MME 4506 Biomaterials

Biopolymers

Most approaches currently pursued within the framework of regenerative medicine are dependent on the ability to synthesize novel materials, assemble materials into appropriate 2D and 3D forms, and precisely tailor physical, chemical, structural and biological properties to achieve desired clinical responses

In these aspects, biodegradable polymeric biomaterials offer the advantages of being able to be eliminated from the body after fulfilling their intended purposes

Polymeric biomaterials, particularly biodegradable synthetic polymers such as the family of *poly(hydroxy esters)* including *poly(lactic acid) (PLA), poly(glycolic acid) (PGA)* and *their copolymers (PLGA),* have been used extensively in medical and surgical applications.

They have high biocompatibility and biodegradability, well established safety as suture materials, and the versatility and flexibility that they offer for producing well-defined highly porous scaffolds with different geometry and structures to meet the needs of specific tissue engineering applications

Their degradation rate can be modulated over a wide range by tailoring the composition, molecular weights, end groups and geometry of the construct

Natural polymers can be classified as proteins (*silk, collagen, gelatin, fibrinogen, elastin, keratin, actin and myosin*), polysaccharides (*cellulose, amylose, dextran, chitin and glycosaminoglycans*) or polynucleotides (*DNA, RNA*)

Natural polymers, because of their similar macromolecular structure to tissues, offer the convenience of recognition from the biological system. This leads to the avoidance of issues related to toxicity and stimulation of a chronic inflammatory reaction

Natural polymers are known to degrade by the effect of naturally occurring enzymes
It is further possible to control the degradation rate of the implanted polymer by chemical crosslinking

On the other hand, natural polymers are frequently known to trigger immunogenic responses and have inadequate mechanical properties for load-bearing applications

They are being used for various biomedical applications such as cardiovascular applications, peripheral nerve regeneration, surgical sutures and drug delivery systems

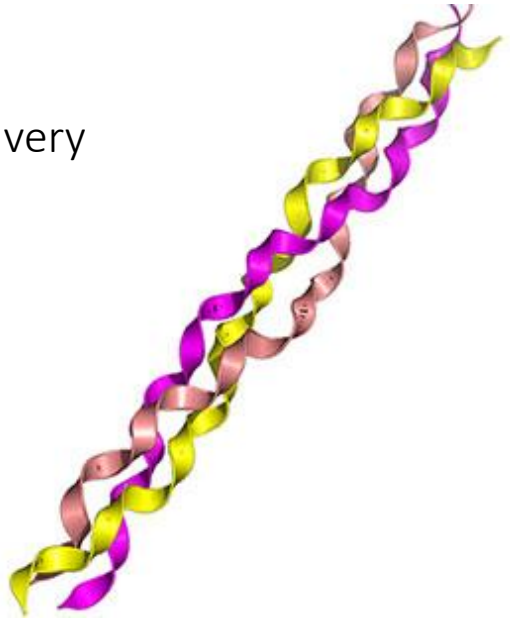
For example collagen gel is being increasingly considered in particular for bone tissue engineering applications

Collagen based biomaterials

Application	Physical State
Sutures	Extruded tape
Hemostatic agents	Powder, sponge, fleece
Blood vessels	Extruded collagen tube, processed blood vessel
Heart valves	Processed porcine tissue
Tendons, ligaments	Processed porcine tendon
Small intestine submucosa	Processed porcine tissue
Burn treatment	Porous collagen-GAG copolymer

Collagen structure (lattice of triple helix) is converted to randomly coiled gelatin by heating above 37 C for bovine collagen

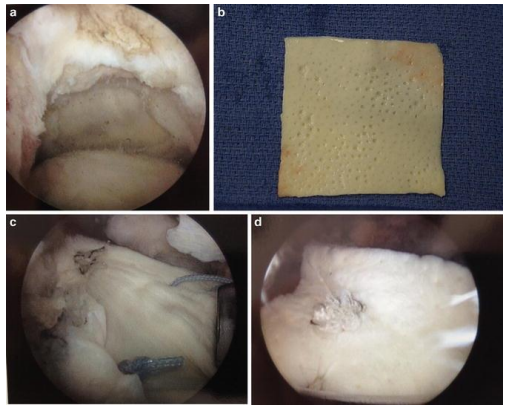
Gelatin is commonly used for tissue engineering and drug delivery
It is much more rapidly degraded than collagen

