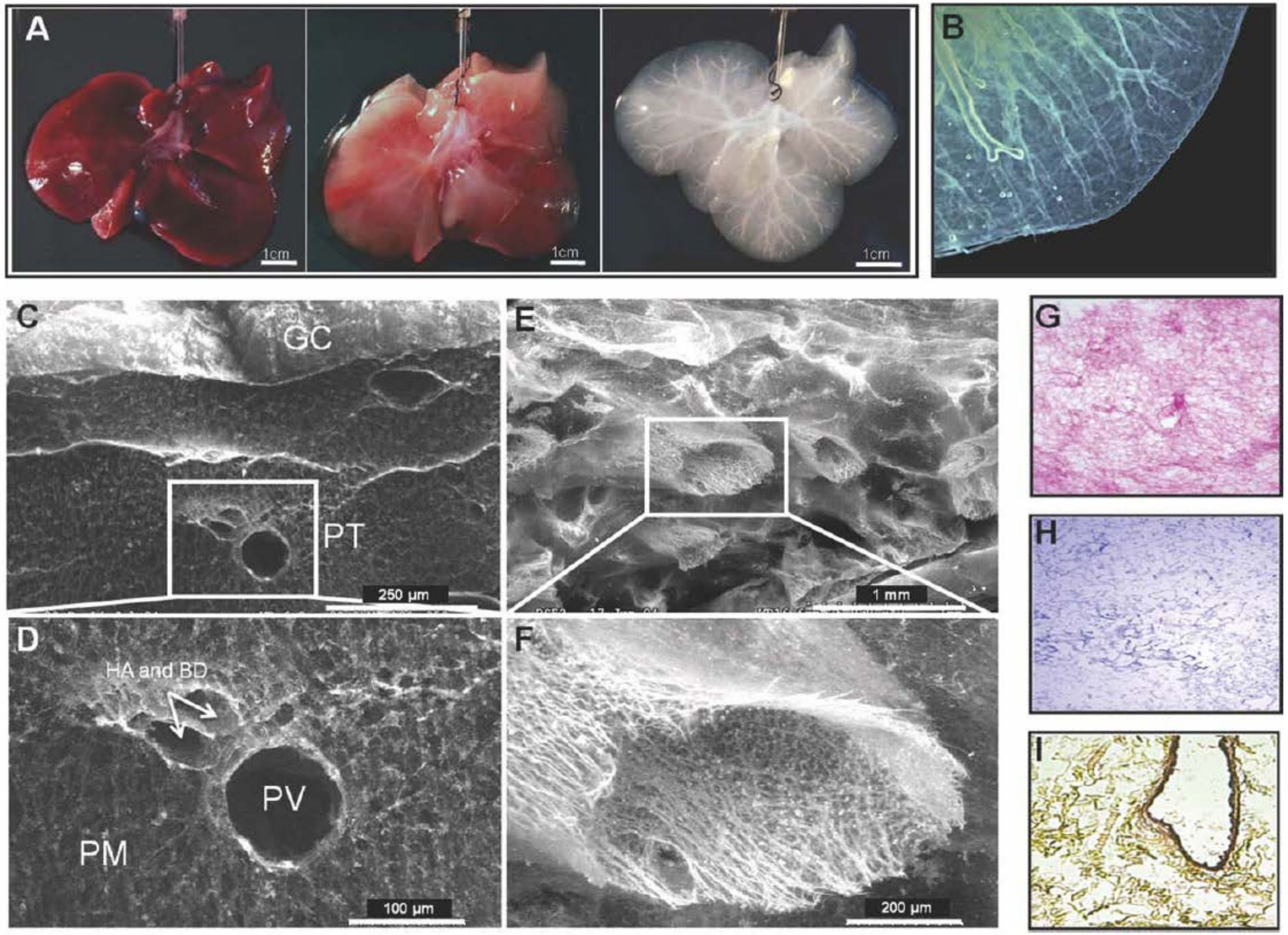
ELLEN SCHMIDT

University alumnus and Miromatrix CEO Jeff Ross holds a decellularized pig heart at his company's space on Tuesday, July 25, in Eden Prairie, Minn. Miromatrix flushes a detergent through a pig organ by way of a major vessel, which preserves the organ's vascularization. The organs are then recellularized to be used as transplants.

Engineering Liver Tissue with Decellularized Donor Organs

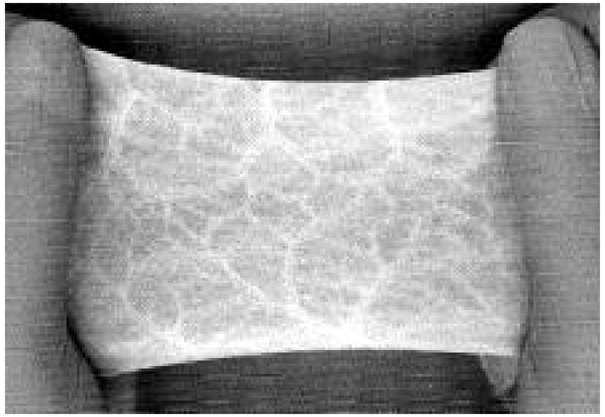


Atala A - 2012 Sci Transl Med

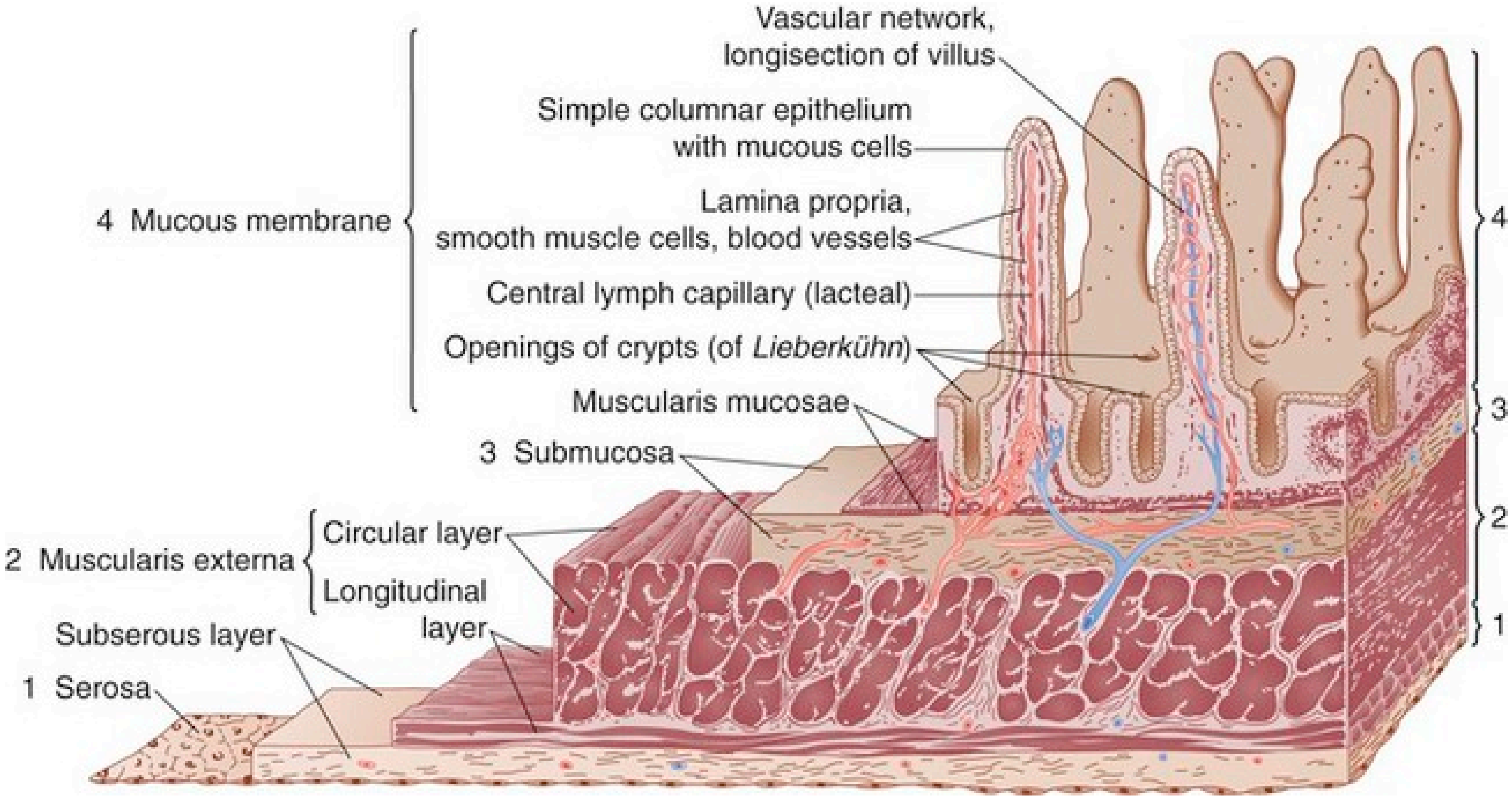


Baptista PM 2011 Hepatology

Small Intestinal Submucosa (SIS)

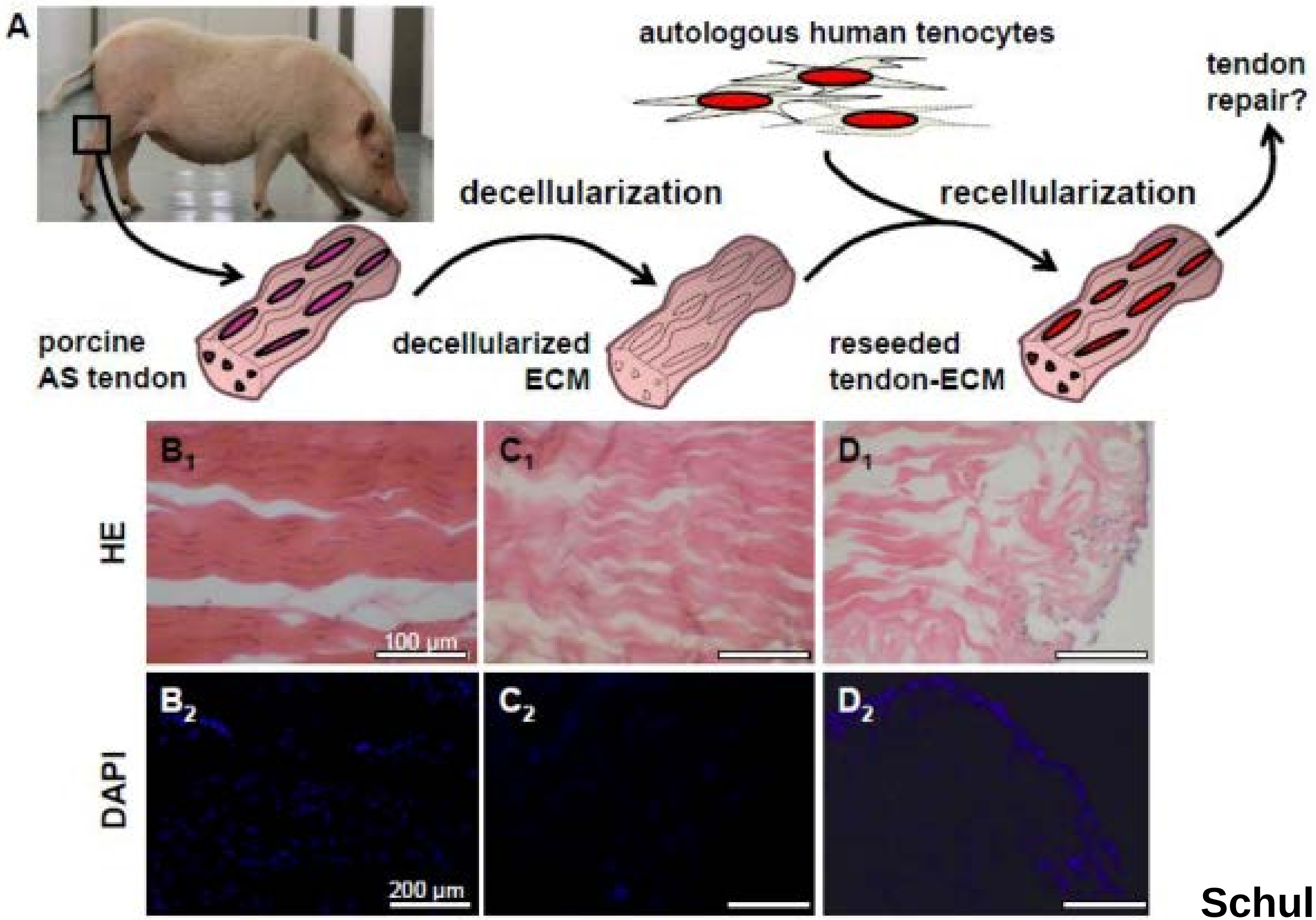


Brown-Etris M 2002 Wounds



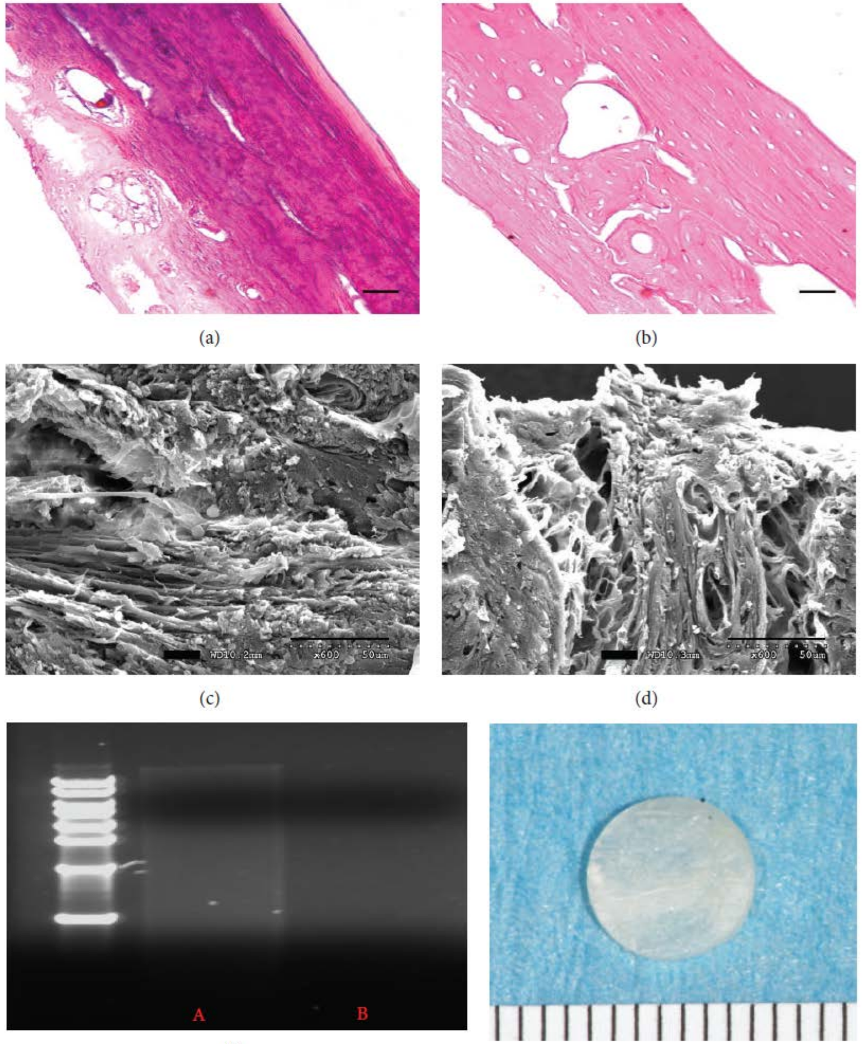
From Sobotta J, Figge FHJ, Hild WJ: Atlas of human anatomy, New York, 1974, Hafner.

