Materials for tissue engineering

Input: from where you can copy?

- extracellular matrix (ECM), a main regulatory and structural component of tissues that is composed of fibrous proteins, proteoglycans and glycoproteins
- mimicking these cues with synthetic analogs of the ECM (scaffolds) has been a major research topic in the tissue engineering field

Materials for TE

- create environments that promote the regeneration of functional tissues and organs
  - chemical signals that direct cell differentiation, migration, proliferation
  - scaffold design and peptide engineering that allow this signaling to be recapitulated
  - new materials, such as DNAbased and stimuli-sensitive polymers,
- immune system, which is often overlooked, has been shown to play a beneficial role in tissue repair

Scaffolds/materials: the first were only static supports

- The first were designed
  - to exhibit some minimum level of **mechanical support**
  - to regulate **diffusion of nutrients and waste products** between the new tissue and surrounding host tissues
- Classic biomaterials (e.g. long-lasting metals, ceramics and polymer composites) have successfully been used to replace the mechanical function of tissues such as teeth, hips, knees, heart valves and intervertebral discs, but they have limited ability to modulate the repair and regeneration of host tissues

Scaffolds/materials: now more dynamic and more close to the natural ones…

- designed with the aim to regulate tissue regeneration by different mechanisms, such as
  - controlling specific cell-binding interactions, releasing growth factors,
  - degrading at a controlled rate
  - responding to environmental cues
- → extensive work to covalently modify existing materials and efforts to synthesize new materials

MUSTs

- The basic promise of in vitro tissue engineering is to integrate the specific cells with scaffolds under appropriate conditions that lead to tissue formation
- → requirements include biocompatibility, physical properties, and biodegradability meeting the target tissue
  - nanotopographic environment is crucial
  - Ex: scaffold's fiber diameter must be smaller than cell size

Properties

- Non-toxicity
- Cell-scaffold interaction
- Angiogenicity
- Porosity
- 3D structure
- Mechanical strength
- Degradation rate
- Degradation product

3D structures

- three-dimensional (3D) micro- and nanostructures in which pore structure, surface area to volume ratio, texture and surface topography are manipulated to control cell shape, alignment and organization
- they have been shown to strongly affect cell phenotype, functional patterning

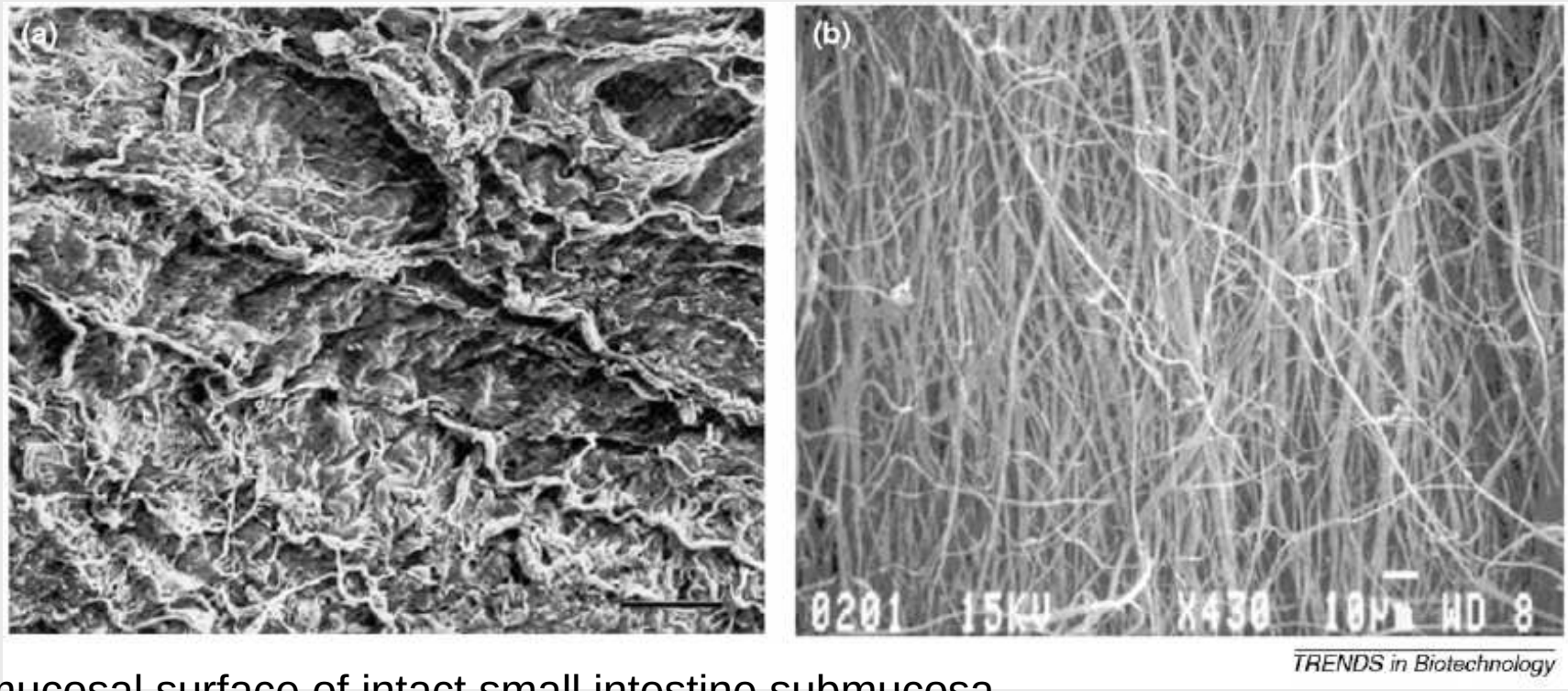
Scaffold materials

- Traditional materials (including functionalization with bioactive molecules and degradation)
- alternative materials
- Materials modulating the inflammatory response via material properties is potentially a powerful tool for driving tissue morphogenesis.

Natural polymers

- Decellularized ECMs derived from animals
  - already developed into commercial products for soft tissue repair, but also potential for immunogenicity, disease transfer and wide variability
- Purified ECM components
  - collagen, hyaluronic acid (HA), fibrin, alone or in combination
- Alginate and chitosan (glycans from brown algae and the exoskeleton of shellfish)
  - popular because of their biocompatibility, easy processing, ability to encapsulate cells and bioactive molecules
  - but weak mechanical properties (not suited for applications that require a high elastic module)
- Silkworm- and spidersilk fibers
  - extensively used in high-strength biomedical applications –sutures
  - used in applications such as bone and ligament repair

ECM-derived materials.



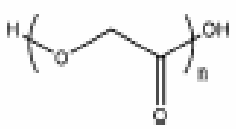
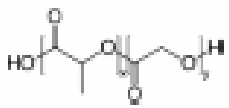
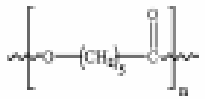
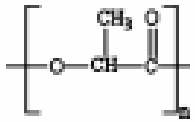
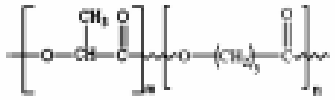
mucosal surface of intact small intestine submucosa.

collagen type I (derived from calf skin) that has been electrospun into fibers

Synthetic polymers

- poly(L-lactic acid) (PLLA), poly(glycolic acid)(PGA), poly(ethylene glycol) (PEG), polycaprolactones, polyorthoesters, polyanhydrides and polycarbonates
- approved by Federal Drug Administration (FDA)
- Co-polymerize → flexibility to tailor the mechanical and degradation properties
  - For example, for anterior cruciate ligament (ACL)–bone interface, a triphasic scaffold consisting of a soft-tissue phase (highly degradable PLGA), a fibrocartilage phase (more-slowly-degrading PLGA), a stiffer bone phase (PLGA and bioactive glass composite), seeded with fibroblasts and osteoblasts, respectively for few days
- Their major limit: cell adhesion is typically mediated by non-specific cell adhesion

Synthetic materials

Polyester	*Chemical Structure*	*Application*	*Remarks*
Polyglycolic acid		Heart[32]	Fast degradation results in limited application
Poly(lactide-co-glycolide)		Nerve,[50,133] skin,[49] heart,[102] vascular graft[51,52]	Controllable degradation rate by varying GA:LA ration
Polycarporlactone		Skin,[61,62] Bone tissue engineering[59,65,67,68,134] heart,[64] vascular graft,[60,135,136] stem cells[68,70]	Very stable polymer with long degradation time
Poly(L-lactide)		Nerve,[11,137] Bone tissue engineering,[40,41,138,139] heart,[102] vascular graft[42]	Very stable polymer with long degradation time
Poly(D,L-lactide)		Bone tissue engineering,[123,139] heart,[102] vascular graft[60]	Amorphous structure with faster degradation rate than PLLA
Poly(L-lactide-co-epsilon-caprolactone)		Vascular graft[12,72,140–143]	Faster degradation than PLLA and PCL
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)		Bone tissue engineering[70]	Highly stable with very long degradation time

PGA

- X bone
- X nervous tissue
- because of its rapid degradation (<20
- days31), electrospun pure PGA has rarely been used as tissue scaffold
- can cause an increase in localized acid concentration and result in tissue damage

PLA

- greater hydrophobicity → less degradability
- for nerve regeneration, bone tissue engineering,vascular engineering and stem cell tissue engineering

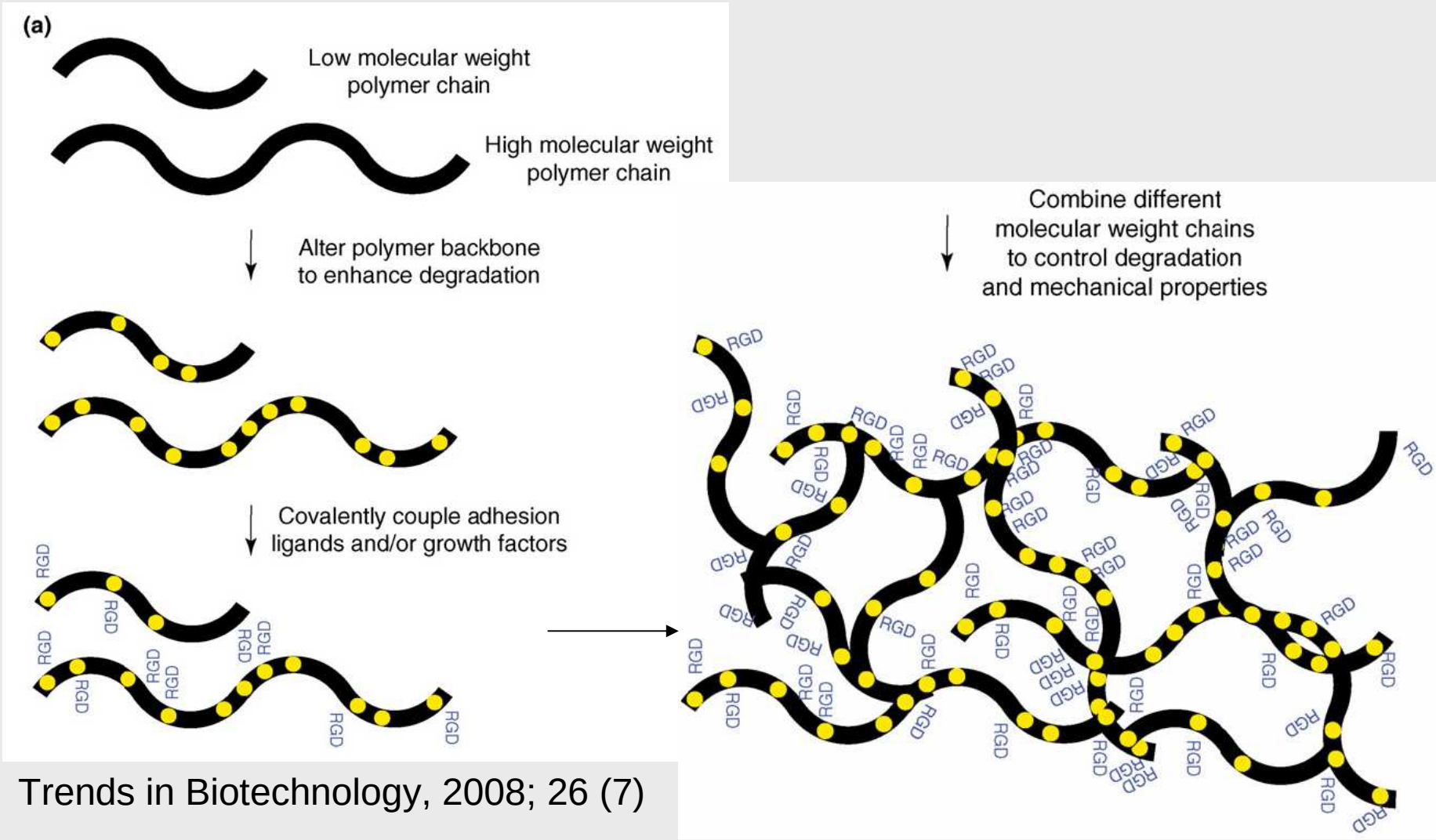
PLGA copolimer

- The most popular popular synthetic polymer in tissue engineering for its excellent biocompatibility and variable degradability (6-8 weeks)
- Microspheres used for drug delivery
- Amorphus, interconnected porous structure
- X skin, nerve, vessel,bone
- For blended PGA/PLA nanofibers, greater PGA content also induces faster degradation.