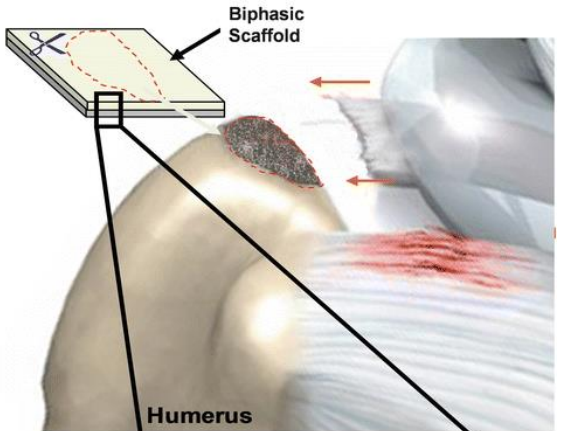


Xenografts are natural polymers obtained from animals

Class	Polymer	Incidence	Physiologic Function
Proteins	Collagen	Connective tissues	Mechanical
	Elastin	Ligaments	Elasticity
	Fibrinogen	Blood	Blood clotting
Polysaccharides	Alginate	Algae	Energy storage
	Chitin	Insects, crustaceans	Shape, form
	Glycosaminoglycans	Connective tissues	Mechanical, water retention
Polynucleotides	DNA, RNA	Cell nucleus	Protein synthesis

Decellularized porcine small intestine submucosa is commonly used to reinforce soft tissue repair such as muscles and tendons





Chitosan

Derived from chitin, present in hard exoskeletons of shellfish like shrimp and crab

Desirable properties

- Minimal foreign body reaction
- Mild processing conditions
- Controllable mechanical/biodegradation properties

Applications

- In the engineering of cartilage, nerve, and liver tissue, wound dressing and drug delivery devices

Alginate

A polysaccharide derived from brown seaweed

Can be processed easily in water, non-toxic, biodegradable, controllable porosity

Forms a solid gel under mild processing conditions

Has been explored for use in:
Liver, nerve, heart, cartilage & tissue-engineering

Mechanical weakness: low strength & poor cell adhesion
Can be overcome by forming composite with ceramics

Synthetic polymers represent the largest group of biodegradable polymers

Compared to natural polymers they exhibit predictable and reproducible mechanical and physical properties such as tensile strength, elastic modulus and degradation rate

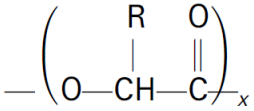
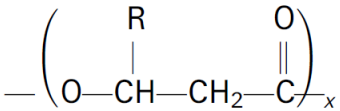
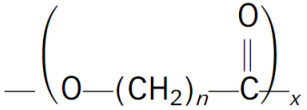
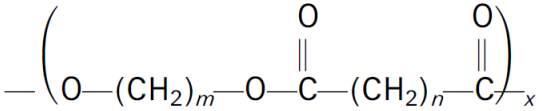
Possible risks such as toxicity, immunogenicity and favoring of infections are lower for pure synthetic polymers with constituent monomeric units having a well-known and simple structure

Moreover synthetic polymers provide the freedom to tailor the properties for specific applications

Some synthetic polymers are hydrolytically unstable and degrade in the body while others may remain essentially unchanged for the lifetime of the patient

Biodegradable polymers have been used to repair nerves, skin, vascular system and bone

A large proportion of the currently utilized synthetic degradable polymers for tissue engineering scaffolds are polyesters

Chemical structure	Polyester	Examples
	Poly(α-hydroxy acid)	Poly(glycolic acid) Poly(L-lactic acid)
	Poly(β-hydroxyalkanoate)	Poly(β-hydroxybutyrate) Poly(β-hydroxybutyrate-co-β-hydroxyvalerate)
	Poly(ω-hydroxybutyrate)	Poly(β-propiolactone) Poly(ϵ-caprolactone)
	Poly(alkylene dicarboxylate)	Poly(ethylene succinate) Poly(butylene succinate) Poly(butylene succinate-co-butylene adipate)