Decellularized Tendon

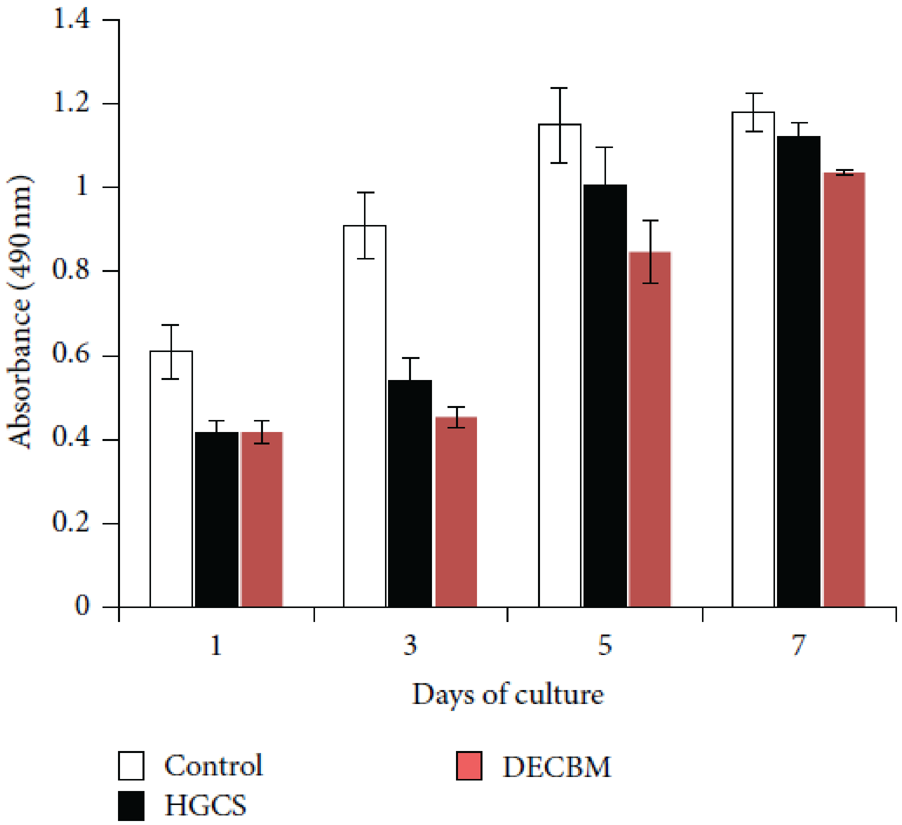


Schulze-Tanzil G 2012 Cells

Decellularized Bone Matrix

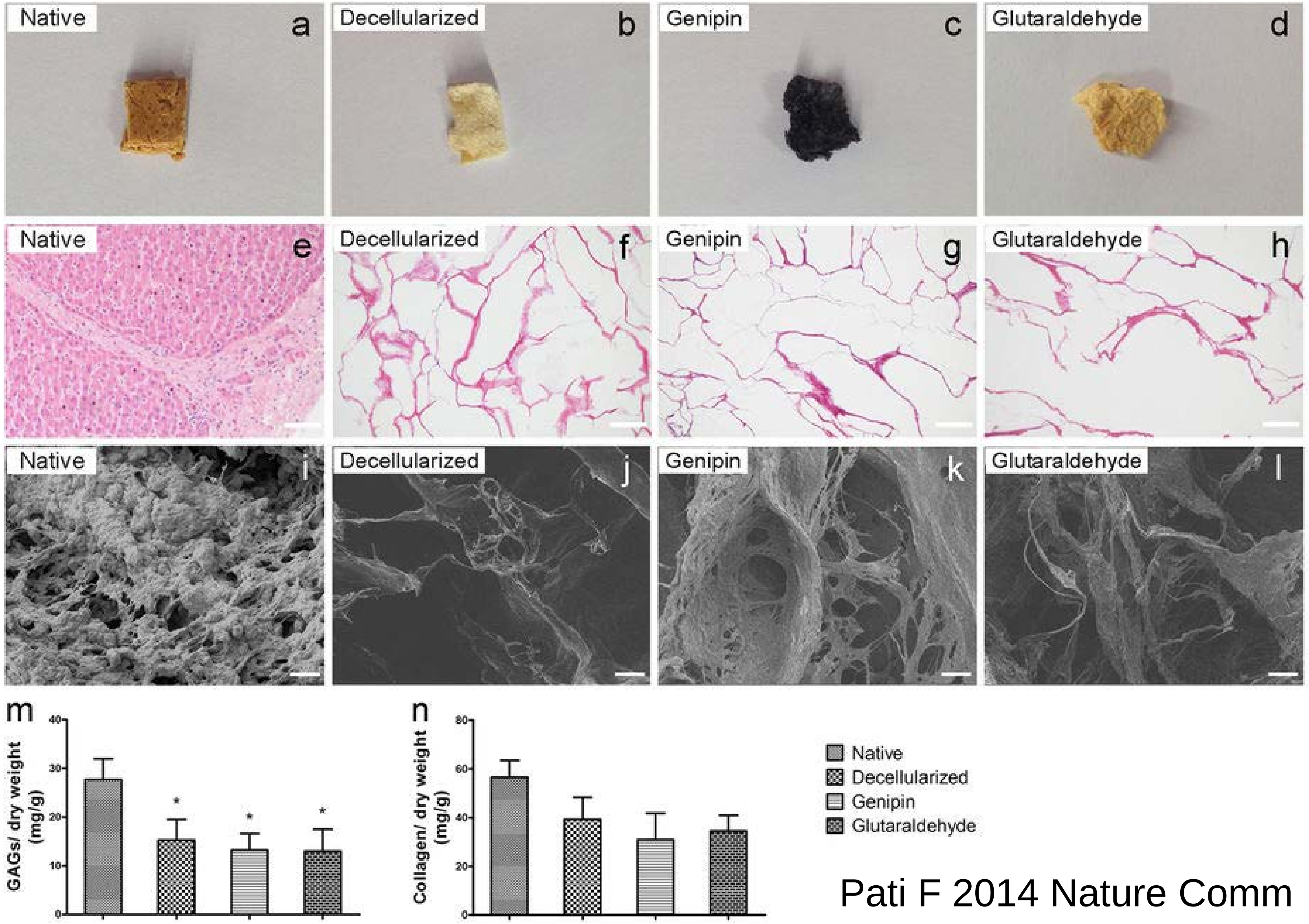


(a) (b) (c) (d) (e) (f)

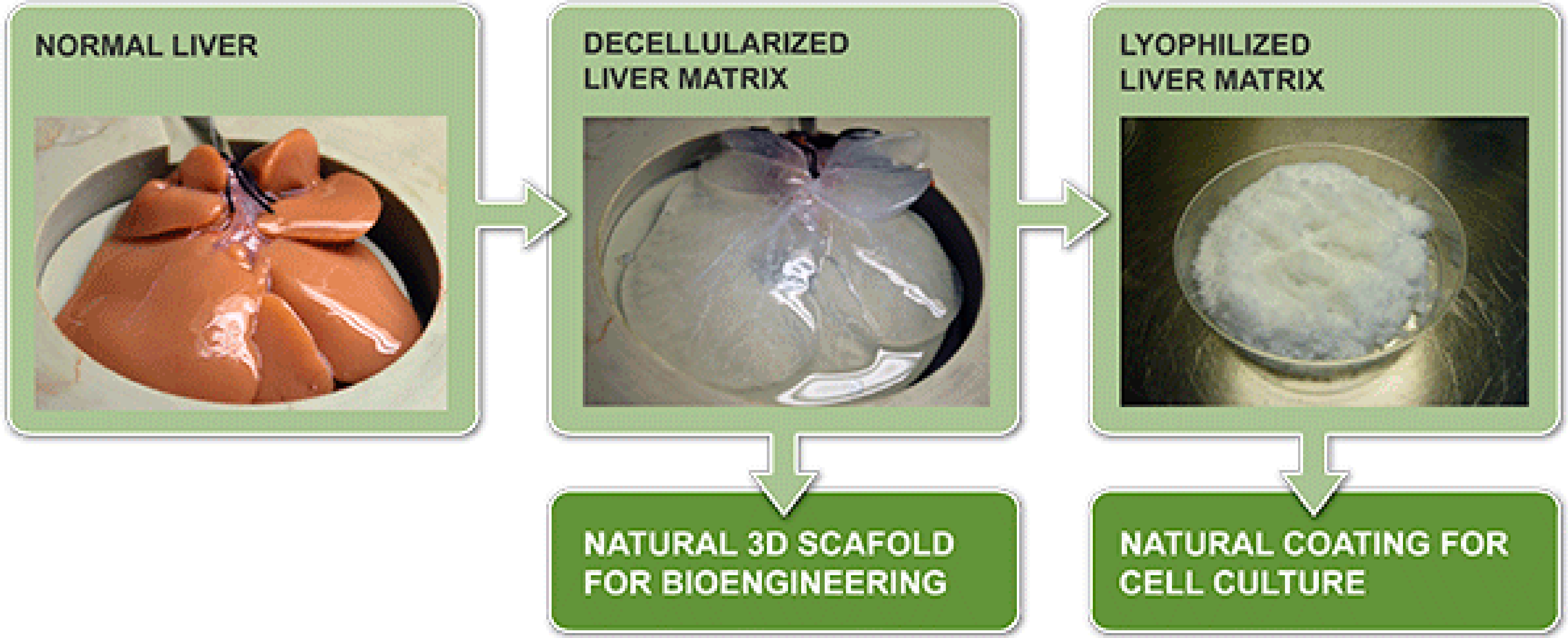


Lee DJ 2014 BioMed Res Int

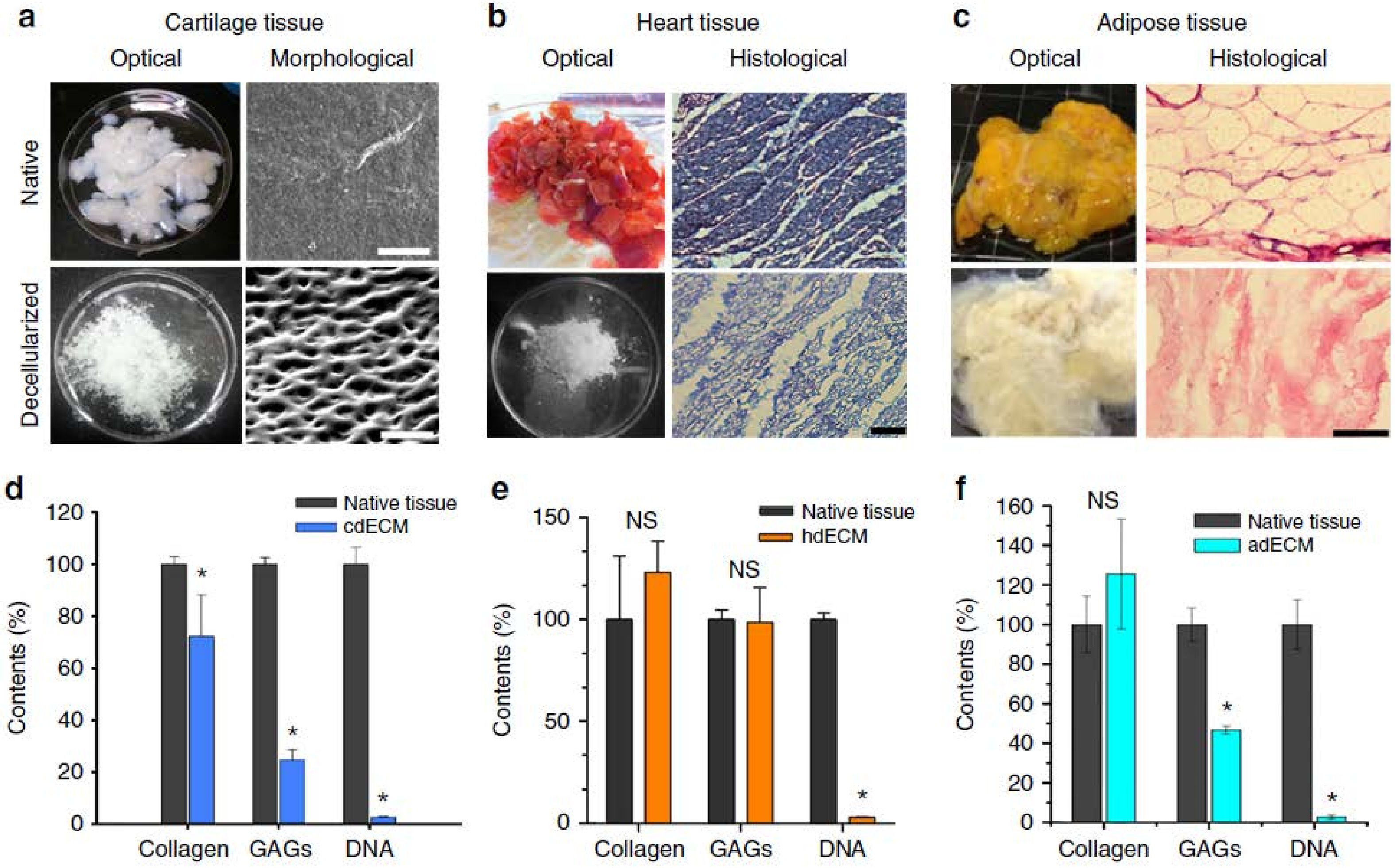
Compromised Stroma Structure after Decellulization



Pati F 2014 Nature Comm


NORMAL LIVER
DECELLULARIZED LIVER MATRIX
LYOPHILIZED LIVER MATRIX
NATURAL 3D SCAFOLD FOR BIOENGINEERING
NATURAL COATING FOR CELL CULTURE

Decellularized Matrix for 3D Printing



3d Organ printing

[Derby B 2012 Science]

KHU

Although organ printing is unlikely to be attainable in the near future, printing and related technologies are of current use in the areas of tissue engineering and regenerative medicine.
Both scaffold material composition and its internal architecture (dimensions of the struct, walls, pores, or channels) control the behavior and well-being of the cells seeded inside

Rapid prototyping methods

- Refer to as 3D manufacturing or solid freeform manufacture
- Produce complex objects from a 3D design file by decomposing the object's shape into a series of parallel slices
- The shape is then fabricated by reproducing these slices a layer at a time, building up the structure

Additive manufacture (AM)

- The method of creating objects by adding material layer-by-layer
- Opposite concept to the conventional machining, which removes material in a subtractive manner