Material functionalization

- Chemical modification of synthetic polymer materials with entire ECM molecules or relevant peptide or glycan fragments for cell adhesion, or association of normally soluble cues (e.g. growth factors)
  - RGD (derived from fibronectin) and YIGSR (derived from laminin) coupled with amide linkages to carboxylic-acid-containing polymers using carbodiimide chemistry
- Nanoscale organization of proteins/peptides on the material might also affect many aspects of cell behavior, including proliferation, migration and differentiation
  - Closely spaced nanopatterned islands of RGD → greater pre-osteoblast focal adhesion kinase phosphorylation and cell spreading, whereas more widely spaced RGD islands → pre-osteoblast differentiation (mechanical stiffness)

covalent modification of traditional polydisperse materials



Trends in Biotechnology, 2008; 26 (7)

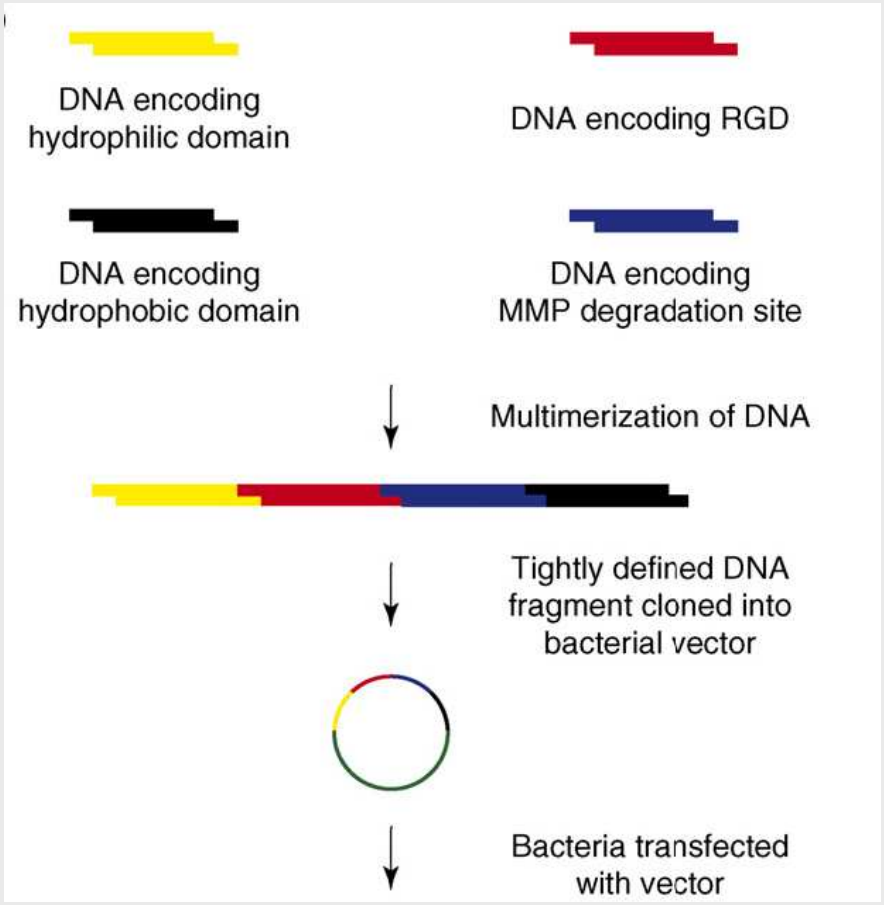
Alternative materials

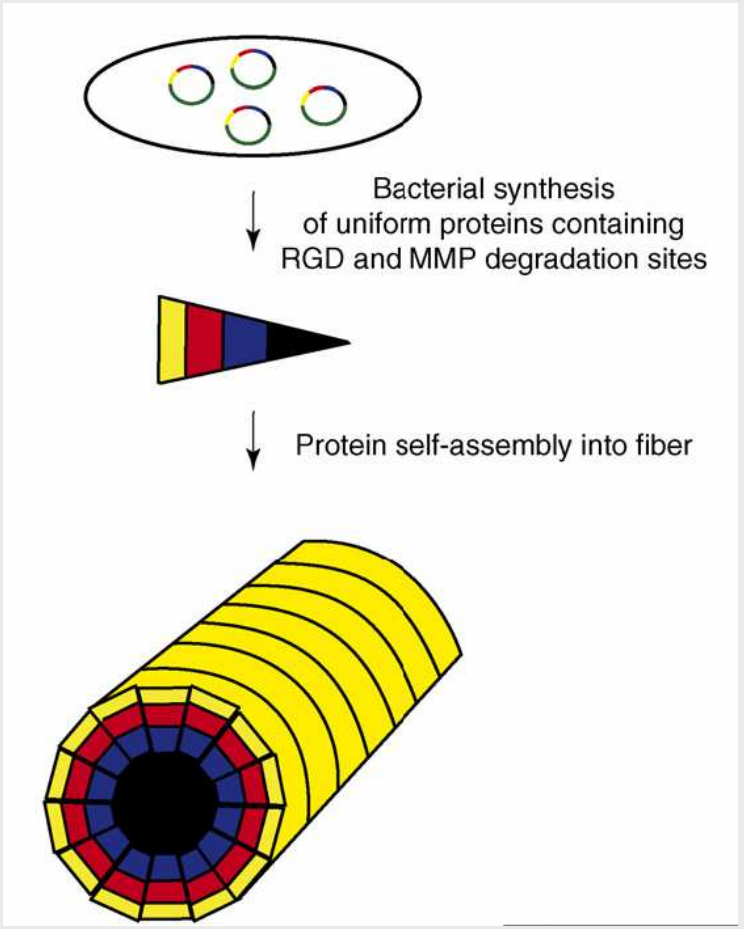
- create ‘designer materials’ with tightly defined physical, chemical and biological properties
- So far: typically at the stage of in vitro or preliminary small animal studies

genetical engineering

- genetical engineering of novel, well-defined and multifunctional materials
  - tailor-made hydrogels, stimuli-sensitive polymers and materials with controlled biorecognition, crosslinking, degradation, structure and mechanical properties
  - Ex: tandem repeats of the collagen II x cartilage
  - Spider silks with β sheet structures → enhanced mechanical properties
  - self-assembly of various peptides into nanofibers for > resistance (bone) or elastic properties,…

recombinant gene techniques





BUT: producing materials via recombinant gene techniques is relatively complex and expensive,

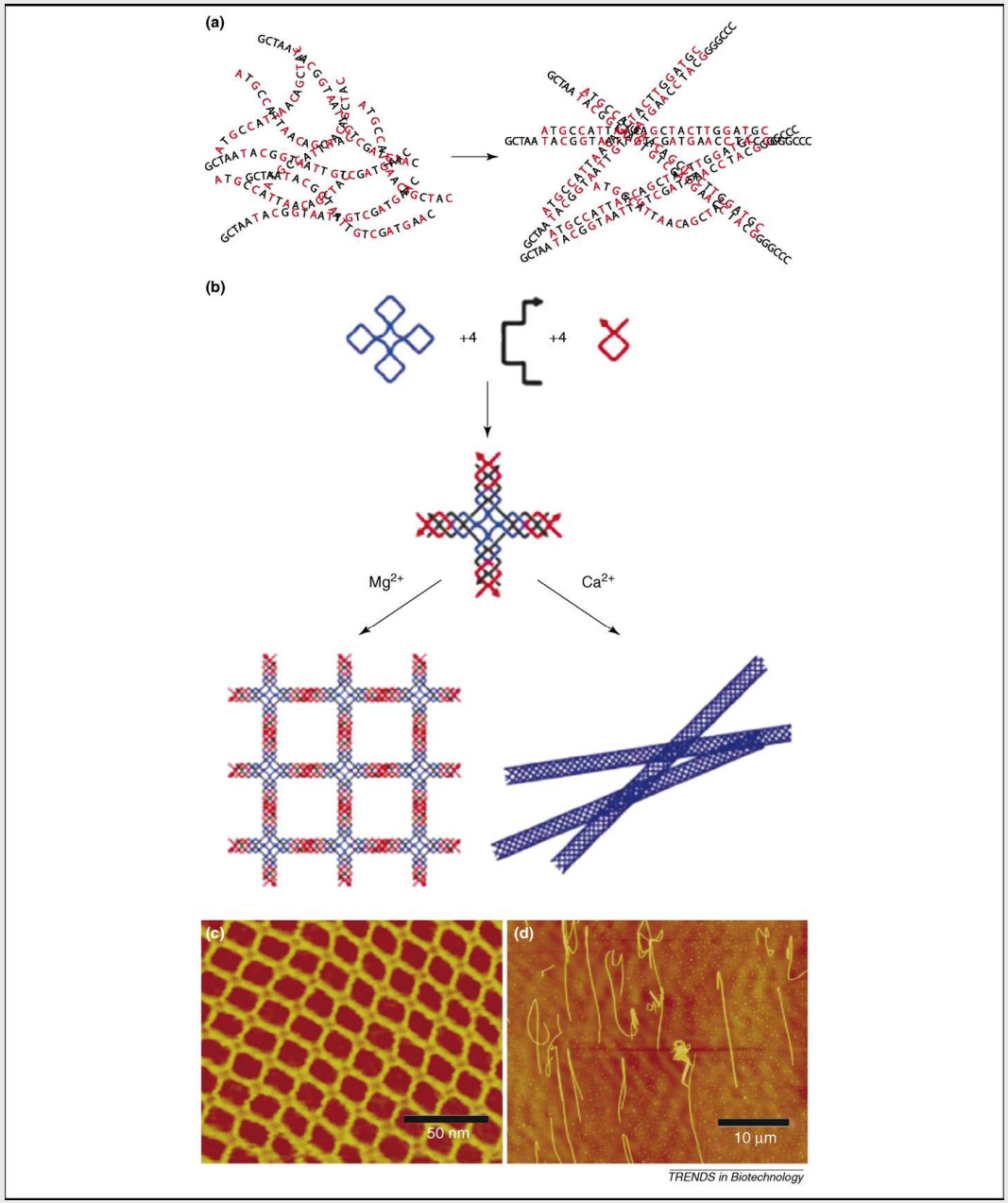
DNA-based materials

- increasingly being investigated as a biomaterial because one can control material properties by defining sequences of building blocks, in this case nucleotides
  - Single-stranded DNA molecules with specific nucleotide sequences can self-assemble into predictable duplex conformations

DNA-based materials

- Self-assembly of DNA-based materials
- specific cations during self-assembly has been shown to provide another level of structural control by manipulating electrostatic interactions that influence the conformation of DNA

DNA-based materials



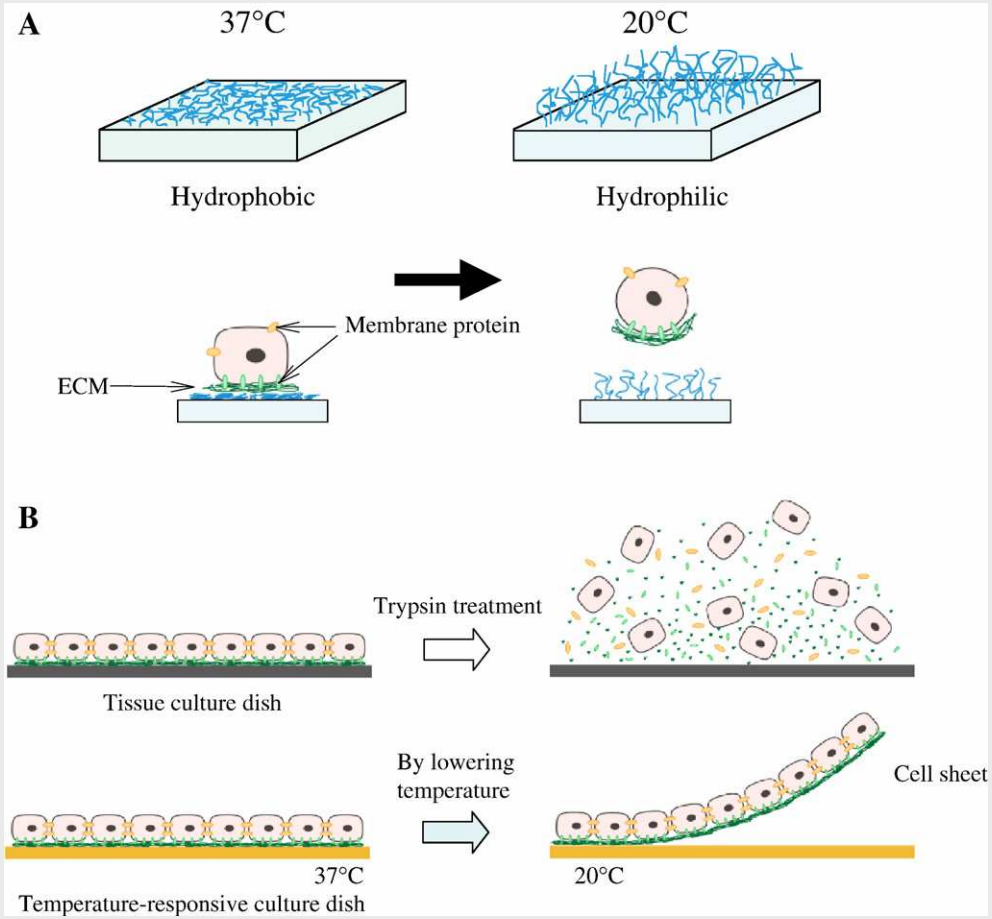
Electrically conductive materials

- Potential for generating electrical signals in muscle and neural tissues and their ability to contract and relax in response to applied voltages.
  - Electroactive polymers (EAPs)(conjugated polymers and dielectric elastomers) are capable of mechanical actuation induced by an external electric field, and consequently they have been studied for applications as artificial muscles
  - Polypyrrole (PPy) and polyaniline-based actuators can function in bodily fluids and require low voltages (<1V) to induce large strains. Used x blood, bladder
  - impart mechanical signals to adherent cells and manipulate their organization and differentiation
  - can control the release of bioactive molecules encapsulated within EAPs
  - improved neurite outgrowth from injured neurons (PPy-NGF)