Manufacture technique

- Selective polymerization
- Selective sintering/melting

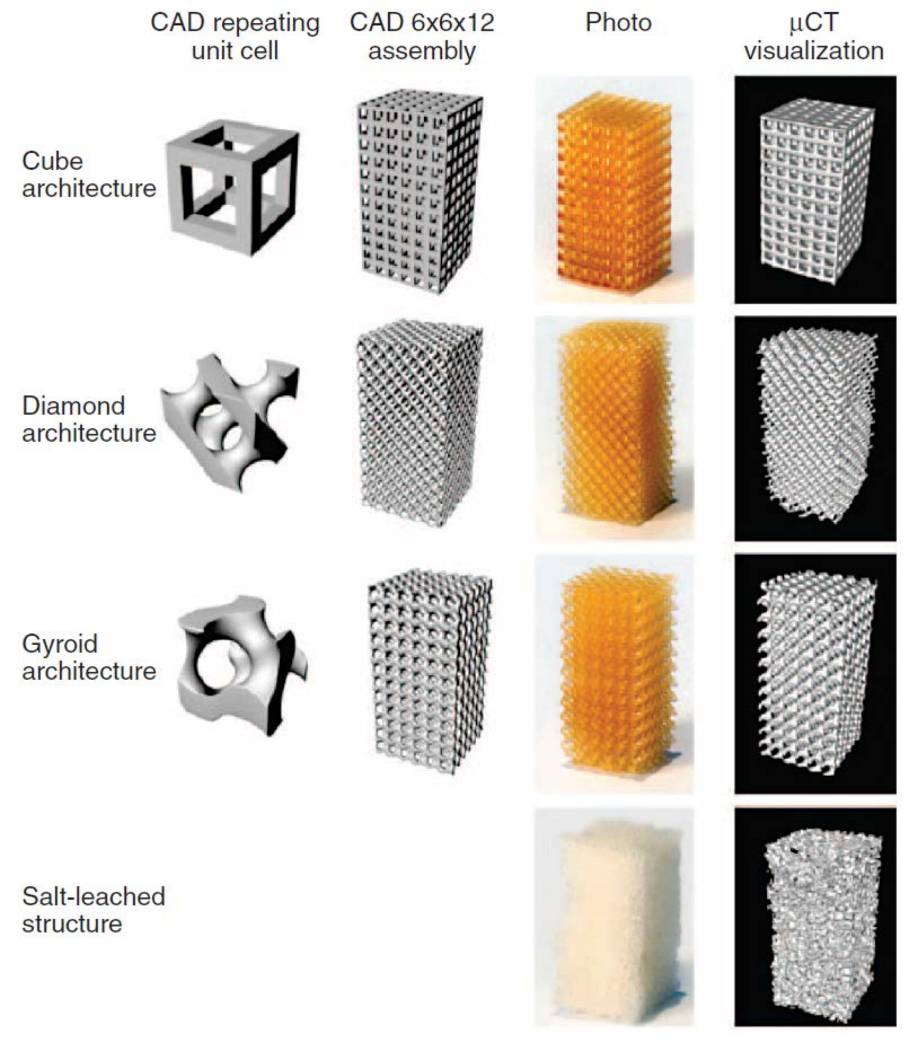
Concept of Bioprinting/Organ printing

Extension of AM methods to build complex scaffold structures

Deposition process must be cytocompatible

- This reduces range of AM techniques
- Need to work in an aqueous or aqueous-gel environment at temperatures from RT to 38^{o}C

Controlling 3D Architectures

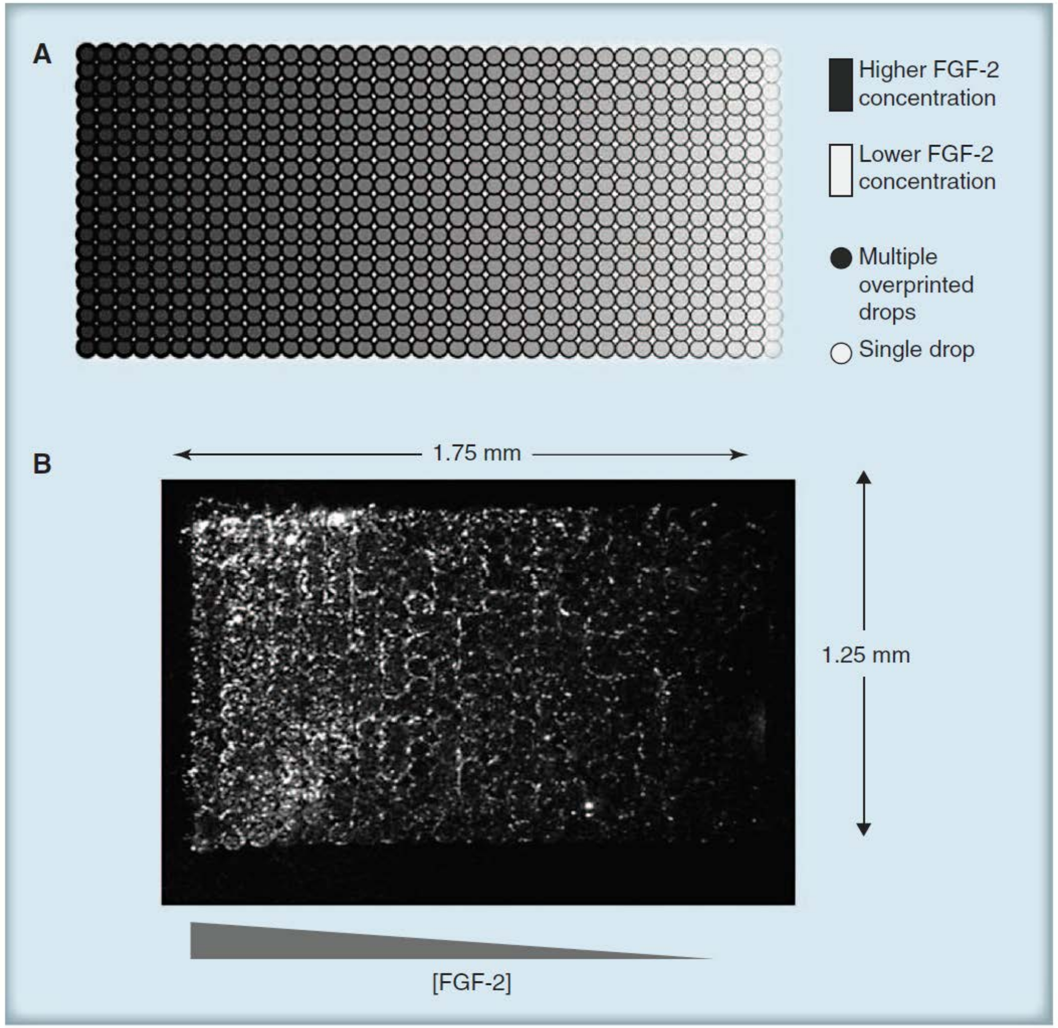


CAD -> Printing

- Frees the designer from the constraints of molds and dies
- Allows the exploration of design variables
- The easy variation of the internal architecture of a scaffold
    - Vary the specific surface area
    - Vary the scaffold mechanical response to external stress
    - Vary flow resistance in a bioreactor
    - Better select and control mechanical properties such as stiffness and strength

Bioprinting

Positioning of biochemicals, biological materials, and living cells



Labeled by streptavidin-quantum dot

Direct Cell Printing

Inkjet printing

- Precise positioning of picoliter (pL) volumes of fluid
- Smallest drop available: 1pL (radius≒6.2μm) (similar to that of whole cells)
- Possible to engineer variation in surface concentration through over-printing at different drop densities

1988, Klebe

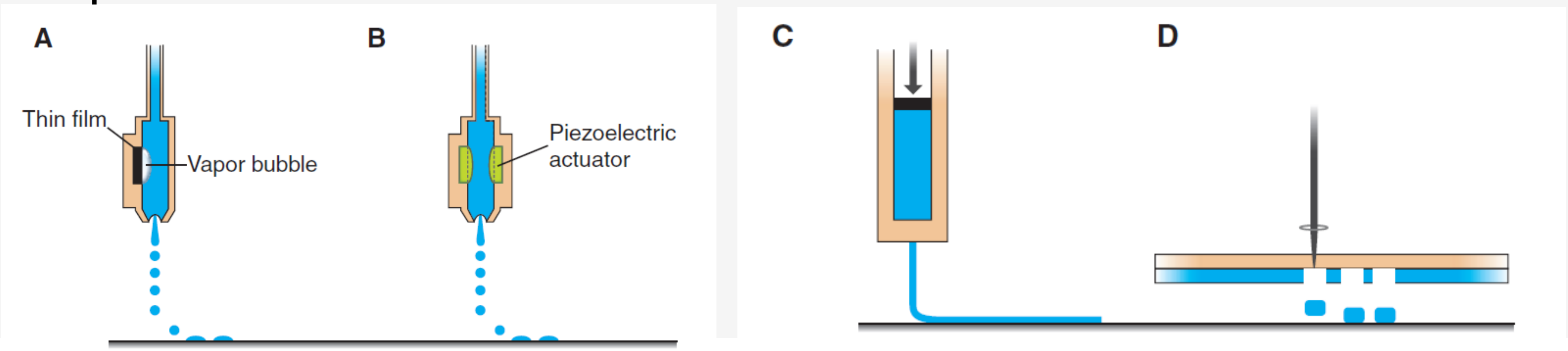
- Used Hewlett-Packard desktop printer to deposit collagen and fibronectin suspensions as well as cells to form 3D simple tissue

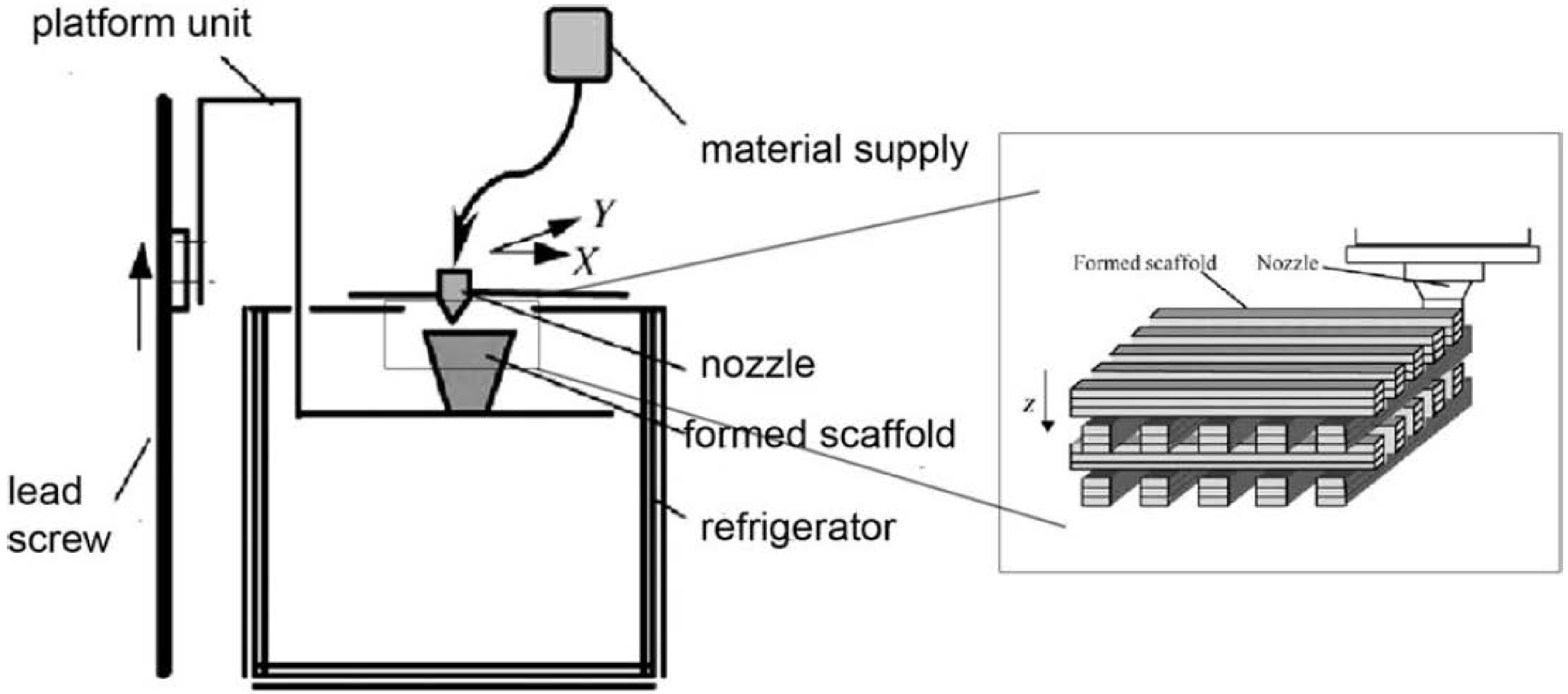
Heating & vaporaziation

Mechanical actuation

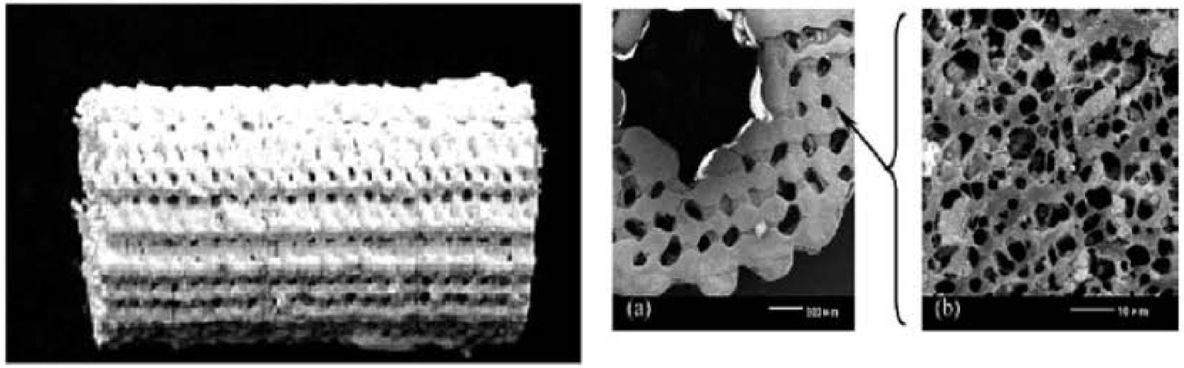
Microextrusion

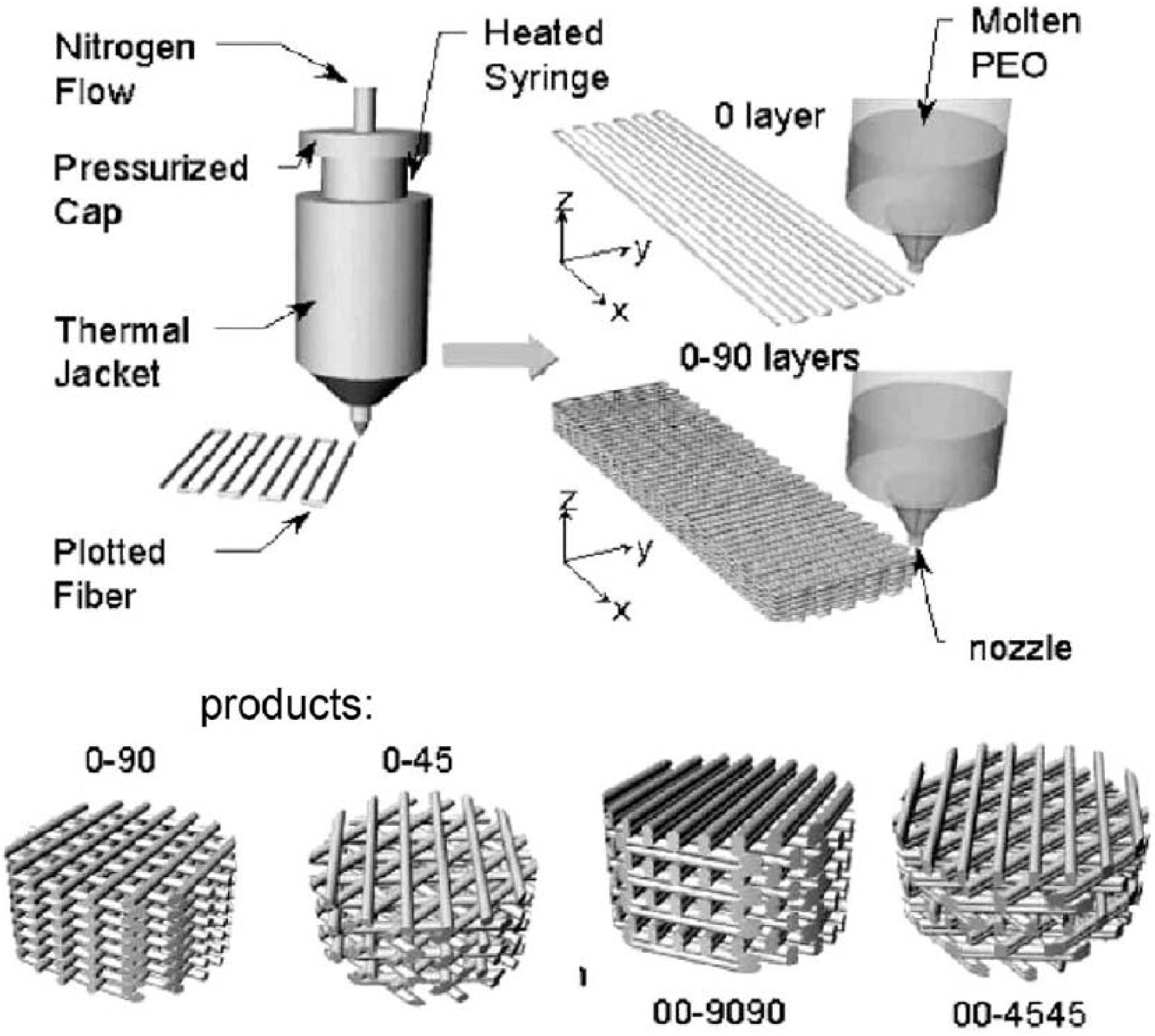
Laser forward transfer





Formed scaffolds:




Nitrogen Flow
Heated Syringe
Molten PEO
0 layer
Pressurized Cap
Thermal Jacket
0-90 layers
Plotted Fiber
nozzle
products:
0-90
0-45
00-9090
00-4545

Types of 3D Printing Process

FDM (Fused Deposition Modeling)

- Printing by extrusion
- Thermoplastic materials
- HDPE, PLA, PCL, PLGA..

SLS (Selective Laser Sintering)

- Granular ink
- Thermoplastic powder
- Metal or ceramic

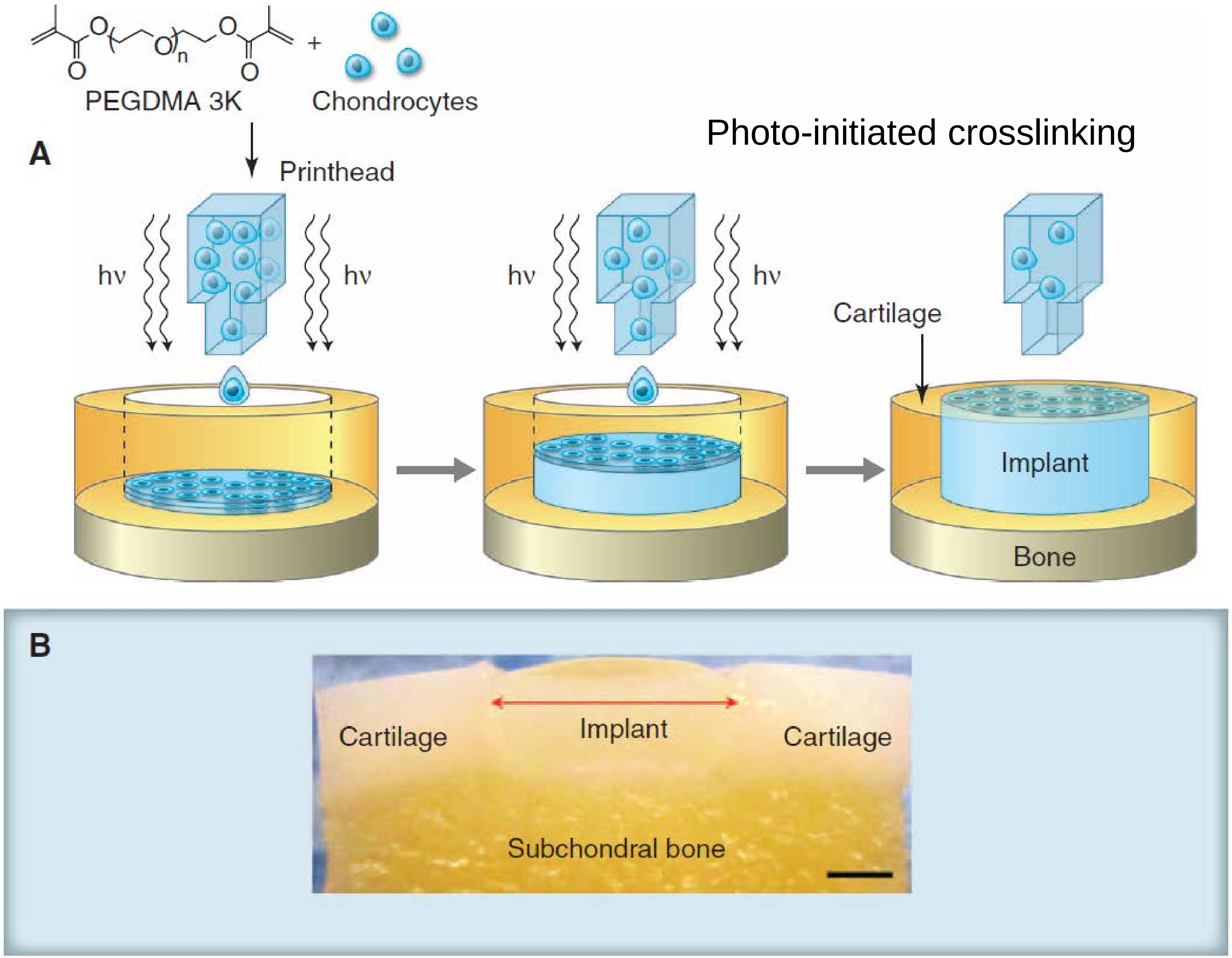
SLA (Stereolithography)

- Photopolymer

DLP (Digital Light Processing)

- Photopolymer
- Light-initiated polymerization

Photo-initiated crosslinking



Limits and Concerns

Typical cell densities used during culture are ~10^7 cells/ml, an individual volume of 100pL (drop radius≒70μm) will contain on average a single cell

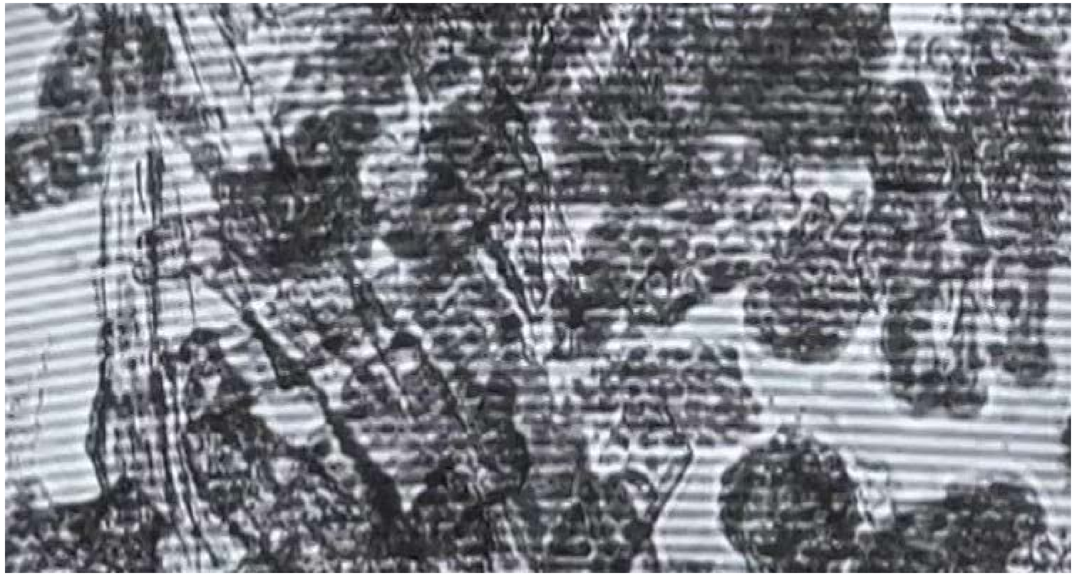
This limits the effective accuracy of deposition for cell placement.

Level of stress that cells experience during deposition process

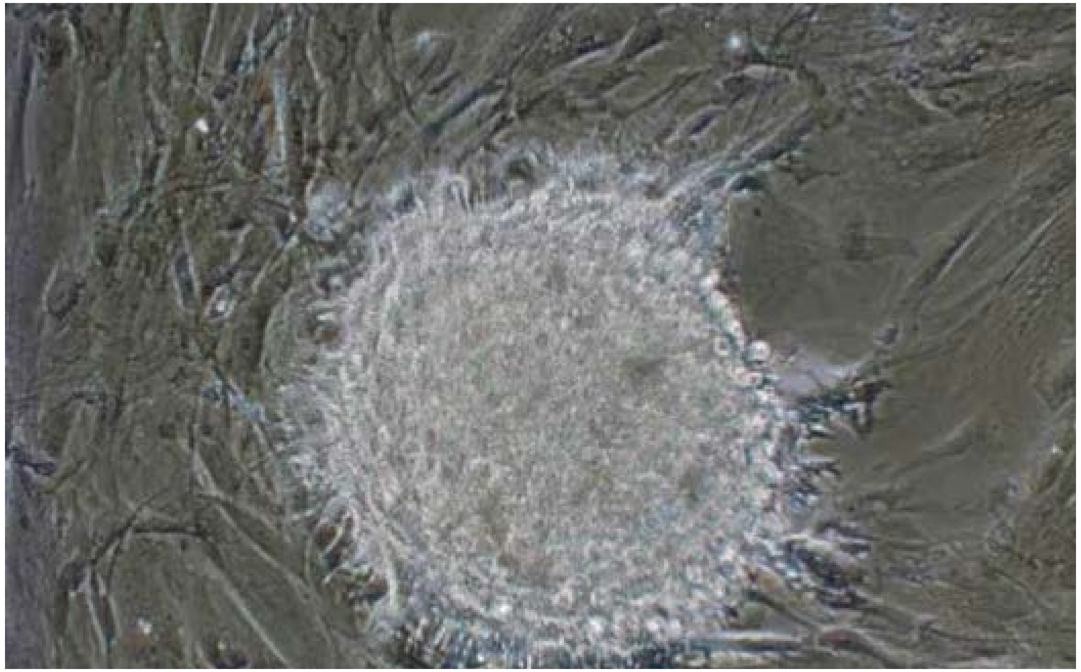
- Inkjet: requires a low viscosity environment to allow efficient drop ejection
- Microextrusion: very wide range of fluid properties but offer a lower spatial resolution
- Laser forward transfer: requires the cells to be immobilized in a gel

Survival rate of inkjet printed cells improved to >95% with appropriate choice of printing conditions.

3D-Printed Muscle Cells



Condensed Muscle Cells



Scaffold-free tissue engineering

[Papers from T. Okano group; Tani G 2010 Pediatr Surg Int]

Conventional TE Approaches

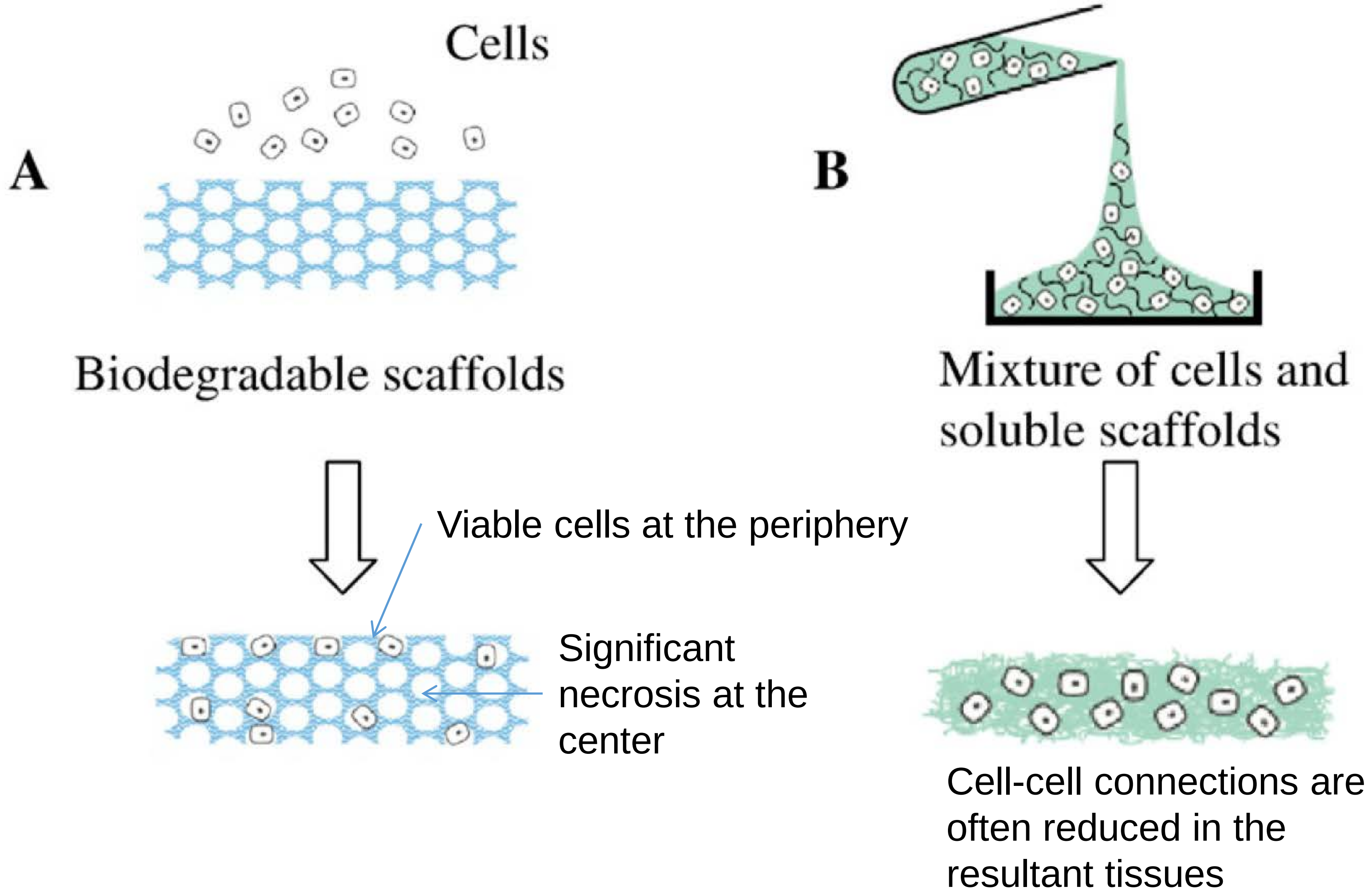
Isolated cells or cell substitutes
Appropriate signaling molecules such as cytokines or growth factors
Extracellular matrix (ECM) proteins