Stimuli-sensitive polymers

- materials that respond to cues in their surroundings (also EAPs)
- **Smart materials**, designed to undergo structural, often reversible, changes in response to environmental factors (e.g. temperature, pH, electric field, solute concentrations, light) → can provide functionalities on-demand, such as phase transition, alteration in shape or **release of encapsulated growth factors or cells**
  - Ex: hydrogel poly(N-isopropylacrylamide) (PNIPAAm) and its copolymers, thermo-sensitive

Smart scaffold: poly (N-isopropylacrylamide) (PIPAAm)



Shape-memory polymers

- They recover large deformations in response to heat, light or solvents, they have the potential to be used as stents or tissue-engineering scaffolds that **could be introduced endoscopically into the body in a compact form and then, once in place, be triggered** to re-assume their more complex, space-filling form.
  - Ex Matrigel

for controlled drug-delivery

- insulin release systems developed to respond to high glucose levels
- growth-factor release systems that respond to the local mechanical environment
- multiple bioactive agents in response to environmental stimuli to optimize tissue morphogenesis
- many of these materials are not biodegradable

How to make scaffolds

Different types of scaffolds can be made with different techniques

Nanofibers for TE

- can be generated using mainly three methods:
- self assembly
  - Solvent extraction from gelated polymer solution to form nanofibrous foam-like structures
- phase separation
  - In ordered structure through weak and noncovalent bonds
- electrospinning

TISSUE ENGINEERING: Part B, 2009; 15 (3)

More advanced techniques to make scaffolds

- Electrospinning
- Hydrogel
- Photolitography (microprinting)

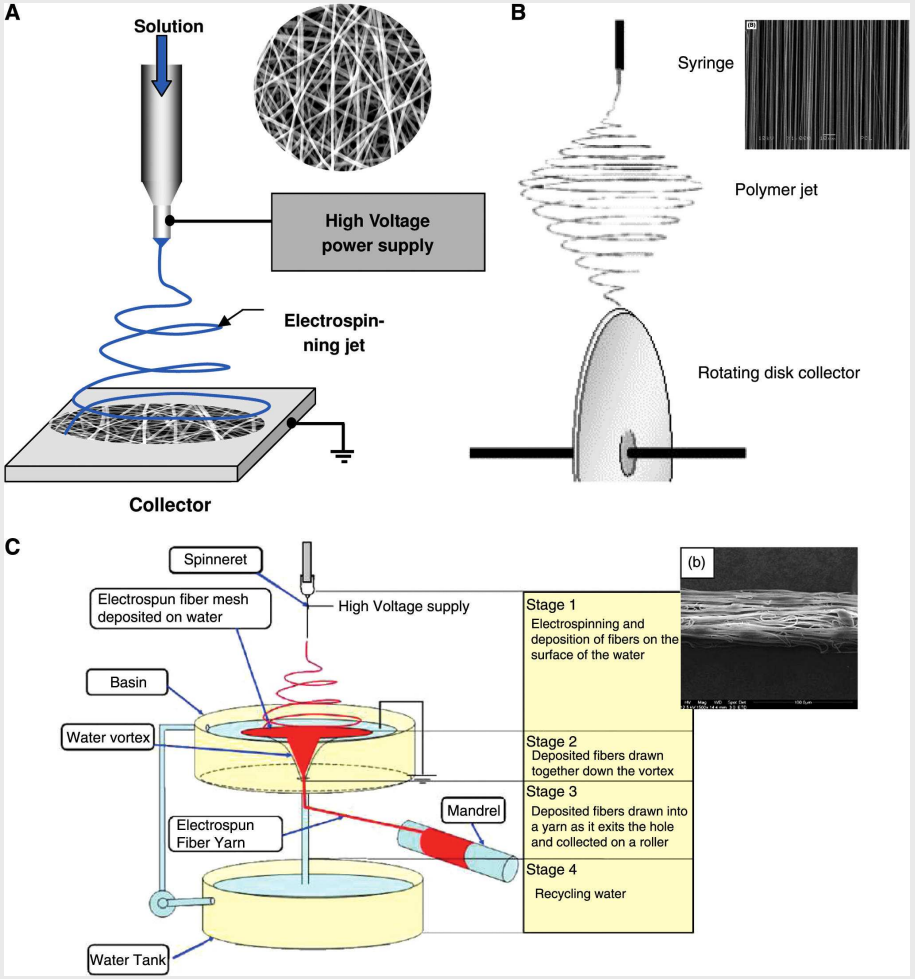
Nanofibers & electrospinning

TISSUE ENGINEERING: Part B
Volume 15, Number 3, 2009

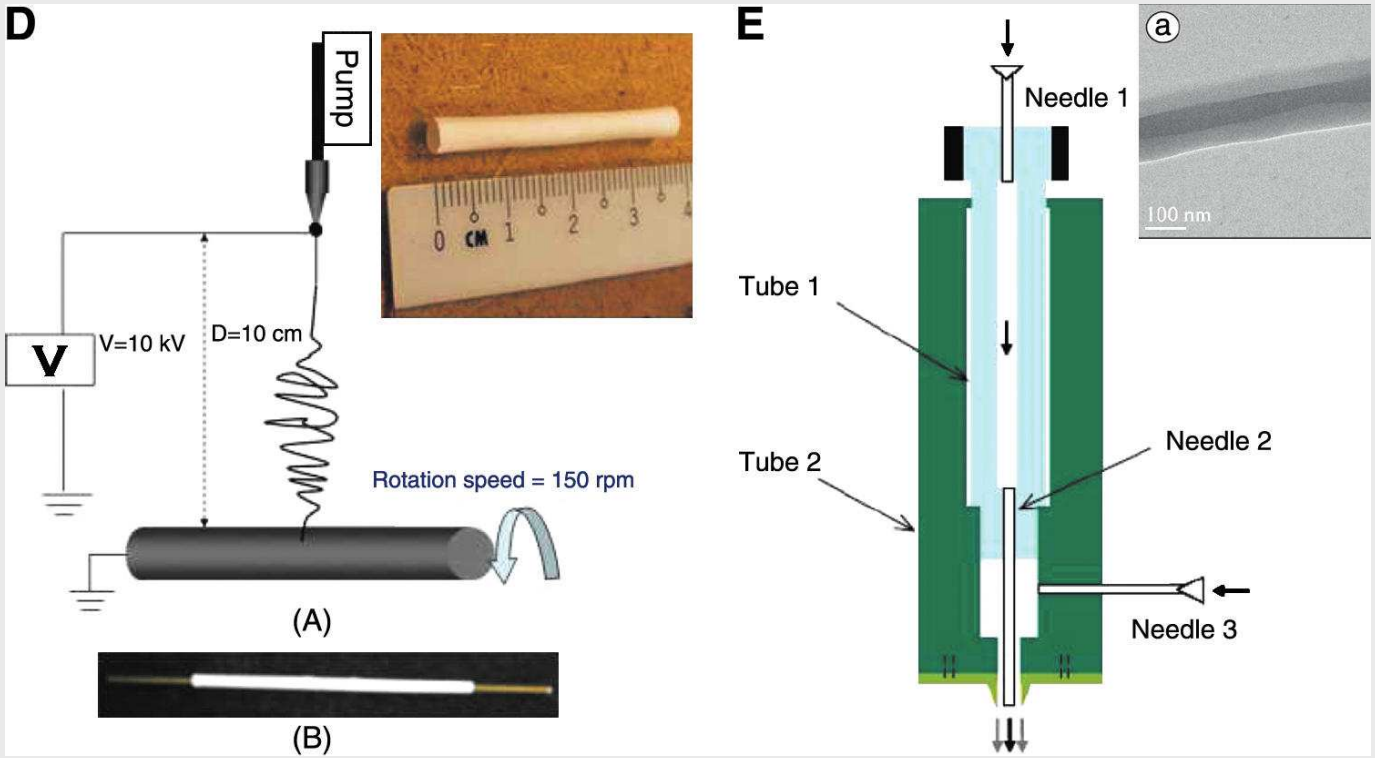
Electrospinning

- static electricity to draw fibers from a polymer solution and deposits the fibers on the surface, where the fibers cure to form a thin, uniform mesh.
- generates continuous, uniform, long fibers with diameters down to the nanoscale dimension
- Advantages: suitable for small quantity production for laboratory research use and mass production for industrial use
- Different nanofibrous structures with different two- or three-dimensional shapes can be generated

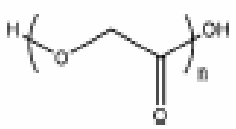
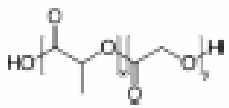
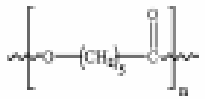
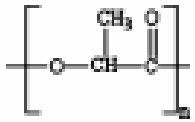
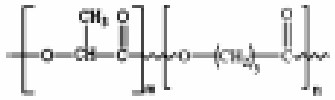
- Biodegradable natural & synthetic (polysters) polymers
- ex_: LA, PGA, PLA,
- Approved by FDA



Electrospinning



Synthetic electrospun nanofibers

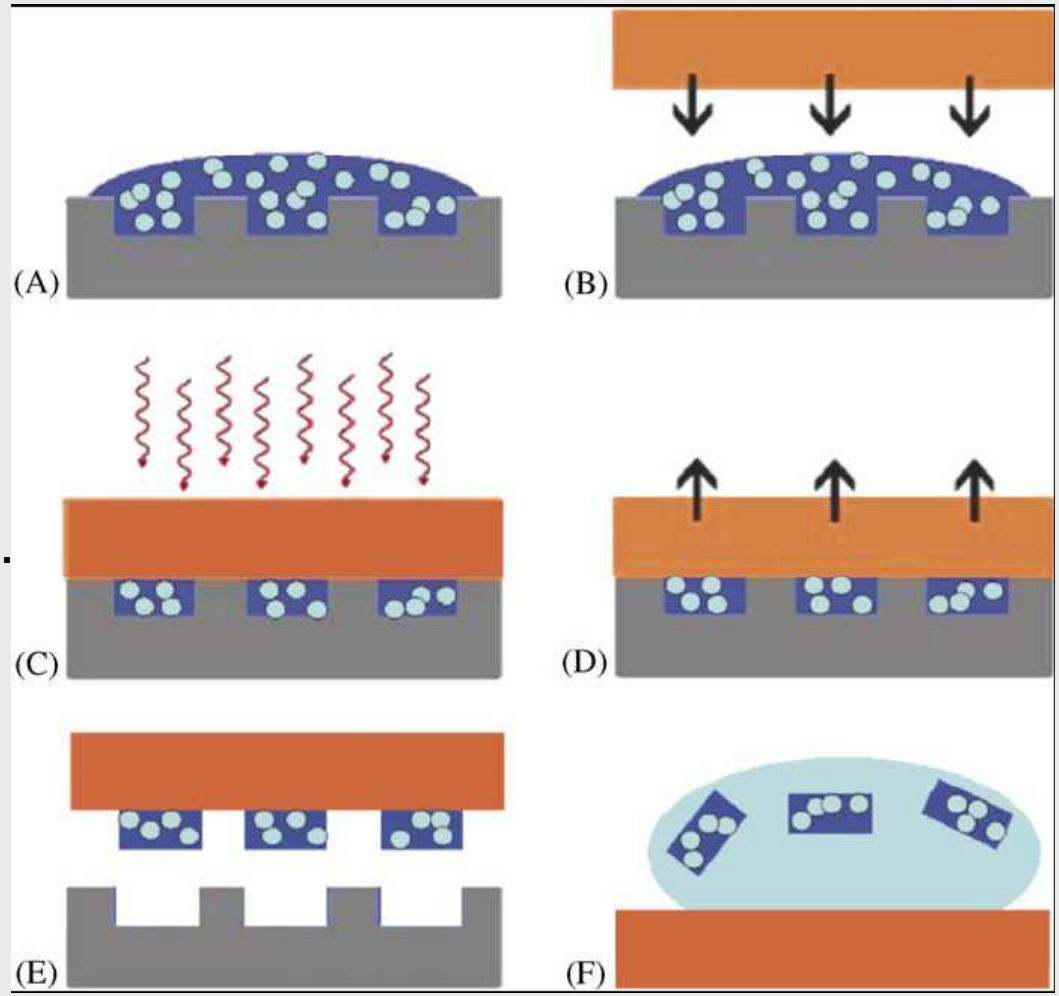
Polyester	*Chemical Structure*	*Application*	*Remarks*
Polyglycolic acid		Heart[32]	Fast degradation results in limited application
Poly(lactide-co-glycolide)		Nerve,[50,133] skin,[49] heart,[102] vascular graft[51,52]	Controllable degradation rate by varying GA:LA ration
Polycarporlactone		Skin,[61,62] Bone tissue engineering[59,65,67,68,134] heart,[64] vascular graft,[60,135,136] stem cells[68,70]	Very stable polymer with long degradation time
Poly(L-lactide)		Nerve,[11,137] Bone tissue engineering,[40,41,138,139] heart,[102] vascular graft[42]	Very stable polymer with long degradation time
Poly(D,L-lactide)		Bone tissue engineering,[120,139] heart,[102] vascular graft[60]	Amorphous structure with faster degradation rate than PLLA
Poly(L-lactide-co-epsilon-caprolactone)		Vascular graft[12,72,140–143]	Faster degradation than PLLA and PCL
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)		Bone tissue engineering[70]	Highly stable with very long degradation time

Hydrogels

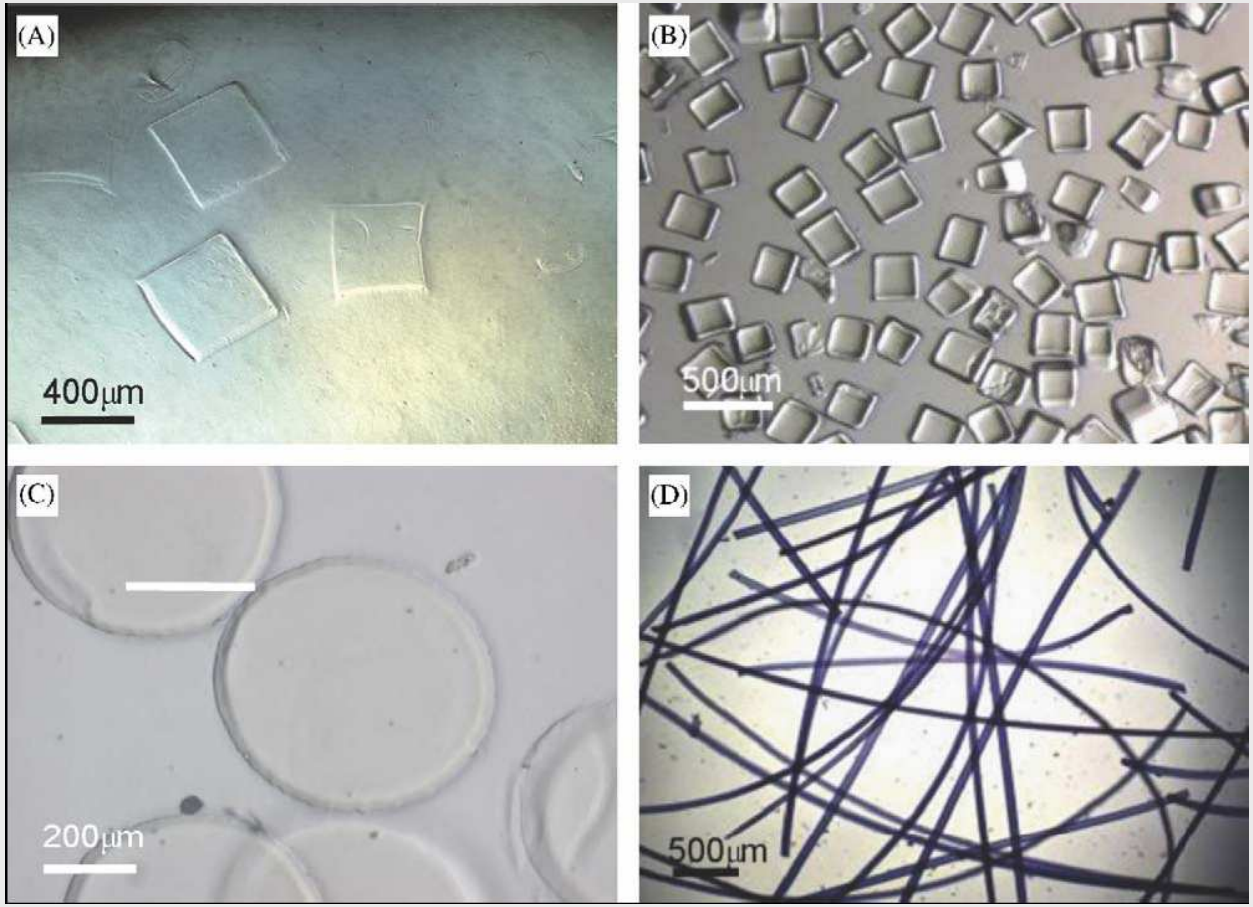
- encapsulate live cells in three-dimensional (3D) microscale hydrogels (microgels) of controlled shapes and sizes
- Cells were suspended in methacrylated hyaluronic acid (MeHA) or poly(ethylene glycol) diacrylate (PEGDA) hydrogel precursor solution containing photoinitiator, micromolded using a hydrophilic poly(dimethylsiloxane) (PDMS) stamp, and crosslinked using ultraviolet (UV) radiation.
- Cells within microgels well distributed and viable. These shape-specific microgels could be easily retrieved, cultured and potentially assembled to generate structures with controlled spatial distribution of multiple cell types.

Process of cell encapsulation and microgel formation.

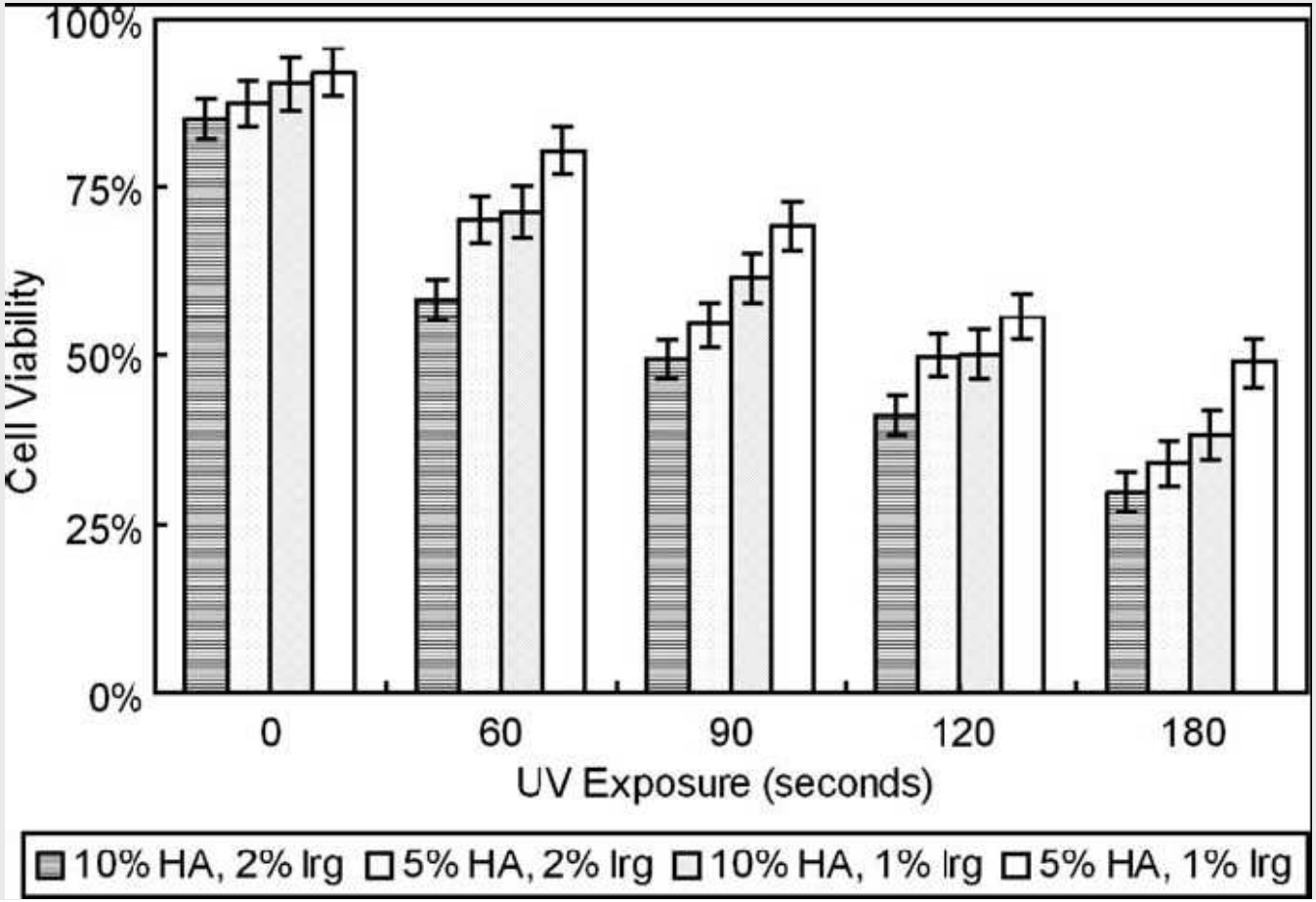
- (A) Cells are suspended in prepolymer solution and deposited onto a plasma-cleaned PDMS pattern.
- (B) A PDMS coverslide is placed on top, forming a
- reversible watertight seal.
- (C) Polymer liquid is photopolymerized via UV light.
- (D) The PDMS coverslide is lifted,
- (E) removing the microgels which are then (F) hydrated and harvested.



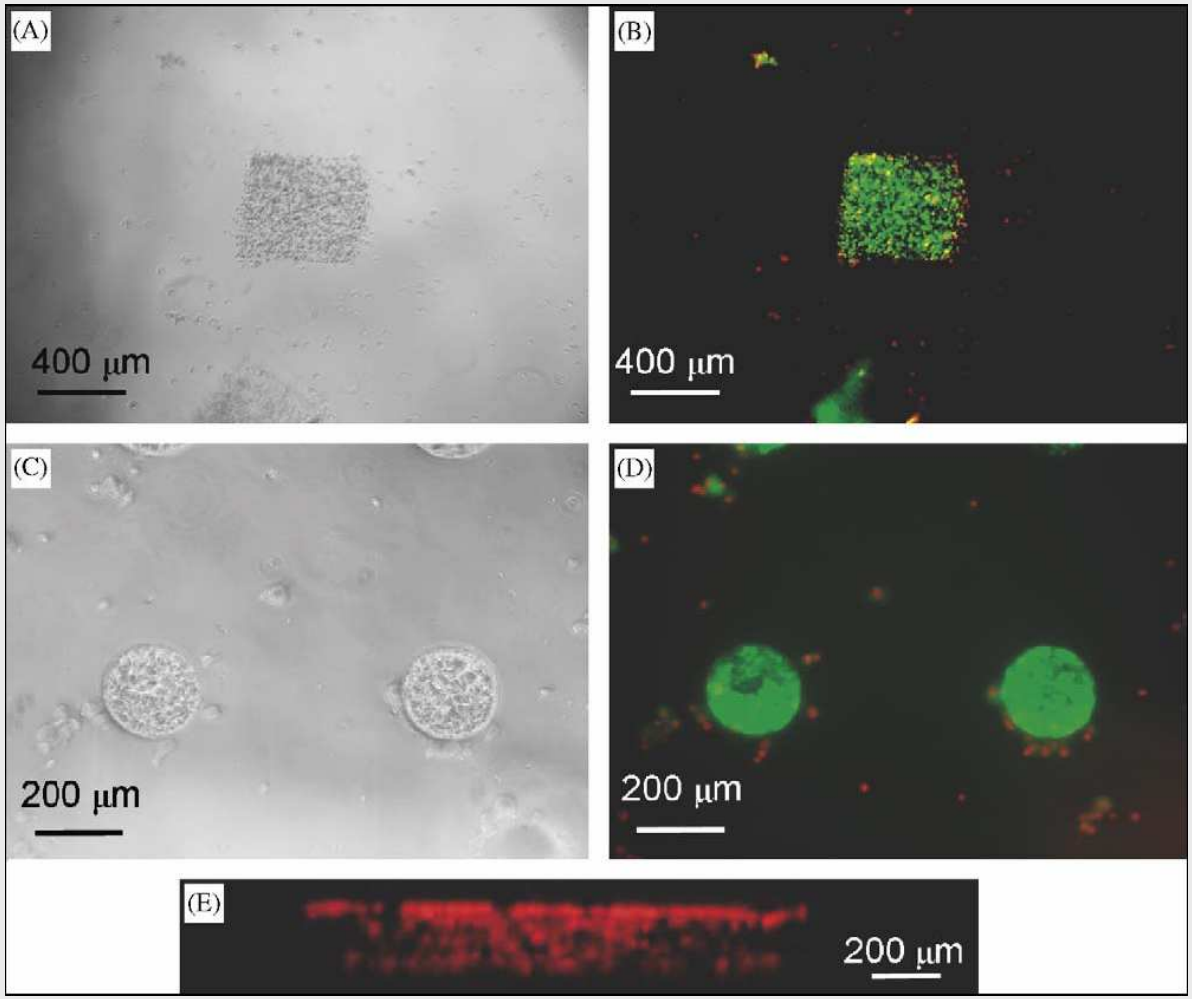
Versatility in microgel shapes.



Optimization for initial cell viability. [macromer (HA) concentrations, photoinitiator concentrations, and UV exposure durations.



Cell encapsulation, viability, and distribution.