Short comings of conventional TE method

(Seeding of cells into biodegradable scaffolds)

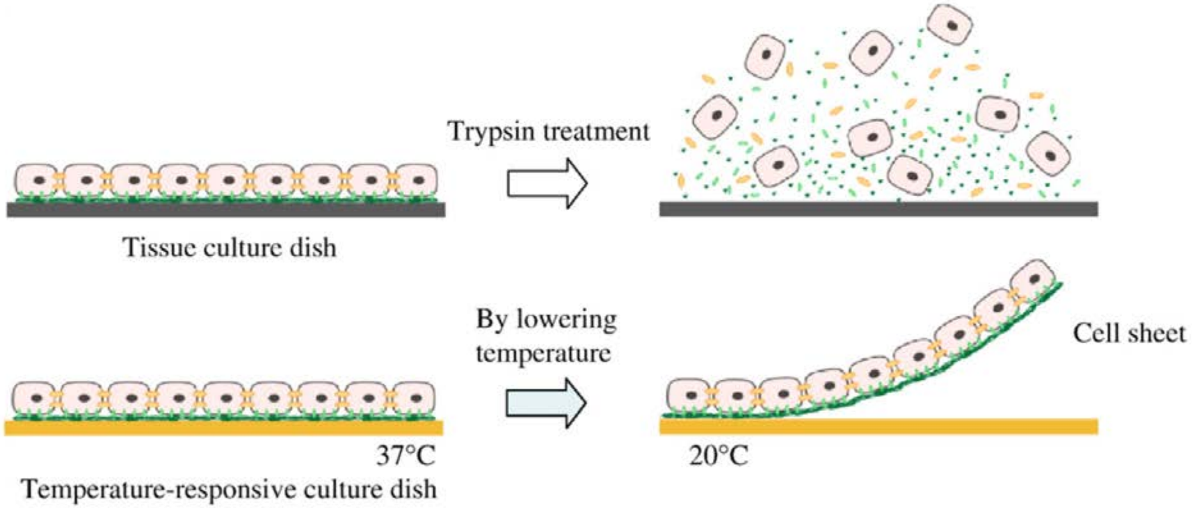
- Inflammatory reactions
- Fibrous tissue formation caused by scaffold degradation
- Stress to the cells

Two conventional approaches



Cell Sheet Engineering

- Constructs 3D functional tissues by layering two-dimensional confluent cell sheets without the use of any biodegradable ECM alternatives

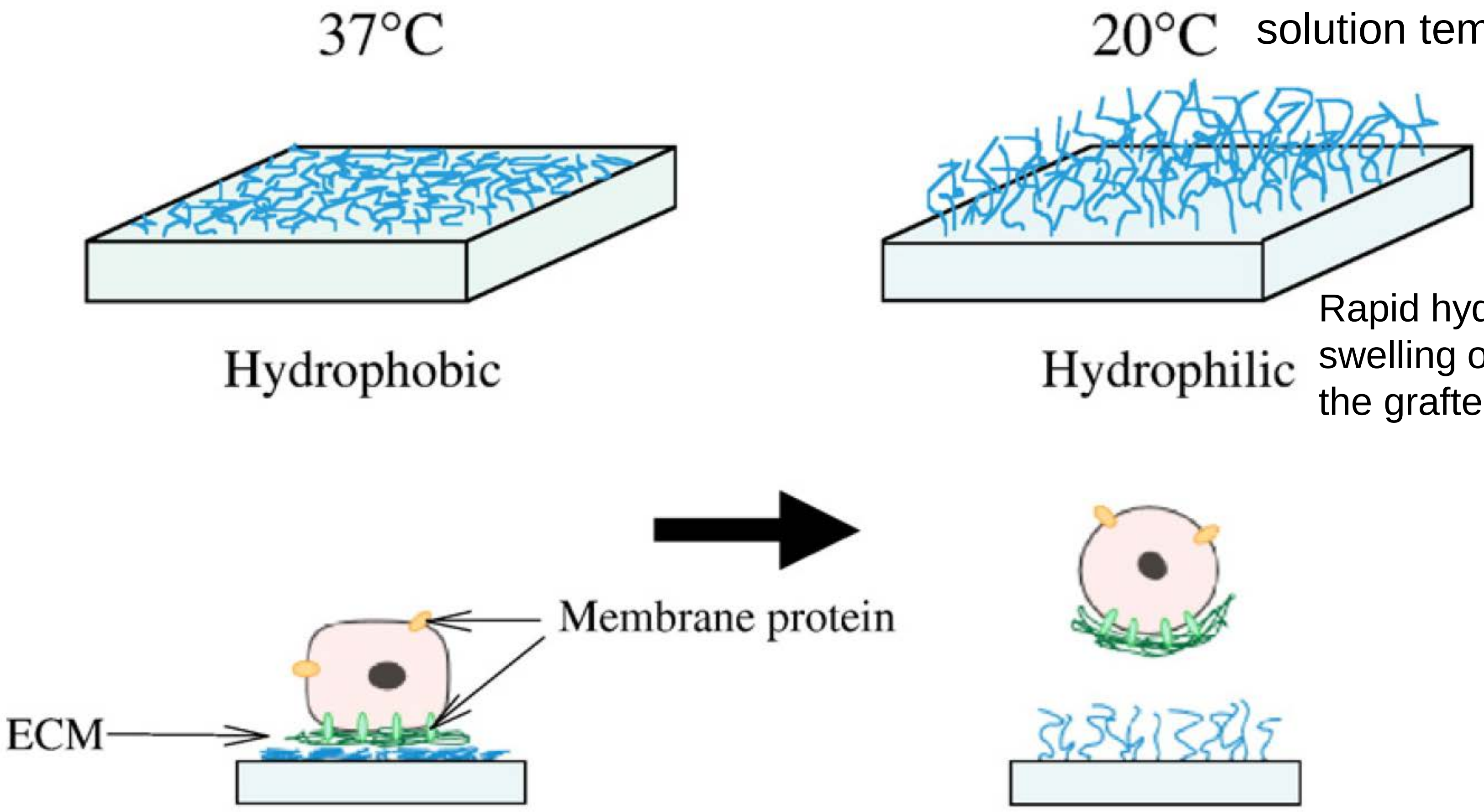


- Cells can be harvested as intact sheets along with their deposited ECM, ion channels, growth factor receptors, and cell-cell junction proteins
- Due to the presence of deposited ECM on the basal sheet surface, cell sheets harvested from temperature-responsive culture surfaces can be directly attached to host tissue without the use of any mediators such as fibrin glue or sutures

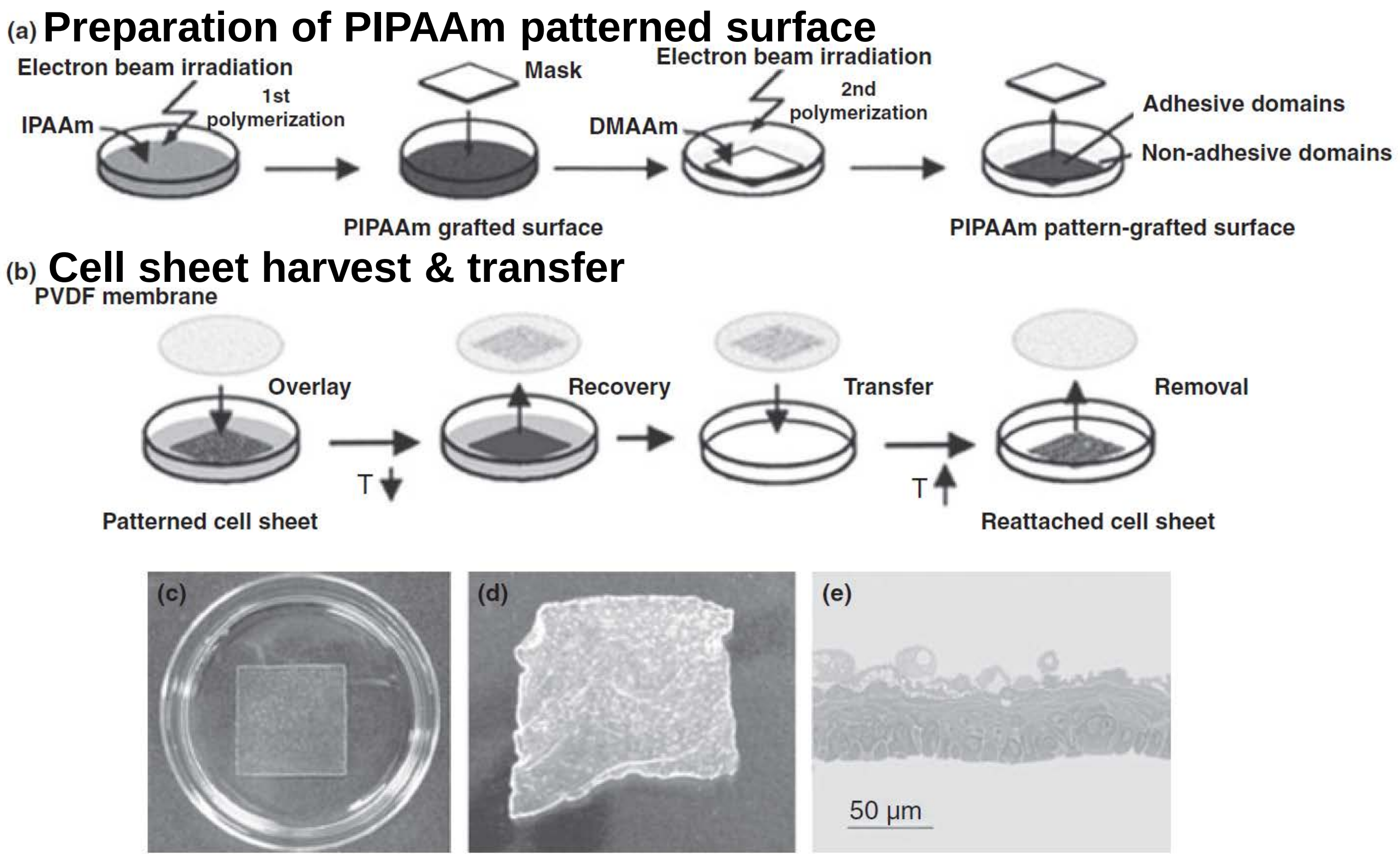
Cell sheets are obtained by using specialized cell culture surfaces

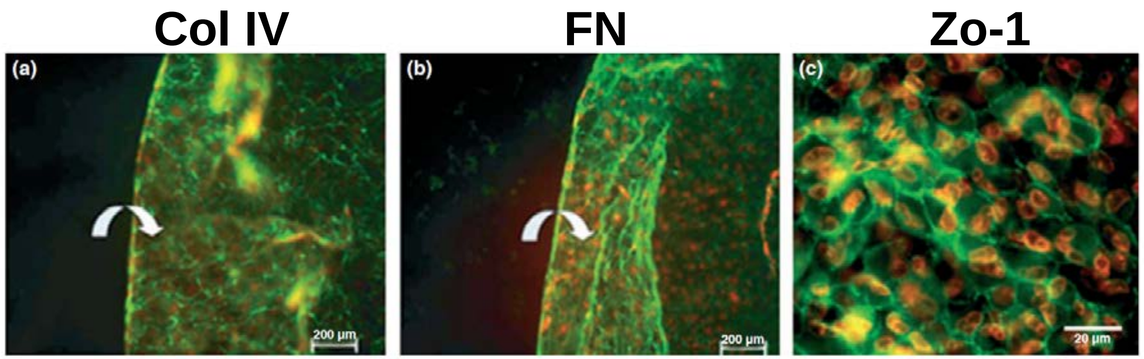
Temperature responsive polymer, poly (N-isopropylacrylamide) (PIPAAm) is covalently grafted to the cell culture surface

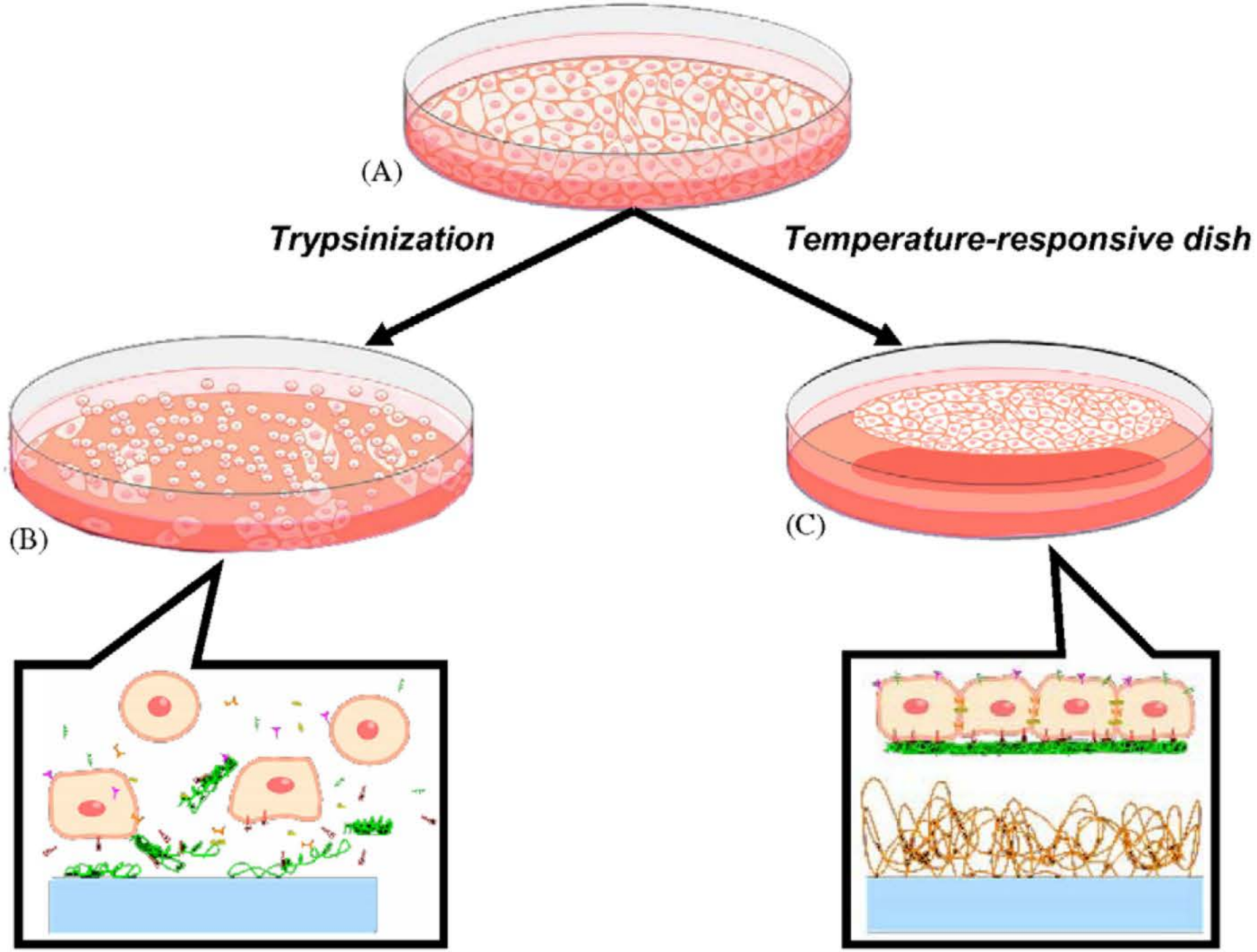
LCST: Lower critical solution temperature

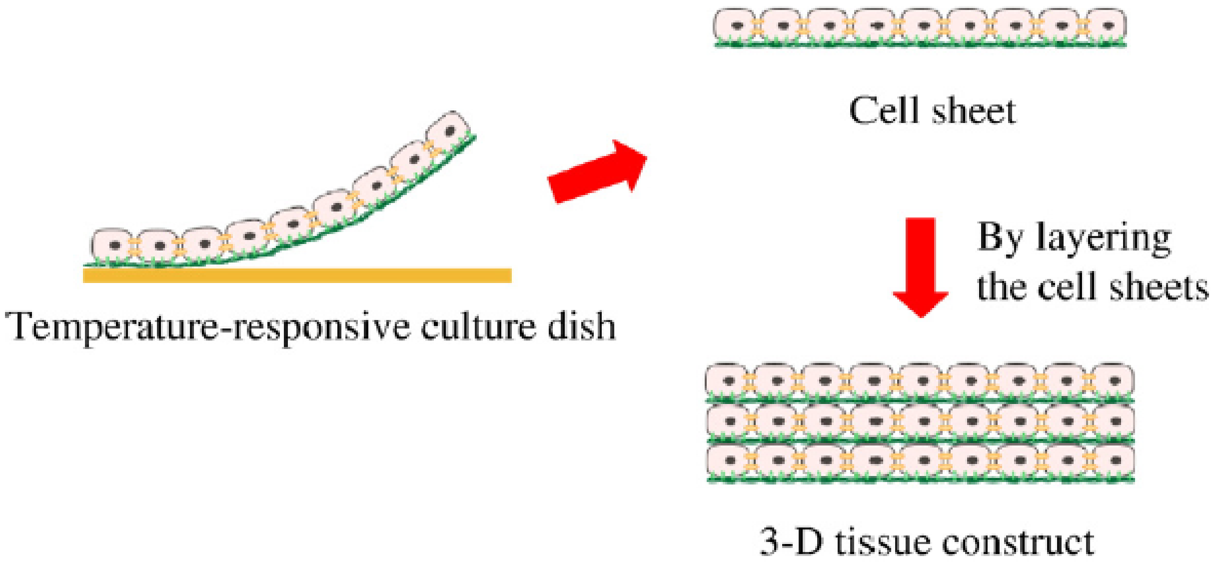


Rapid hydration and swelling of the grafted PIPAAm


(a) Preparation of PIPAAm patterned surface
Electron beam irradiation
1st polymerization
IPAAm
Mask
PIPAAm grafted surface
Electron beam irradiation
2nd polymerization
DMAAm
Adhesive domains
Non-adhesive domains
PIPAAm pattern-grafted surface
(b) Cell sheet harvest & transfer
PVDF membrane
Overlay
Recovery
Transfer
Removal
T
T
Patterned cell sheet
Reattached cell sheet
(c)
(d)
(e)
50 µm

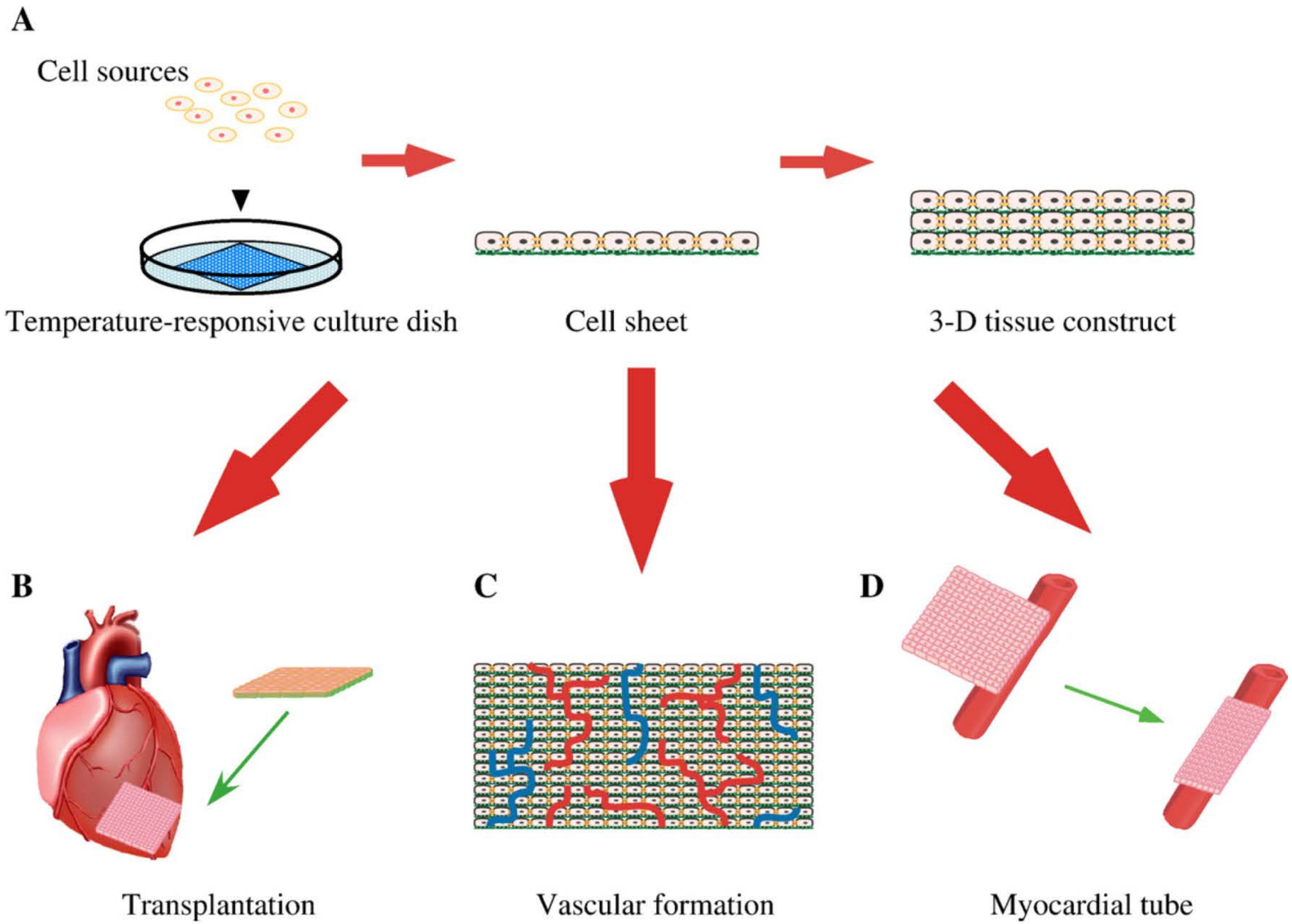

Col IV
FN
Zo-1
(a)
(b)
(c)
200 µm
200 µm
20 µm


(A)
Trypsinization
Temperature-responsive dish
(B)
(C)



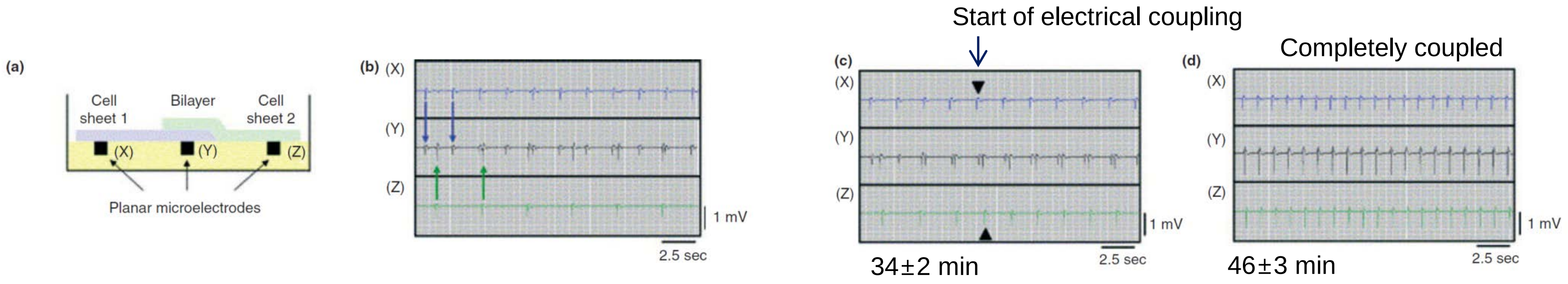
- Cell-dense thick tissues are constructed by layering these cell sheets
- Cell sheets have been fabricated including…
    - Keratinocytes
    - Retinal pigment epithelial cells
    - Corneal epithelial cells
    - Corneal endothelial cells
    - Oral mucosal epithelial cells
    - Urothelial cells
    - Periodontal ligament cells
    - Aortic endothelial cells
    - Cardiac myocytes
    - Kidney epithelial cells

Cell sheet for heart tissue repair



Monitored by multiple-electrode extracelluar recording system…

- Neonatal rat cardiomyocytes sheets were layered and began to pulsate spontaneously and simultaneously
- In vivo, layered cardiomyocyte sheets were transplanted into dorsal subcutaneous tissues of nude rats and after 3 weeks, surface electrograms originating from the grafts were detected independently from host electrocardiograms



Dye transfer assay..

The formation of the gap junction occurred rapidly between the layered cardiomyocyte sheets

Layered skeletal myoblast sheets transplanted to infarcted rat hearts were shown to…..

- Enhance left ventricular contraction
- Reduce fibrosis
- Prevent left ventricular dilatation

Several problems remain unresolved

The potential source of tissue-specific precursors
Obtaining and amplifying the progenitors into sufficient numbers of transplantable cells
Thickness limit for layered cardiomyocyte sheets in subcutaneous tissue is approximately 80 um (3 layers)