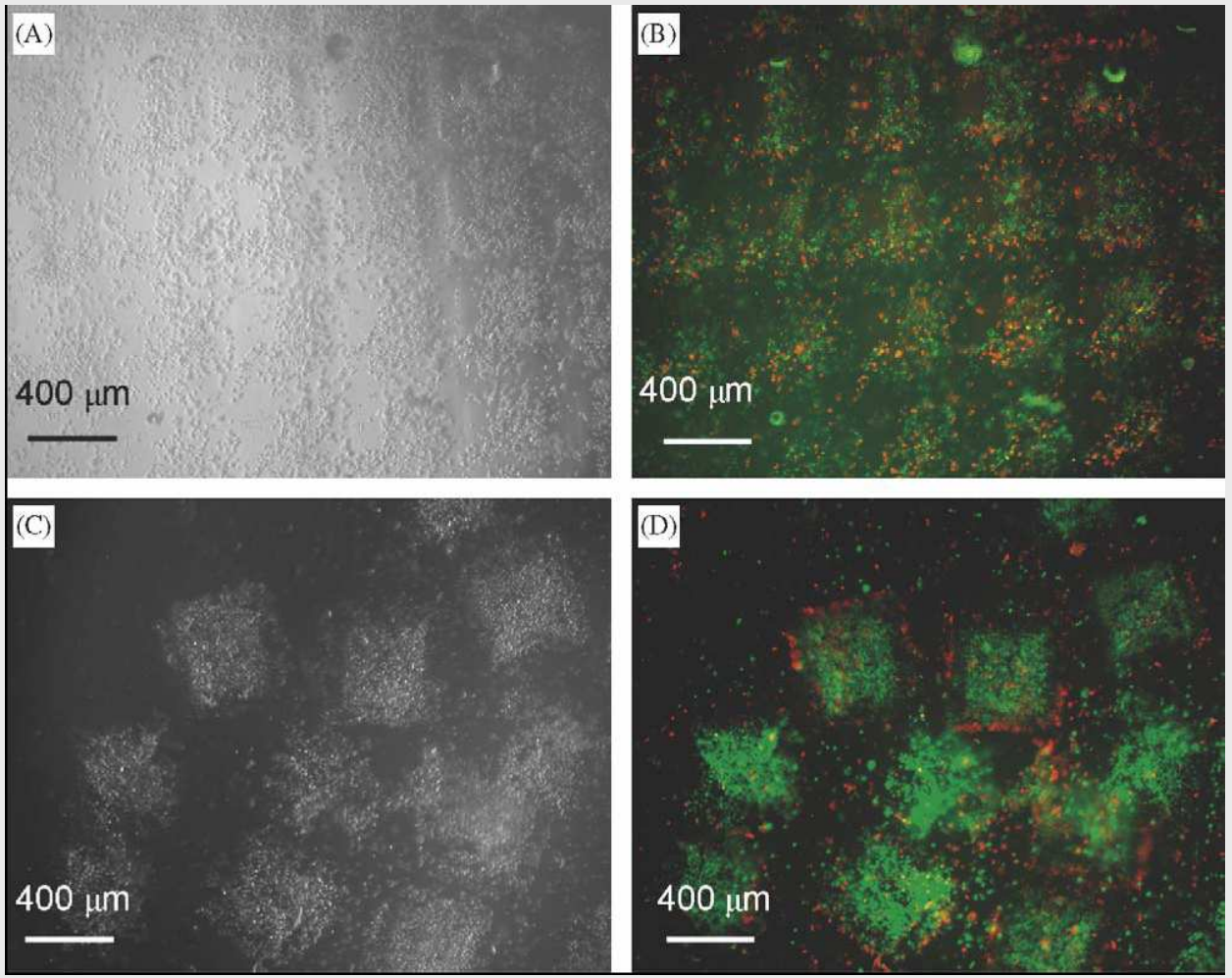


Green =
Metabolized calcein

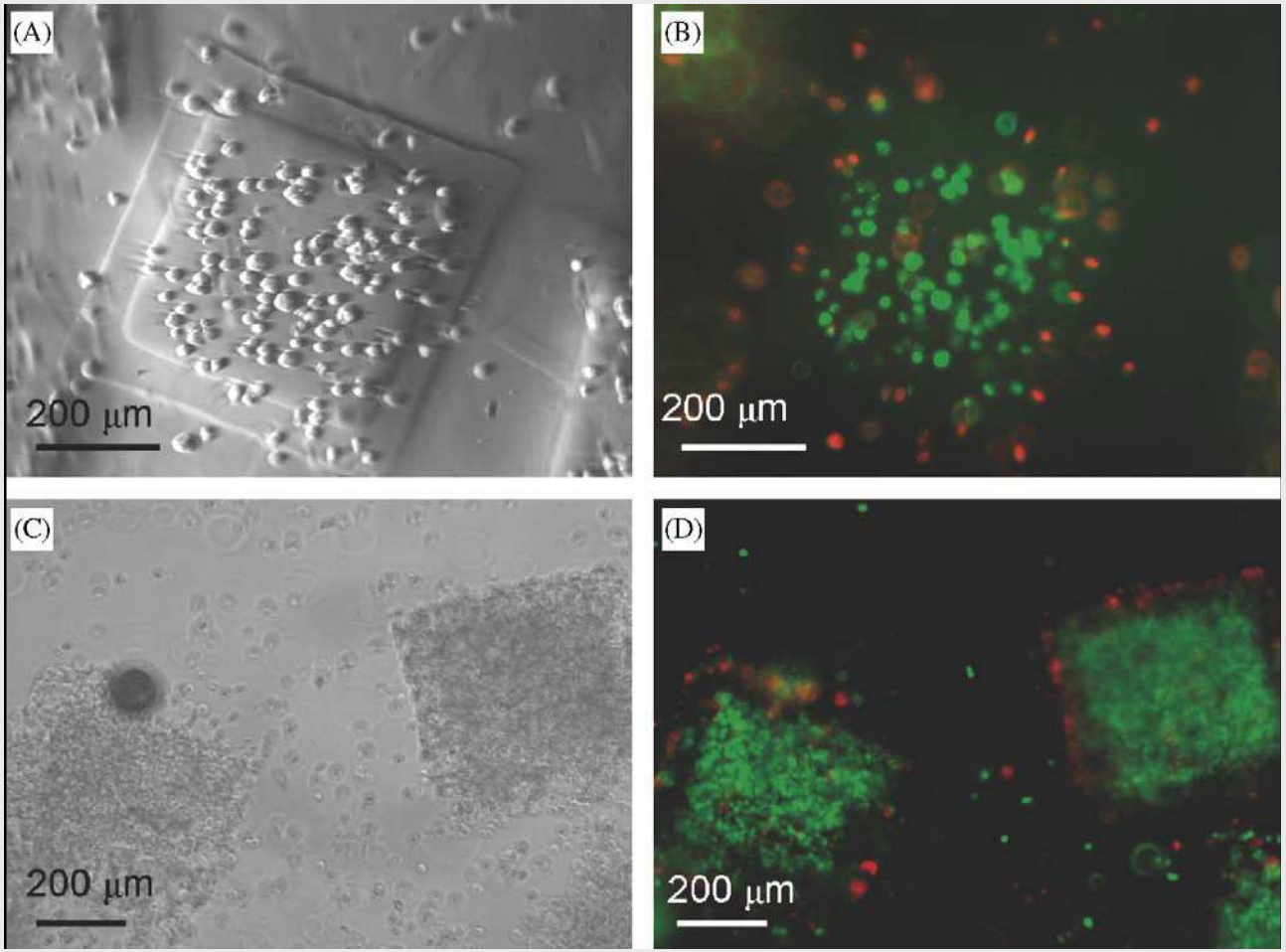
Red = dead cells

Rodhamine x
Cell distribution

Harvesting microgels. Removal of the PDMS coverslide and subsequent hydration

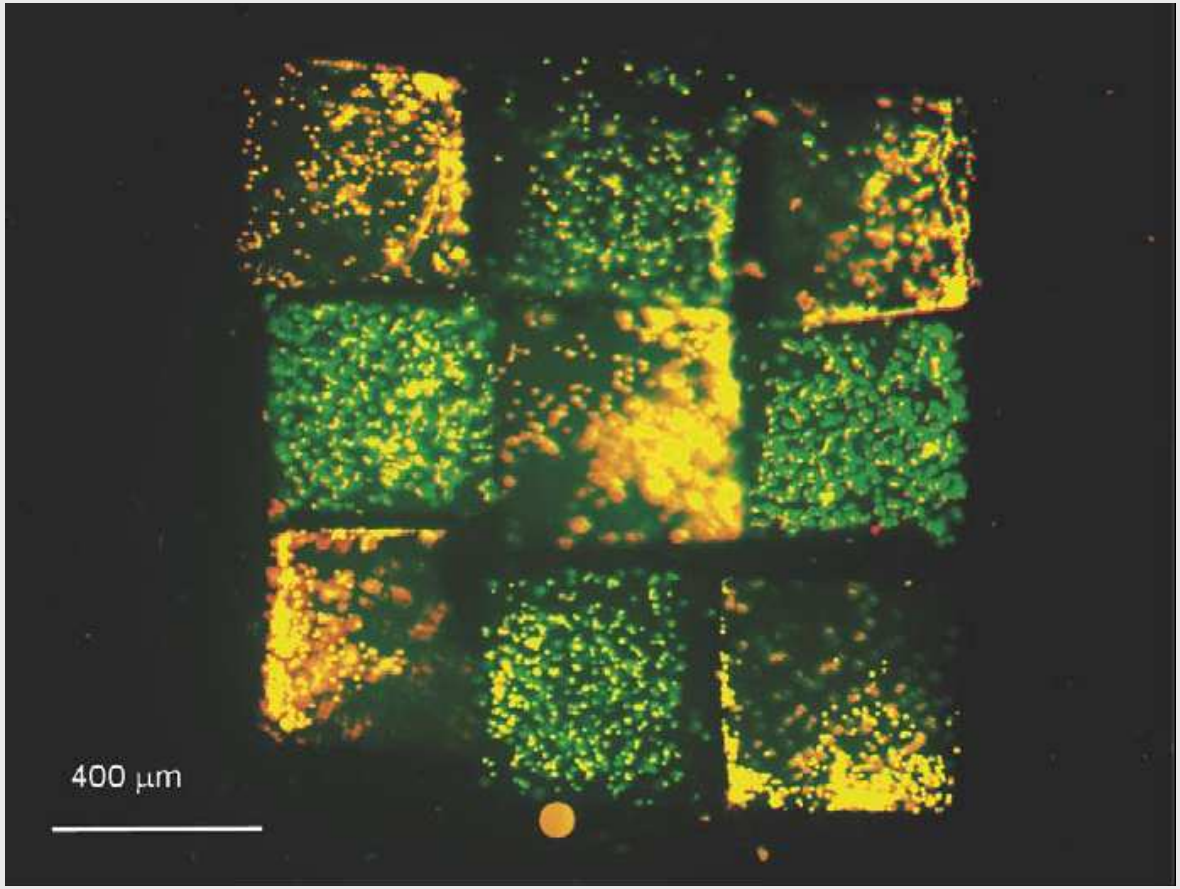


Variation in cell density.



Microgel arrangement and assembly.

Rhodamine (red) and FITC (green) stained cells were encapsulated in separate HA microgels and subsequently arranged in an alternating checkerboard pattern.



- developed a micromolding technique for encapsulating live cells in microscale photocrosslinkable hydrogels of controlled 3D shapes.
- cells successfully encapsulated in both MeHA and PEGDA, homogenously at various cell densities
- → for tissue engineering applications that require controlled spatial distributions of cells.

Fabrication of transferable micropatterned-co-cultured cell sheets with microcontact printing

Biomaterials. 2009 Oct;30(29):5427-32

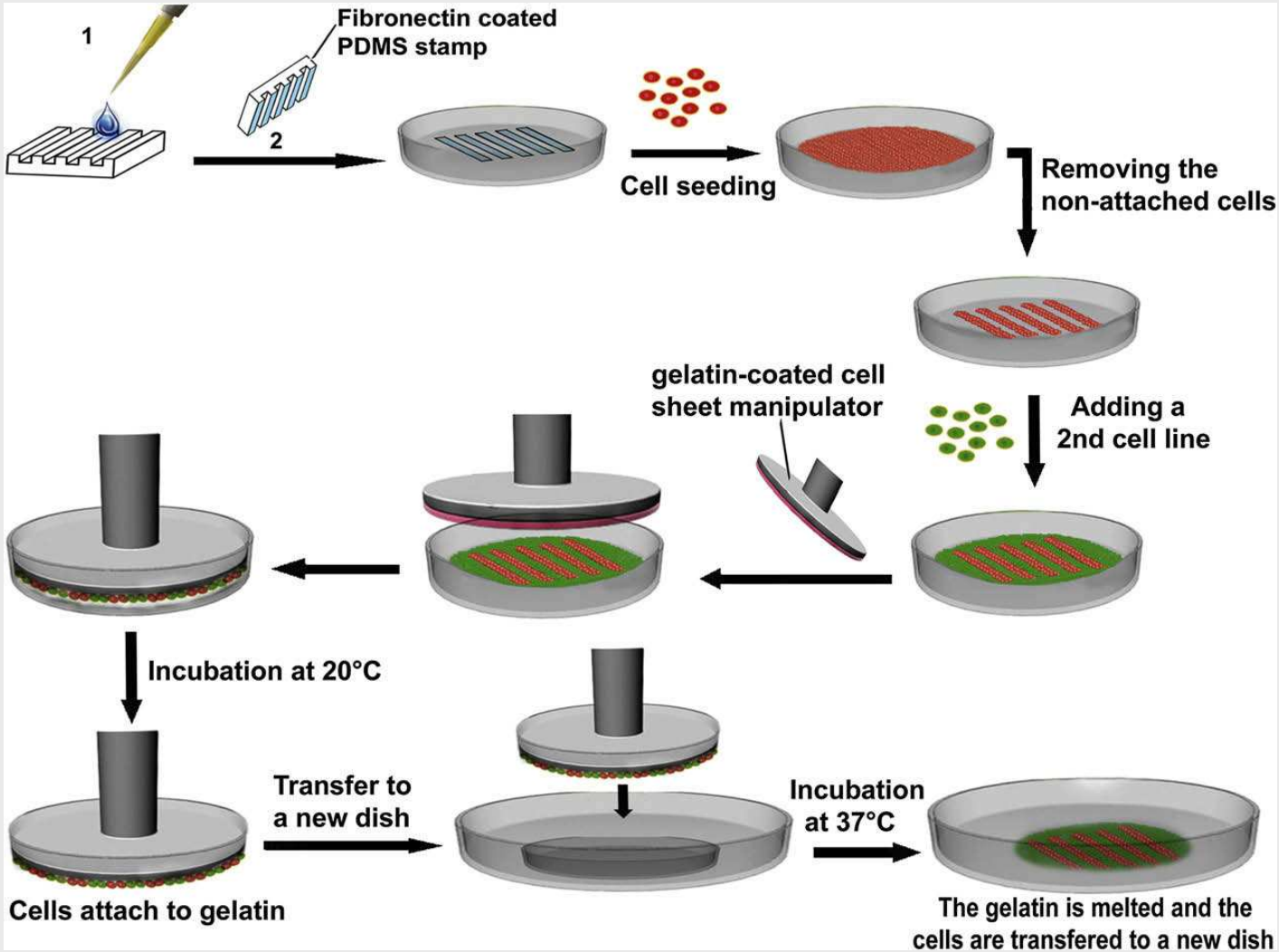
For fabrication of thick tissue constructs having a complex microarchitecture