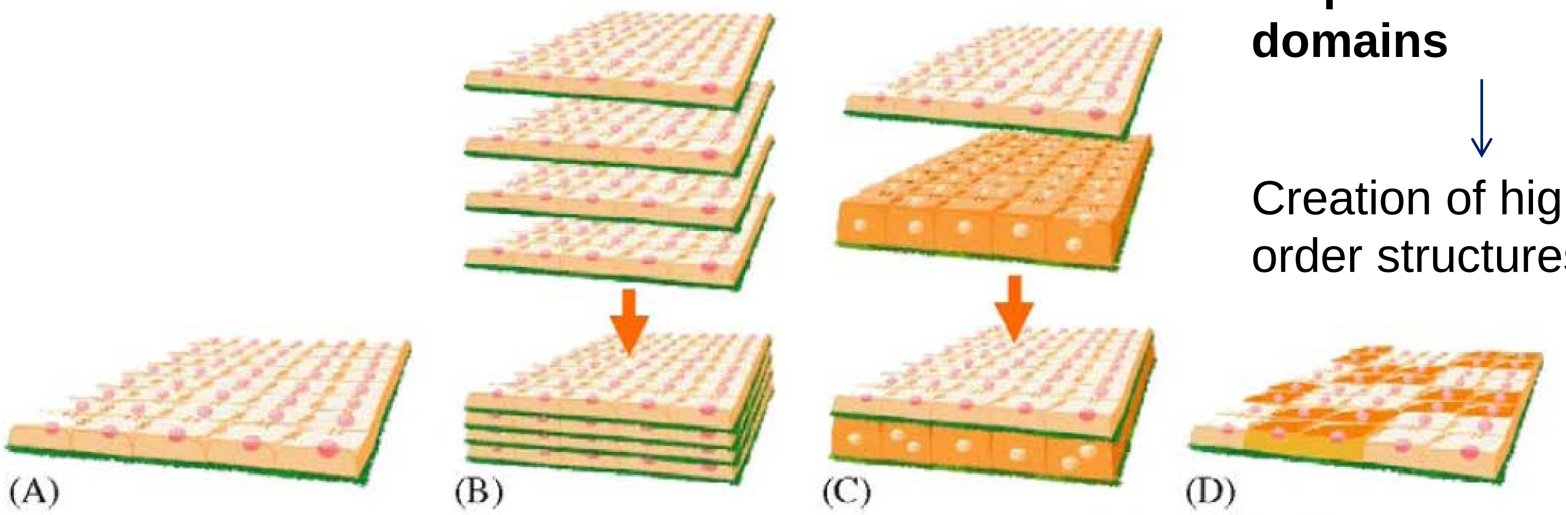
Homotypic stratification

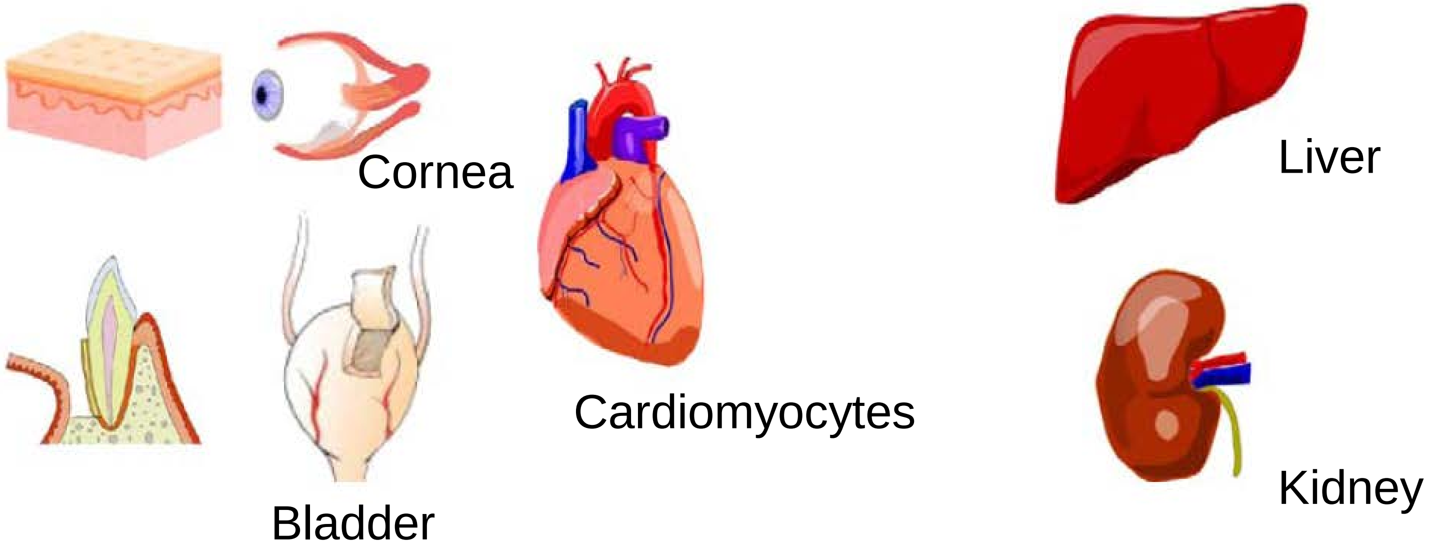
Heterotypic stratification

Dual Temperature-responsive domains

↓

Creation of higher order structures

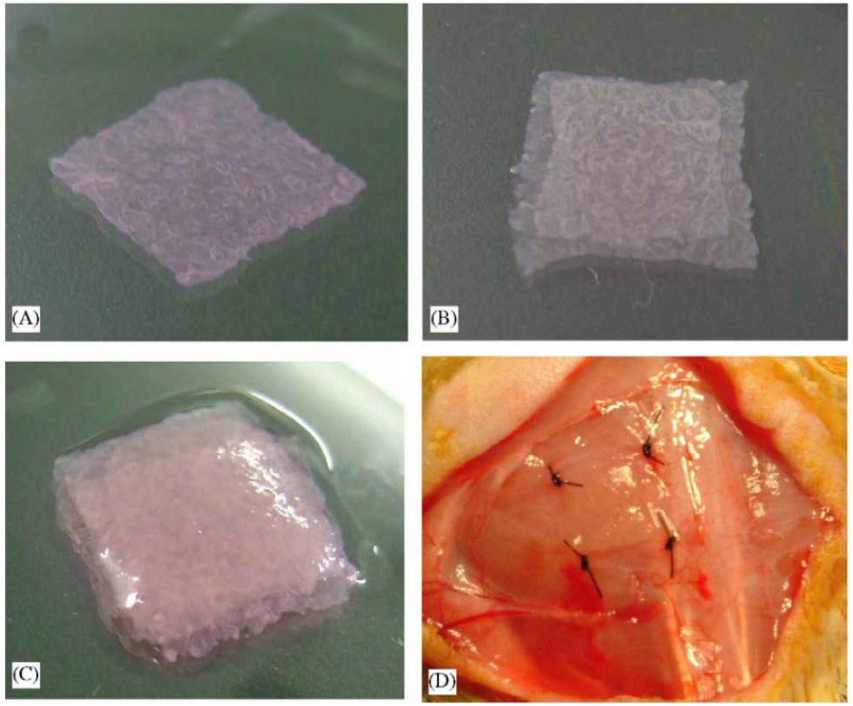




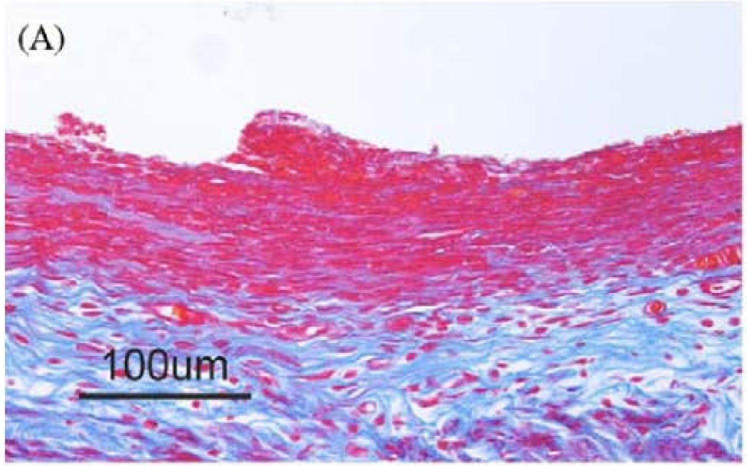
1 Layer

2 Layers

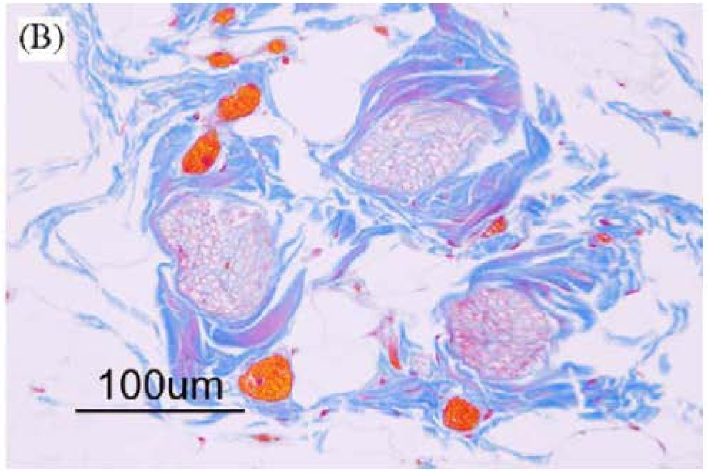
5 Layers

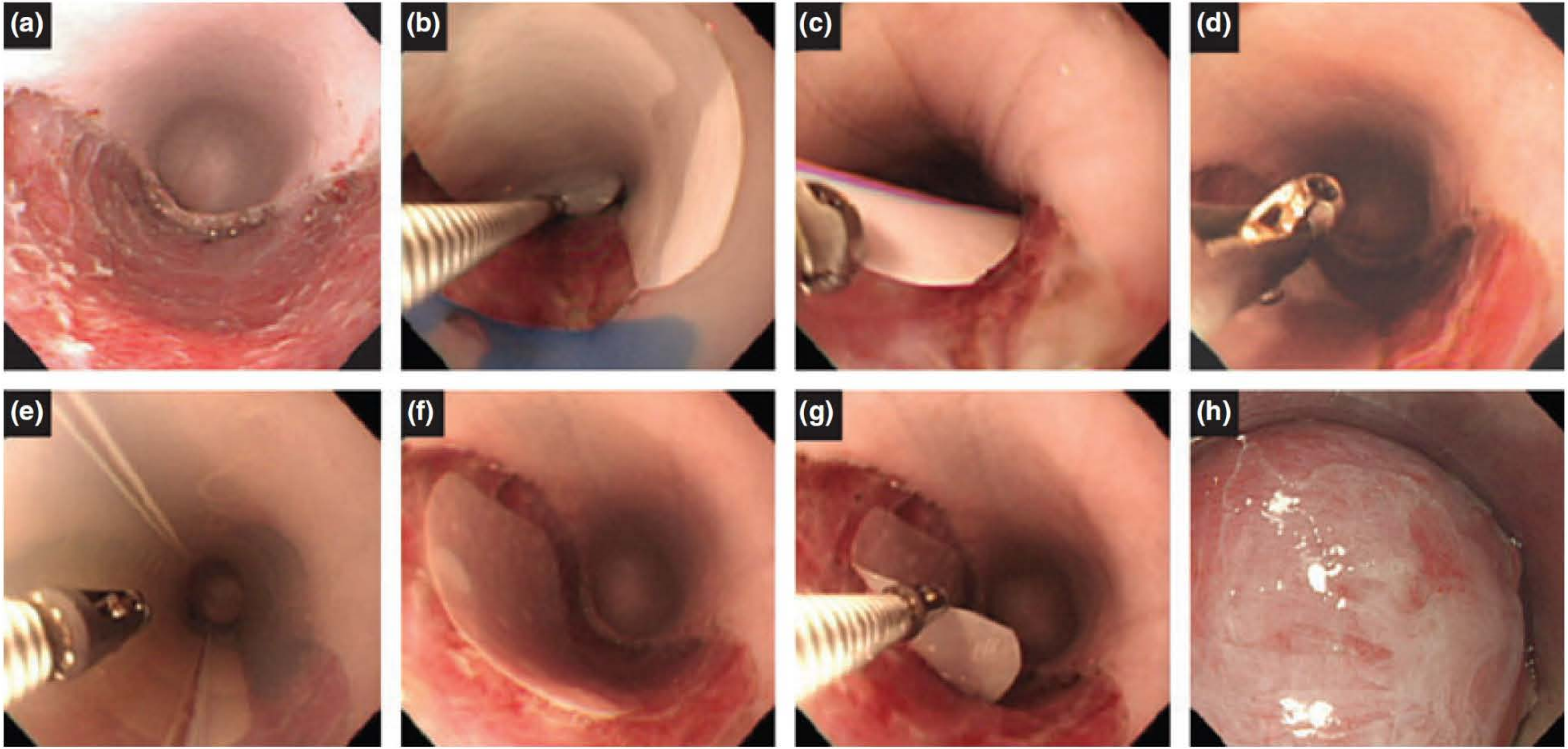


Cell sheet transplanted

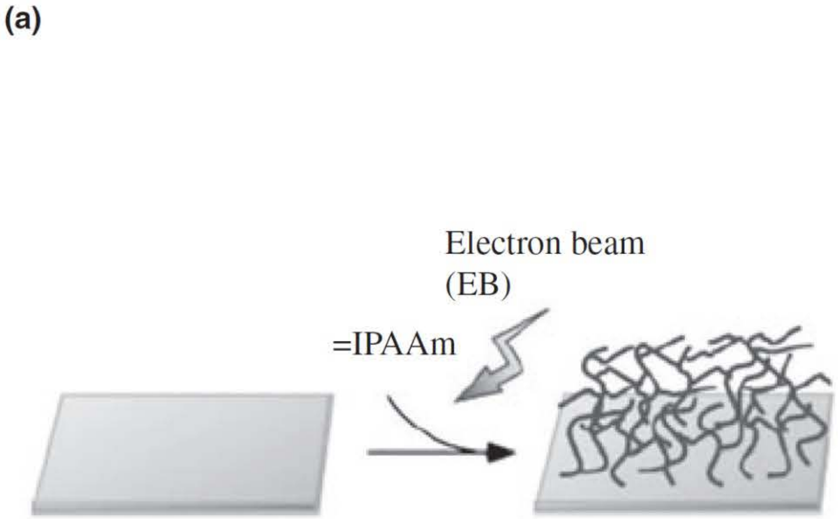


Injection of smooth muscle cells

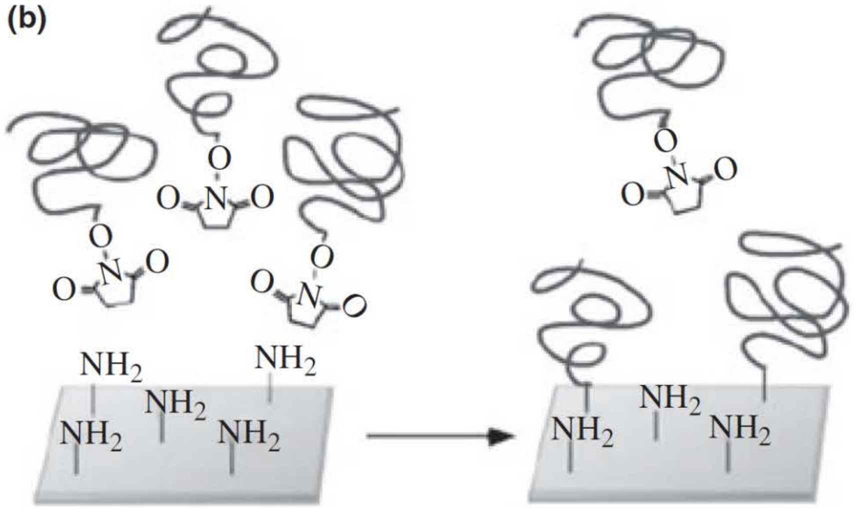



(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

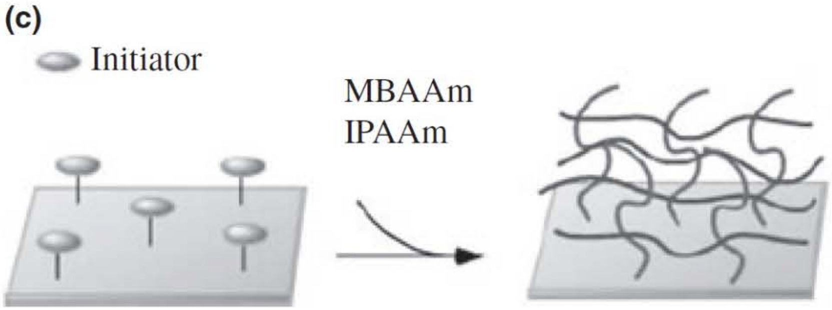
Electron beam irradiation



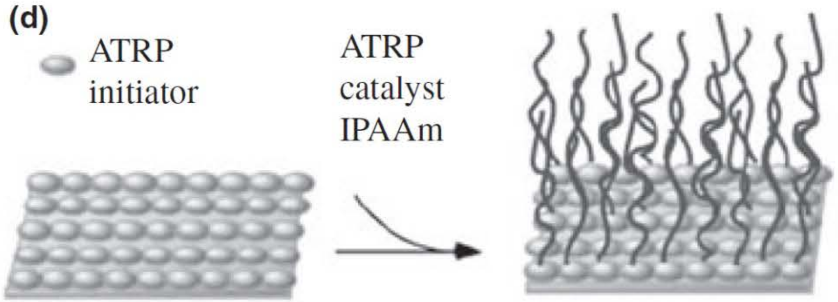
Grafting to approach



Grafting from approach

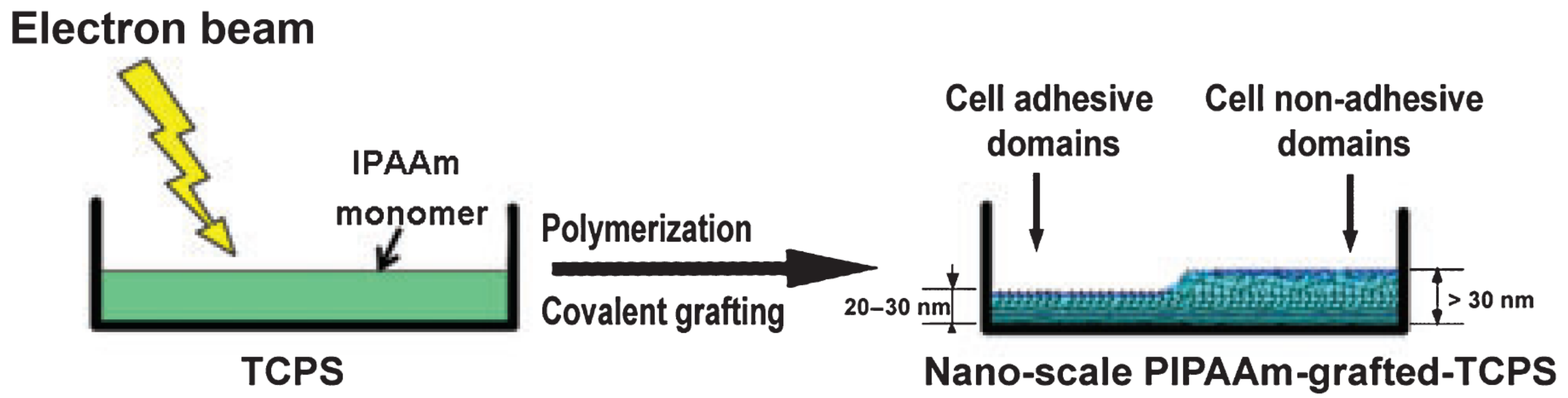


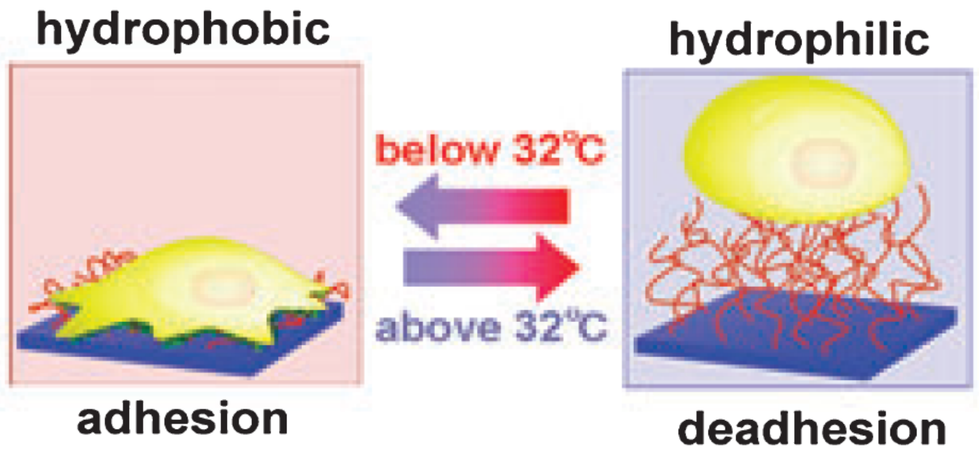
Surface-initiated living radical polymerization