Microcontact printing

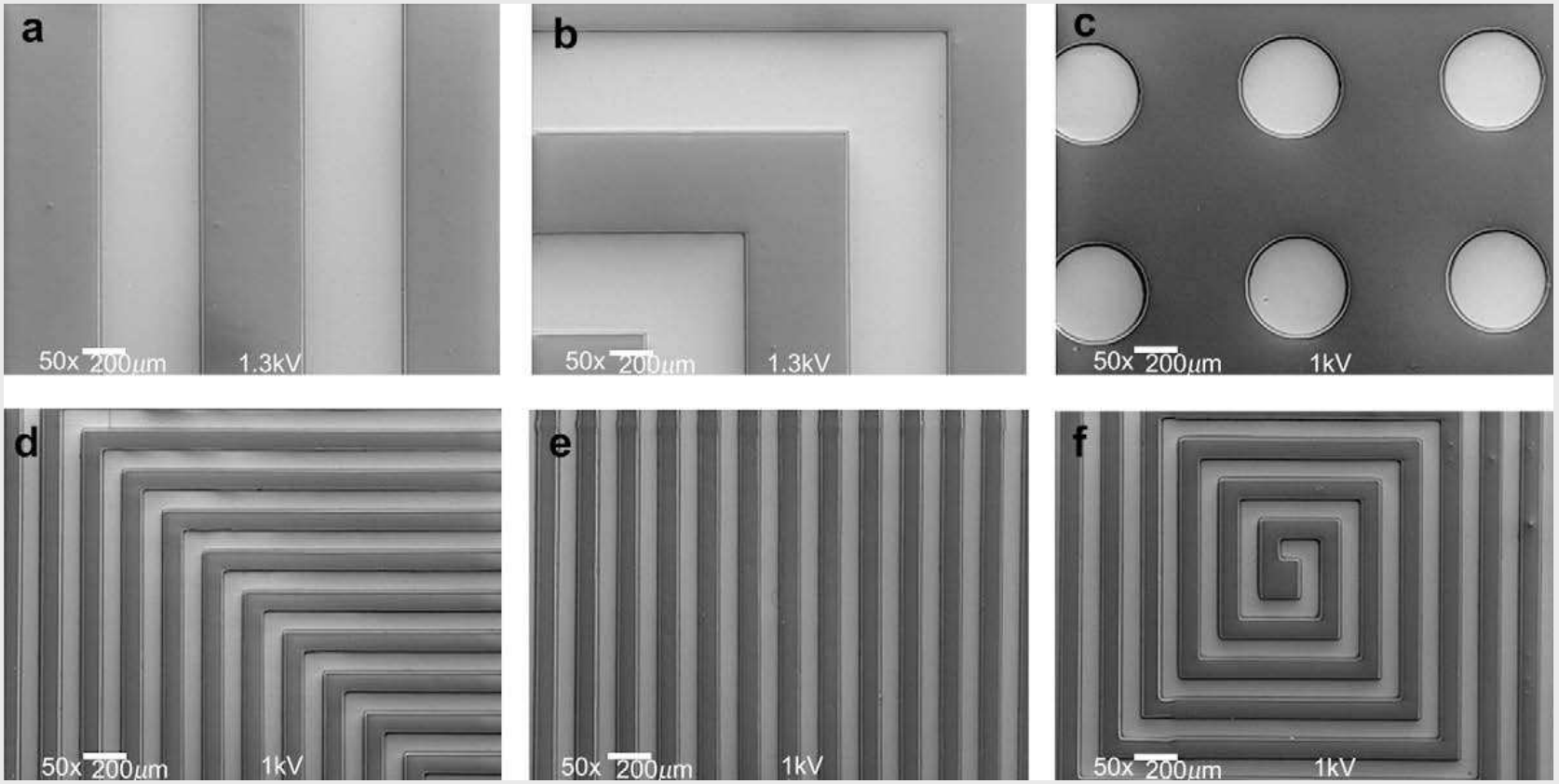
- rapid prototyping manner
- compared with our previous work to obtain micropatterned cell sheets, expensive masks for electron beam radiation, electron beam sources, and laborious grafting of two kinds of temperature-responsive polymer on the surfaces were eliminated in the present method
- 5 cell layers were repeatedly stratified, and thick cell sheet constructs were fabricated

- PDMS stamps were treated with oxygen plasma to make the surface temporally hydrophilic, coated with fibronectin (30 min at 80 C)
- transfer of fibronectin onto temperature-responsive dishes
- seeded hepatocytes in FCS- medium, 3 h at 37 C
- plated endothelial cells
- cell sheet harvest and transfer

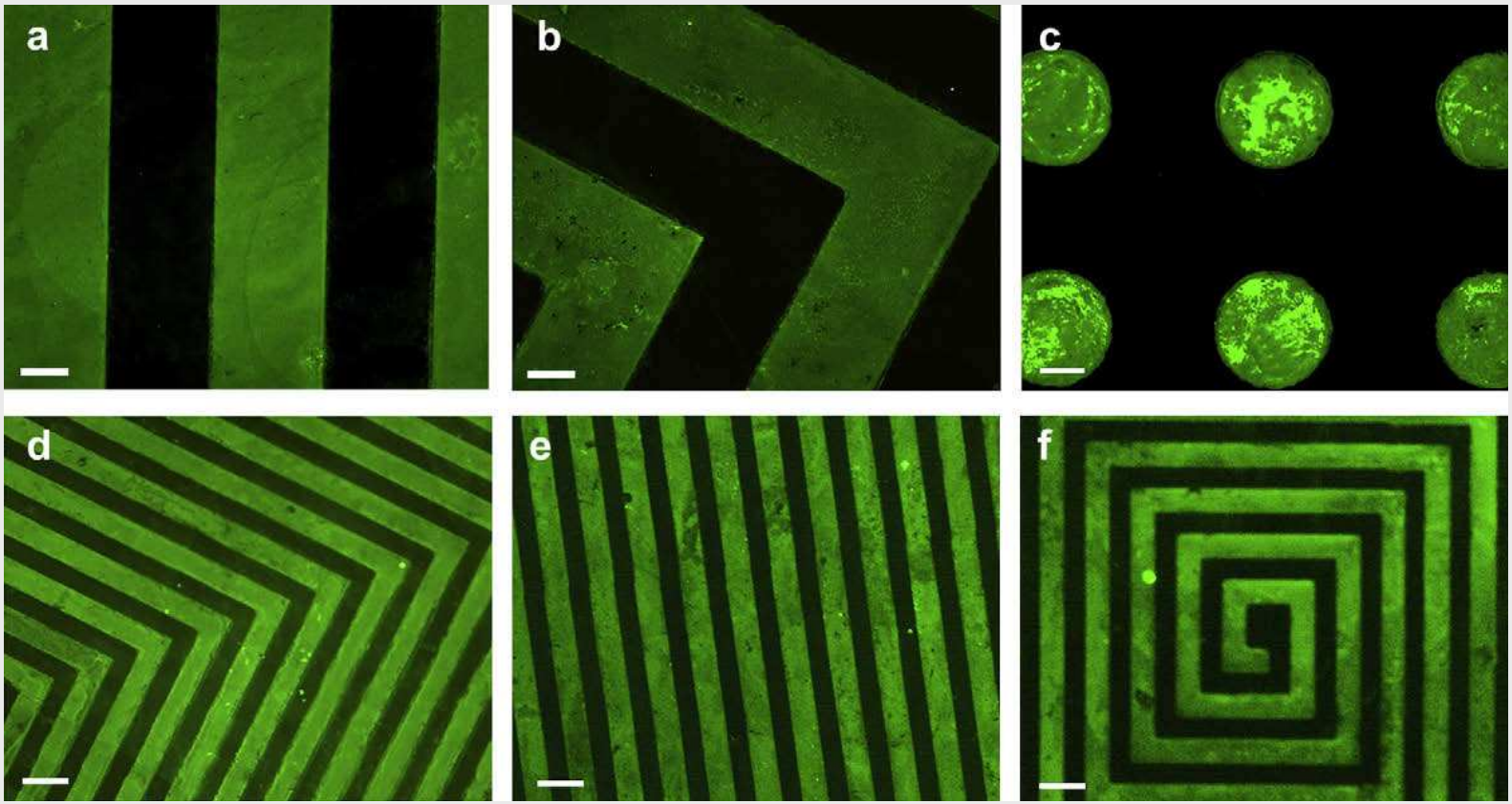
Transferable micropatterned cell sheets for tissue engineering



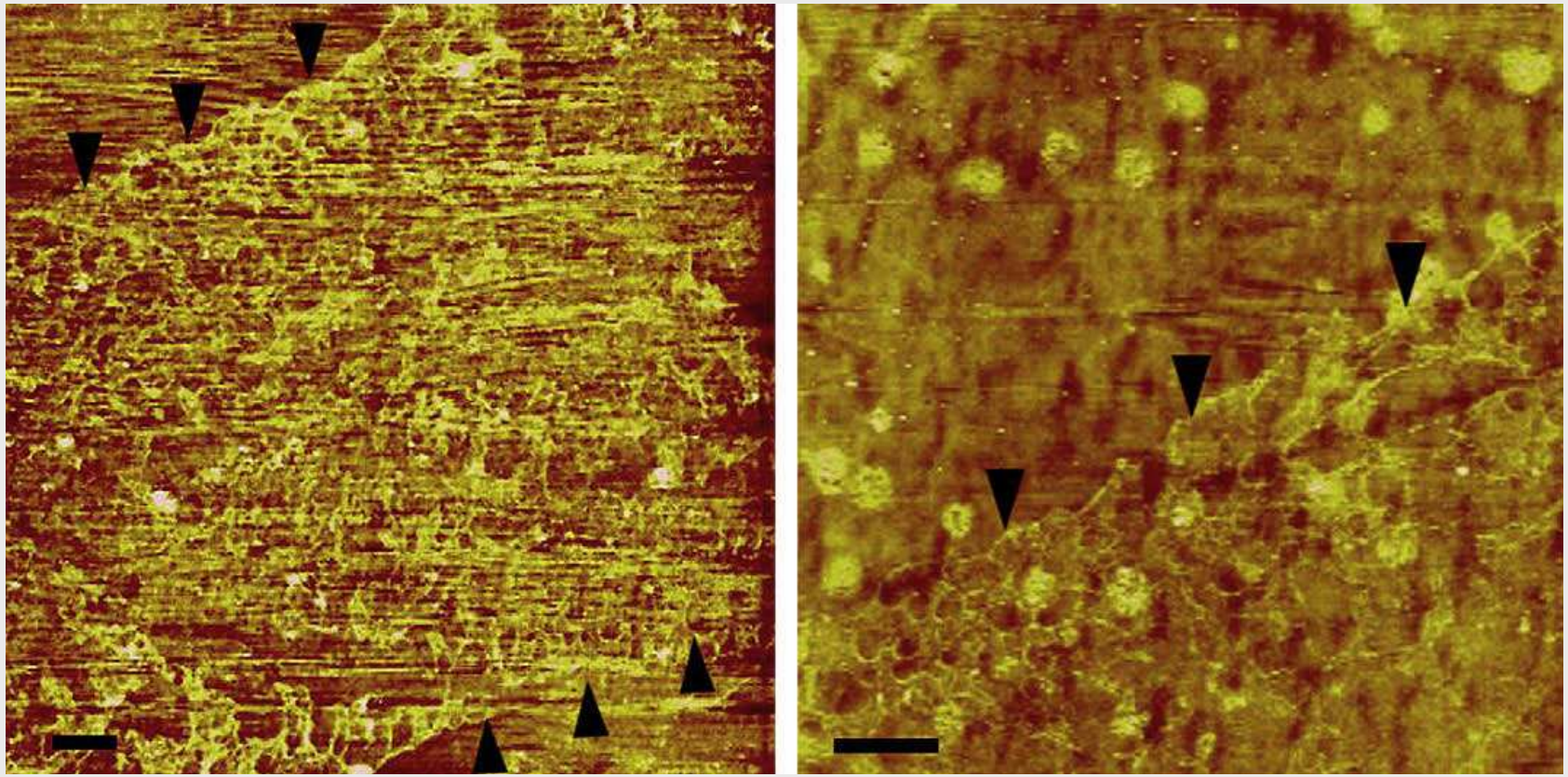
SEM images of micropatterned photoresist on siliconwafers



Immunofluorescent micrographs of temperature-responsive surfaces microcontact-printed with fibronectin

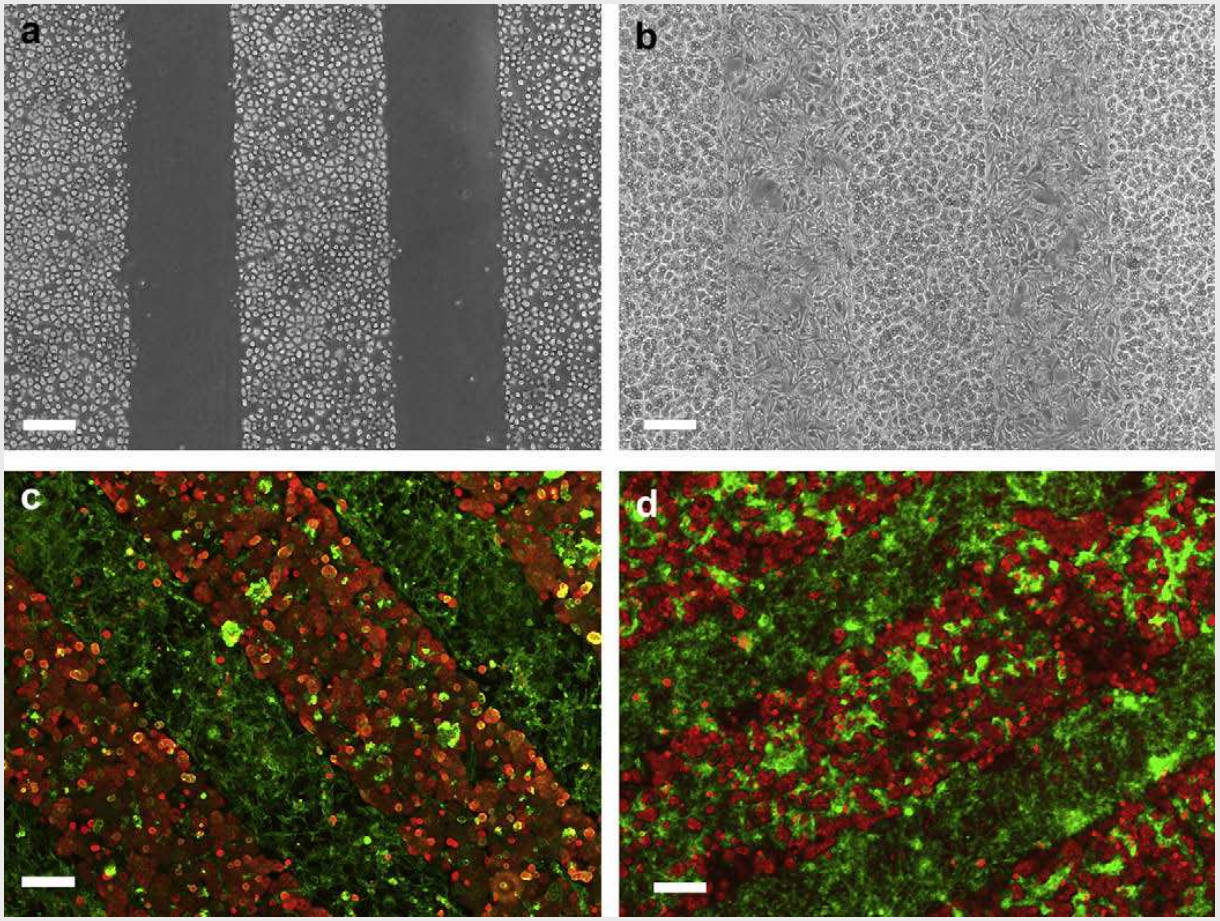


AFM images of temperature-responsive dish surfaces printed with 100-mm width fibronectin stripes



Microscopic views of micropatterned-co-culture of hepatocytes and endothelial cells.

Hepatocytes
FCS-



co-cultured
Hepatocytes
endothelial cells

Red
Hepatocytes
Green
endothelial cells

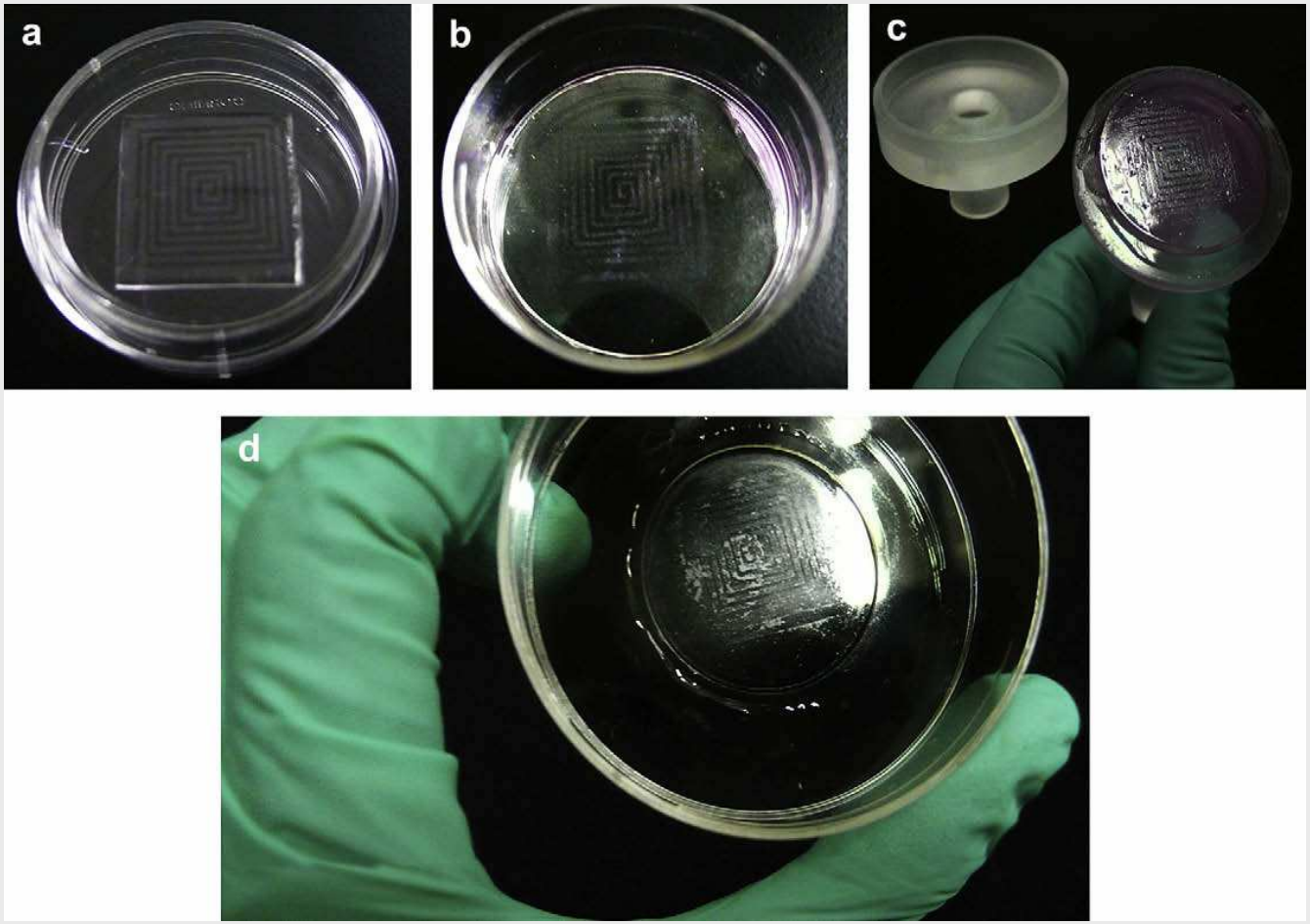
before

After cell sheet transfer

PDMS stamps

Micropatterned hepatocytes cells

Harvest of cell sheets with gelatin-coated stamps



Cell sheets transferred onto larger dishe

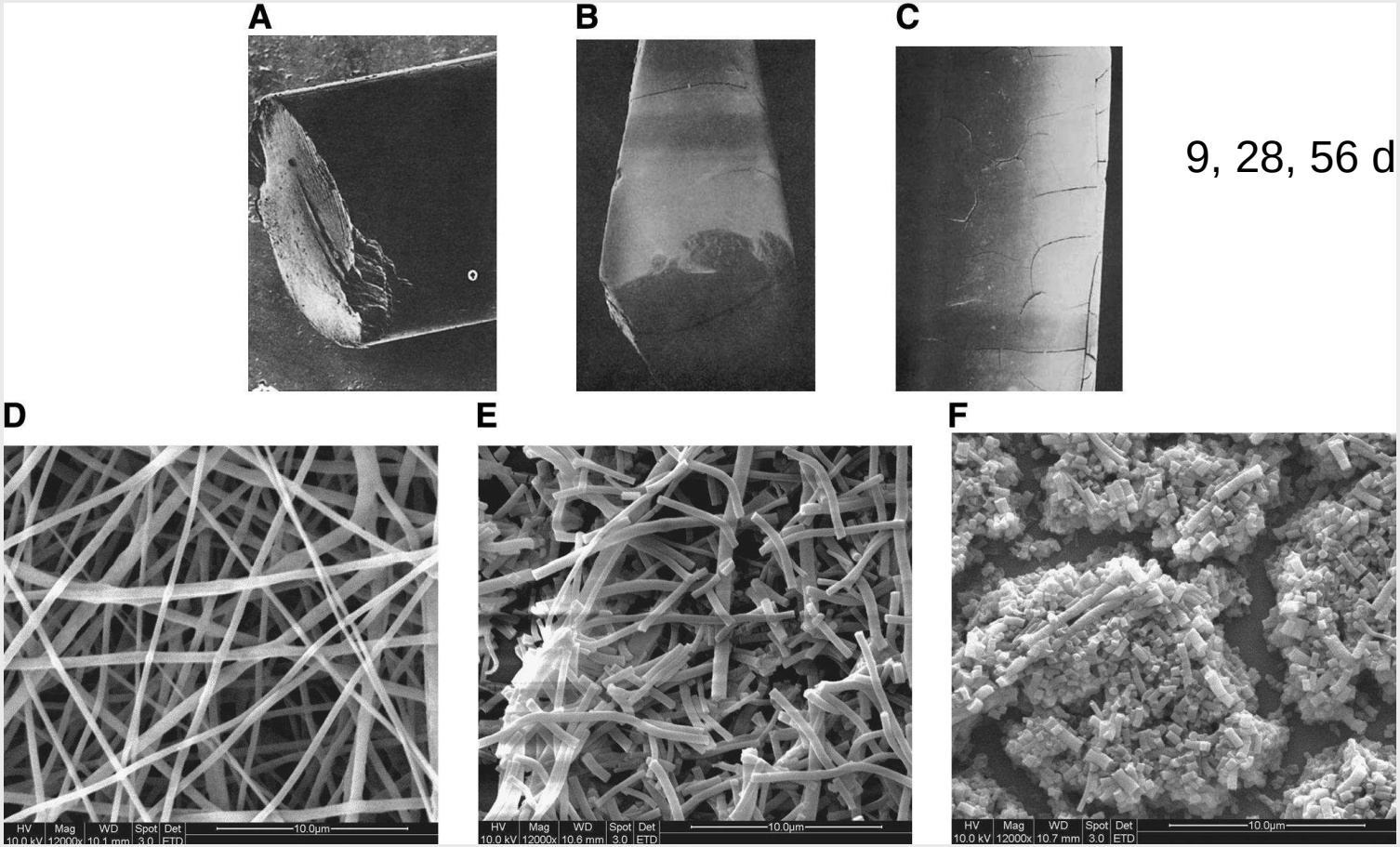
- useful for the fabrication of thick tissue constructs having a complex microarchitecture

Material degradation

Control of material degradation

- To facilitate scaffold remodeling and replacement by resident and host cells, enable the infiltration of blood vessels and control the release of matrix-associated growth factors and morphogens to enhance tissue regeneration
  - addition of side chains to sensitize or desensitize the polymer to hydrolysis
  - chemical alteration of main chains to generate controlled numbers of functional groups in the polymer backbone that are susceptible to hydrolysis (e.g. partially oxidizing alginate)
  - the co-polymerization of macromers that have different degradation profiles
  - Ex alginate hydrogel to deliver VEGF-A165 x ischemic hindlimb
- Warning: undesired inflammatory responses or toxicity in the body