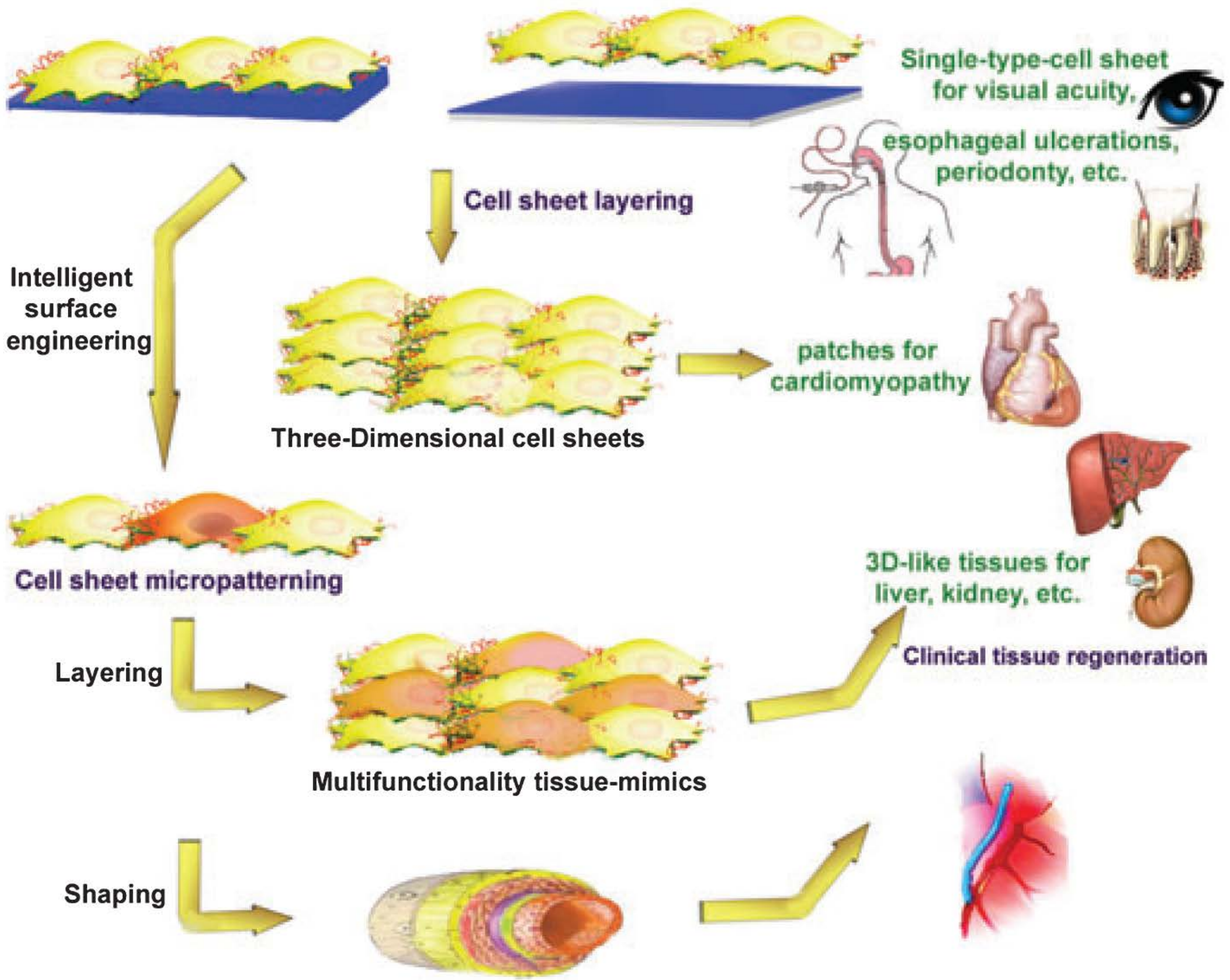
- The grafted polymer thickness is controlled by monomer concentrations and radiation energy. The typical thickness of the EB-grafted PIPAAm polymer is <100nm.

- By adjusting polymerization reaction conditions and duration, it becomes possible to culture cells to confluence and to detach them as contiguous cell sheets from PIPAAm brush surfaces at relatively elevated and relatively reduced temperatures, respectively.
- A density of approximately 1.4 ug/cm (ca. 20nm in thickness) is optimal for the temperature-responsive cell adhesion/detachment control of various types of cells within a margin of 0.8-2.2 ug/cm.


Electron beam
IPAAm
monomer
TCPS
Polymerization
Covalent grafting
Cell adhesive
domains
Cell non-adhesive
domains
20–30 nm
> 30 nm
Nano-scale PIPAAm-grafted-TCPS

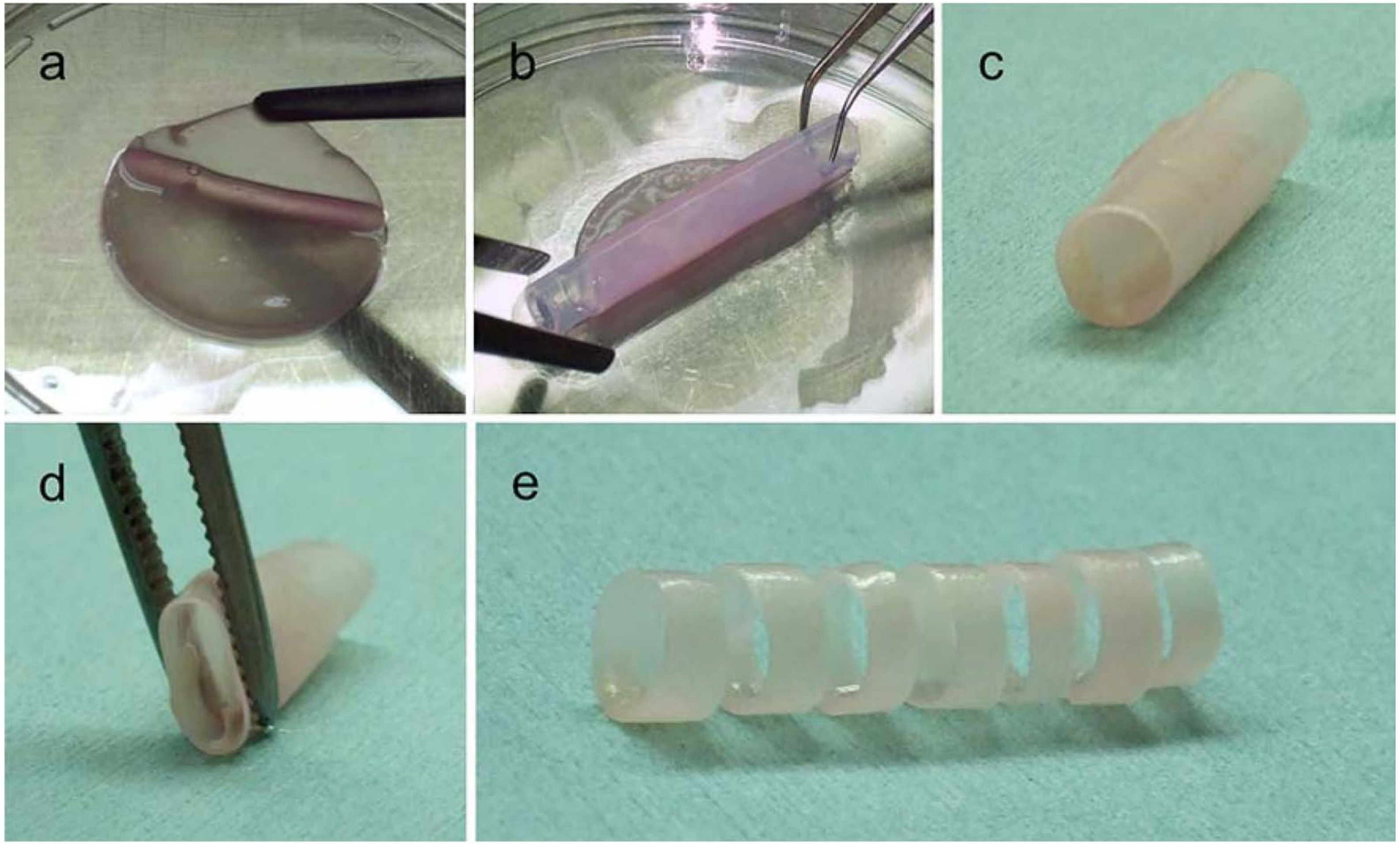

hydrophobic
adhesion
below 32°C
above 32°C
hydrophilic
deadhesion

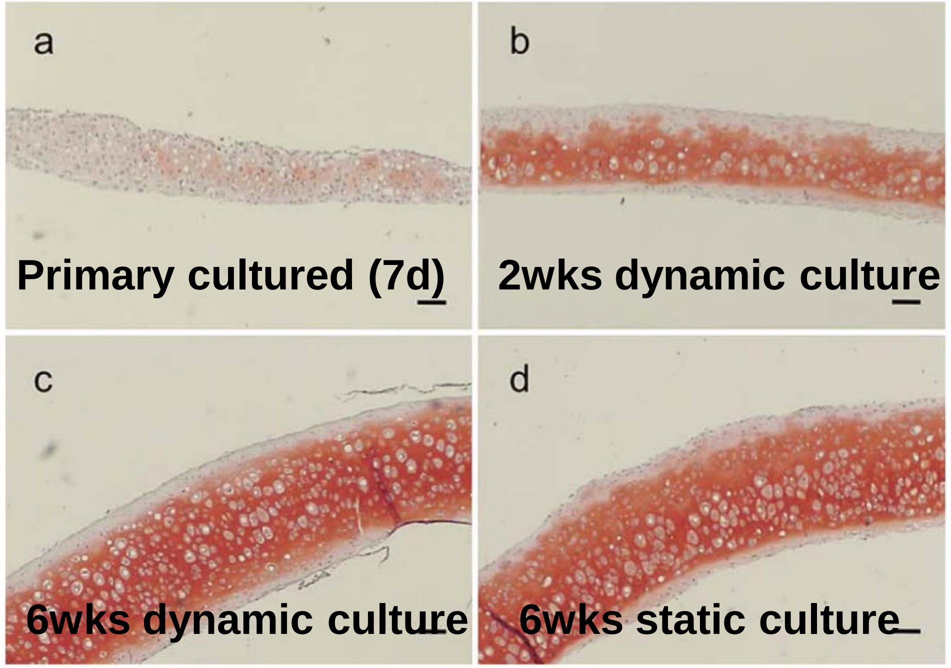
NANOTECH-BASED CELL SHEET ENGINEERING

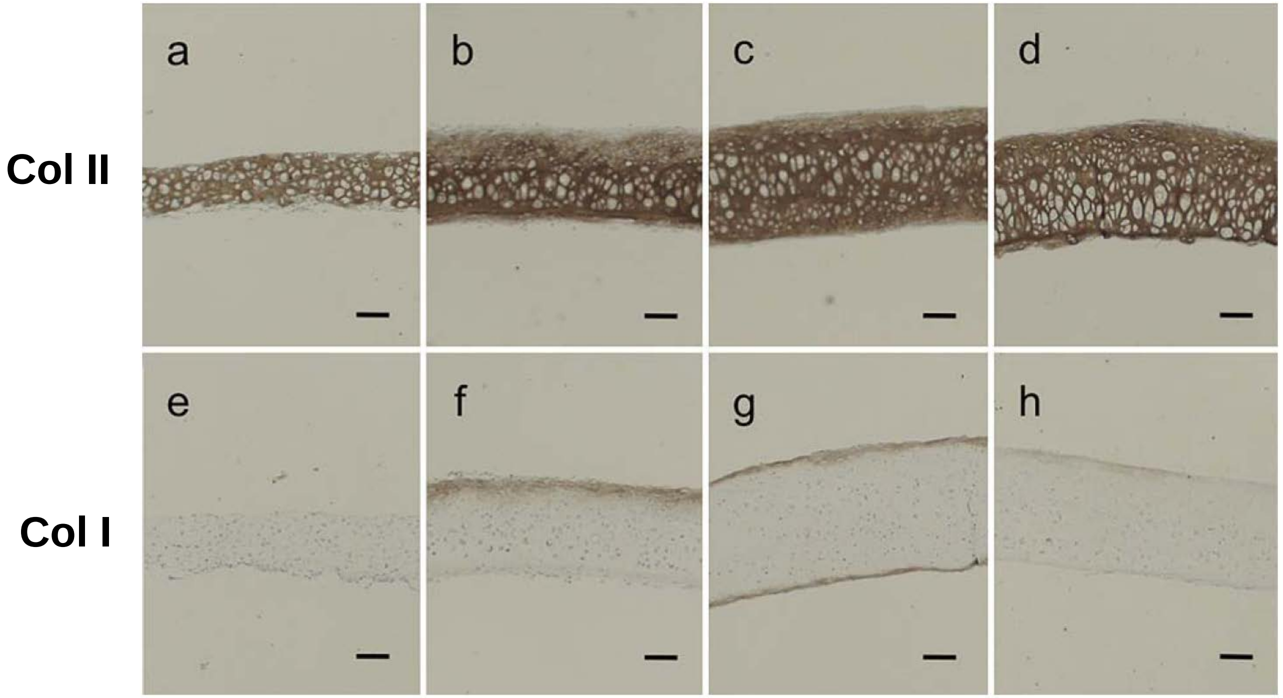


Scaffold-free Cylindrical Cartilage

Primary culture for 7days **Loop around a sterile silicon tube**




a
b
Primary cultured (7d)
2wks dynamic culture
c
d
6wks dynamic culture
6wks static culture


Col II
Col I
a
b
c
d
e
f
g
h