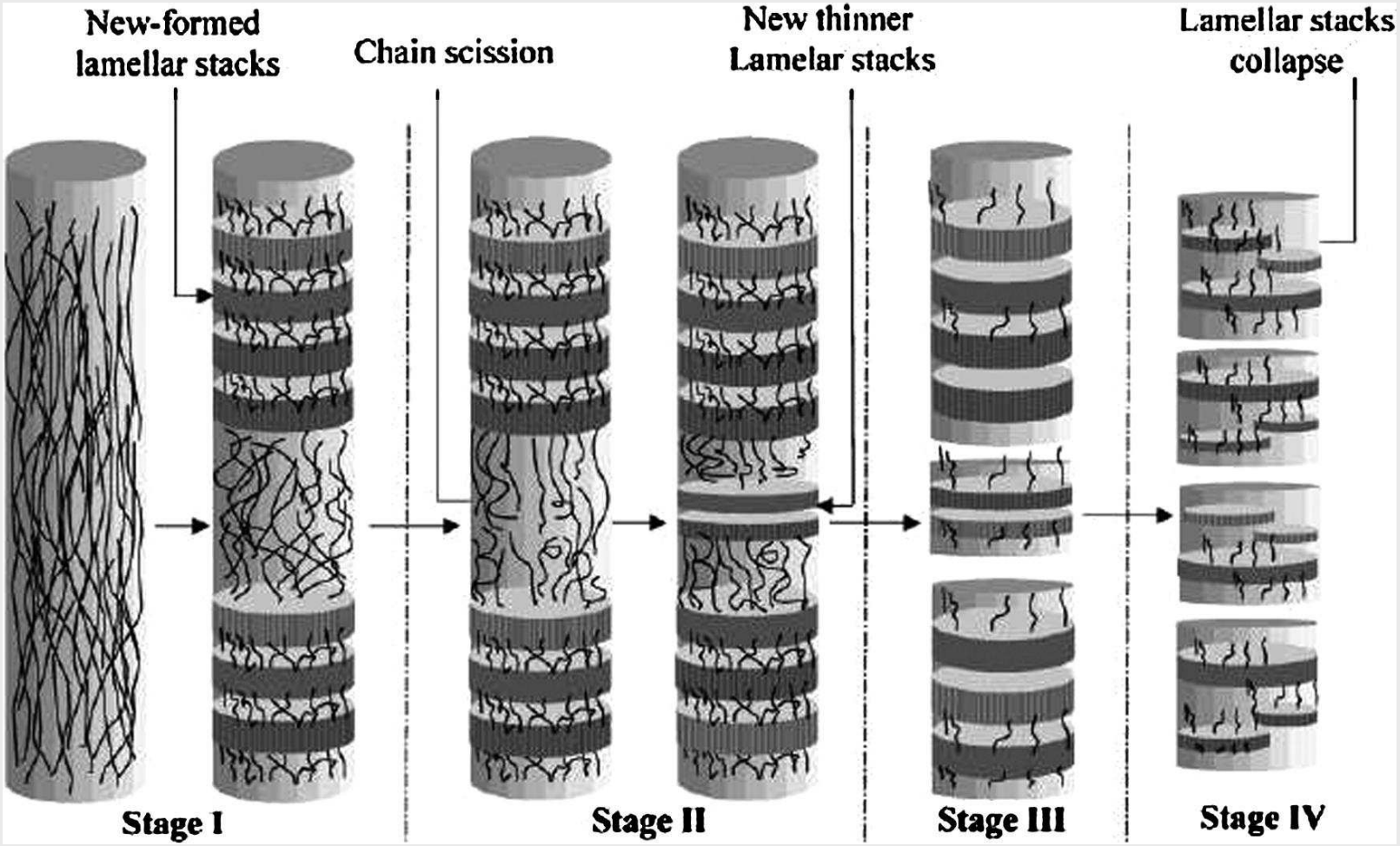
PGA rods and nanofibers degradation in acqueous solution



9, 28, 56 d

3,15,20 d

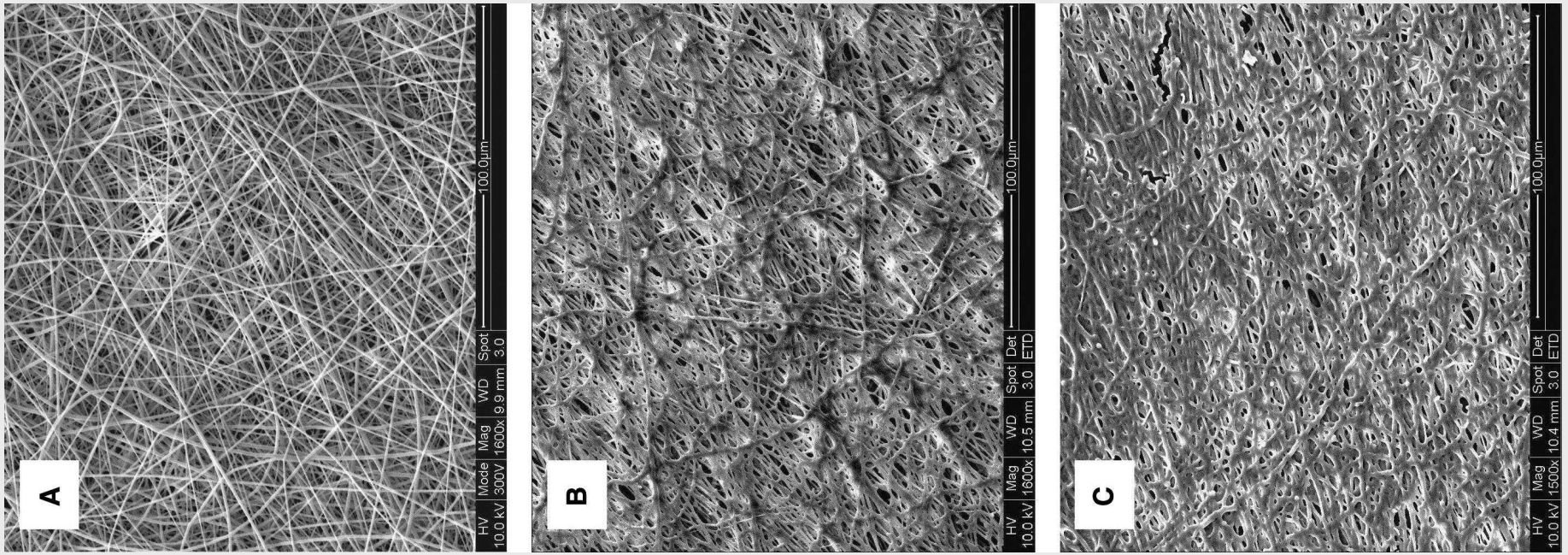
Model of in vitro degradation of electrospun PLGA (10:90) nanotubes



in vitro degradation of electrospun PLGA

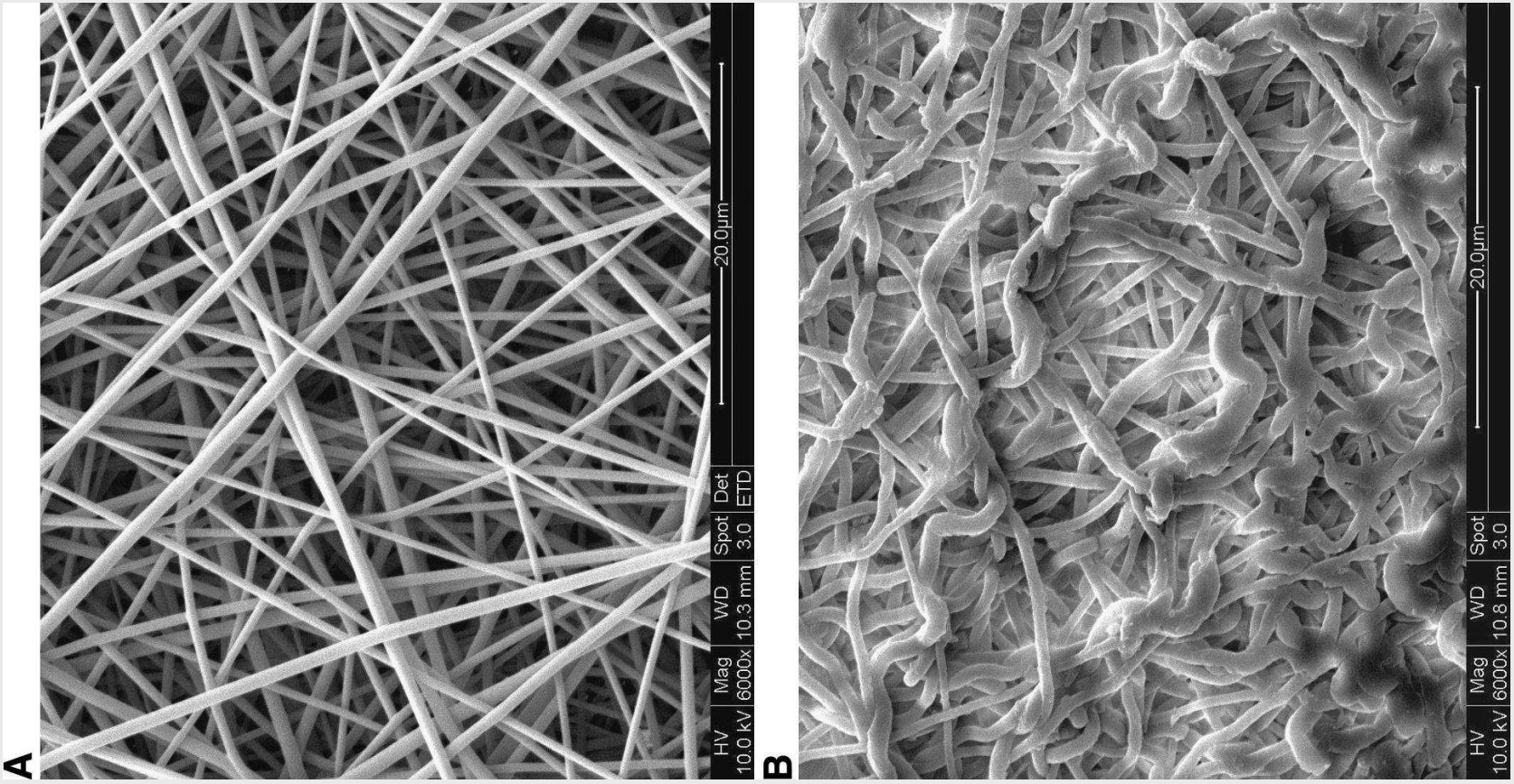
- A four-stage model of structure and morphology changes of electrospun poly(lacide-co-glycolide) (PLGA) (10:90) membranes during in vitro degradation.
- Stage I: thermally induced crystallization from amorphous PLGA (10:90) nanofibers and lamellar stacks are formed.
- Stage II: the mobility of polymer chains within large amorphous gaps increases after chain scission, cleavage-induced crystallization occurs, and thinner lamellae and lamellar stacks form.
- Stage III: mass loss rate is accelerated, large amorphous gaps disappear, and nanofibers start to break down.
- Stage IV: lamellar stacks start to collapse, and accelerated mass loss is observed.

PLGA nanofibers degradation in phosphate buffered saline at 37℃



(A) 0, (B) 15, (C) 30 days

Shrinkage of electrospun nanofiber of PLGA (75:25) in the aqueous medium for 24 h.

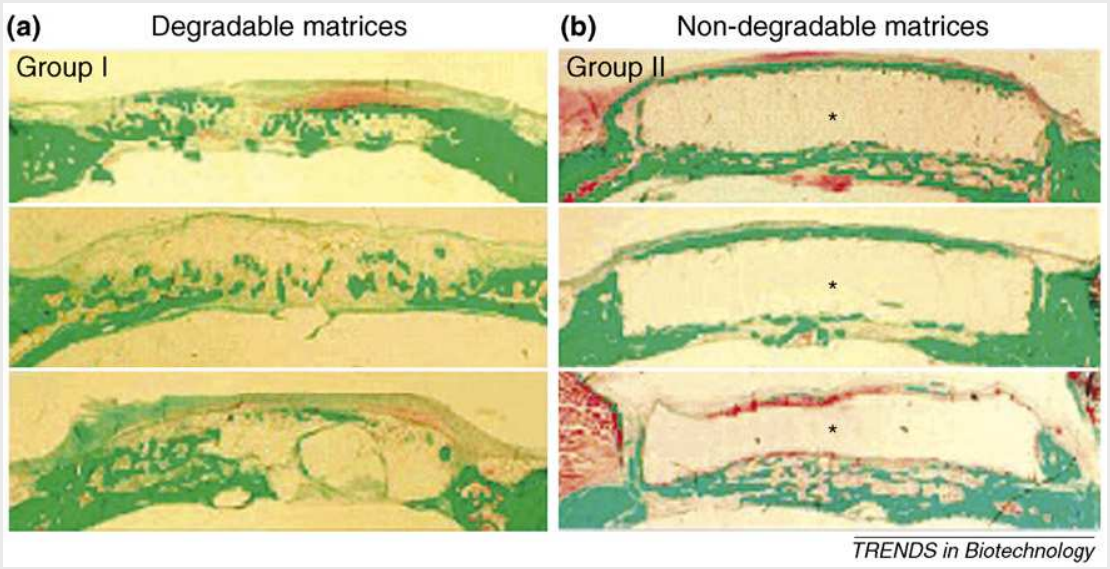


weight-size could be reduced by approximately 80%, fiber diameter increases, and porosity decreases.

Degradability is important

- co-polymerization of PEG polymers with proteins encoding RGD peptides and plasmin and matrix metalloproteinase (MMP) degradation sites (here Group I) .

Implanted in calvaria in vivo



Materials for controlling inflammation and the immune response

- immune system generally regarded as a negative regulator of wound healing
- but recently suggested that immune responses could be actively modulated to drive regeneration
  - role of acute inflammation → help x vascularization; tissue regeneration, Macrophages produce GFs
  - HA exhibits anti-inflammatory behavior and has also been associated with scarless wound healing in the fetus
  - Dendritic cells increase expression of the pro-inflammatory cytokines TNF-α and IL-6 when cultured on PLGA

Materials can modulate the immune response.