Since tissue engineering scaffolds do not have to be removed surgically once they are no longer needed, degradable polymers are of value in short-term applications and can circumvent some of the problems related to the long-term safety of permanently implanted devices

The human body already contains highly regulated mechanisms for completely removing monomeric components of lactic and glycolic acids. For this reason PLA and PGA have been used in numerous biomedical products and devices, such as degradable sutures and fixation plates

Table 4.2 Physical properties of synthetic, biocompatible and biodegradable polymers used as scaffold materials

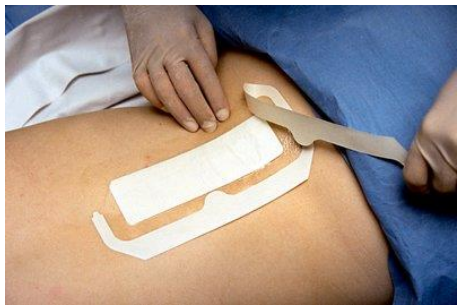
	P3HB	PGA	PDLLA	PLLA	PLGA	PCL
Melting temperature (°C)	170–175	225–230	nd	173–178	nd	58
Glass transition temperature (°C)	−4–10	35–40	55–60	60–65	45–55	−72
Young's modulus (GPa)	1.1–3.5	7–10	1.9–2.4	1.2–3.0	1.4–2.8	0.4
Elongation (%)	2–6	15–20	3–10	5–10	3–10	>70
Water contact angle (°)	70–80		60–70	70–80		66
Crystallinity (%)	55–80	55–56	0	37	0	
Degradation period (months)	>18	6–12	12–16	>24	1–12	>24

P3HB, poly(3-hydroxybutyrate); PGA, poly(glycolic acid); PDLLA, poly(DL-lactic acid); PLLA, poly(L-lactic acid); PLGA, poly(lactic-co-glycolide); PCL, poly(ϵ-caprolactone).

A wide range of physical properties and degradation times can be achieved by varying the monomer ratios when using copolymers such as lactide/glycolide copolymers, or hydroxybutyrate-hydroxyhexanoate copolymers

Polyhydroxyalkanoates (PHA) are a class of microbial polyesters
Only two of the PHA polymers are produced commercially in biological production plants i.e. poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-hydroxyvalerate)

Applications of PHA in medicine are being expanded to include wound management, vascular system devices, orthopaedics and drug delivery systems



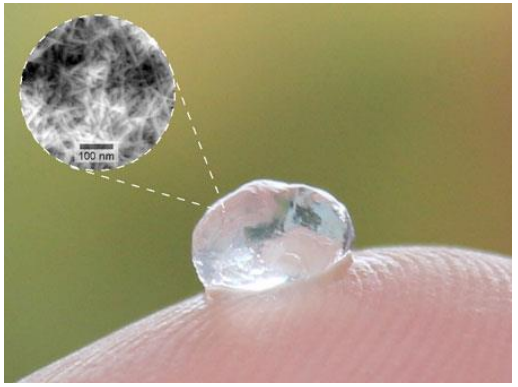
<u>Advantages of PLA and PGA</u>
Can be processed easily
Their degradation rates, physical and mechanical properties are adjustable over a wide range by using various molecular weights and copolymers

<u>Disadvantages</u>
Undergo a bulk erosion process and can cause scaffolds to fail prematurely
Abrupt release of acidic degradation products during degradation can cause a strong inflammatory
response.

These polymers on their own are mechanically inadequate for applications in hard tissue repair and load-bearing sites. For this reason their combination with stiff inorganic phases in composites are required

Hydrogels are crosslinked networks of hydrophilic polymers that are highly swollen with water
Hydrogels may be natural or synthetic, physically or chemically crosslinked

They are environmentally responsive and can swell up to 95% with water



They have similar properties to tissues

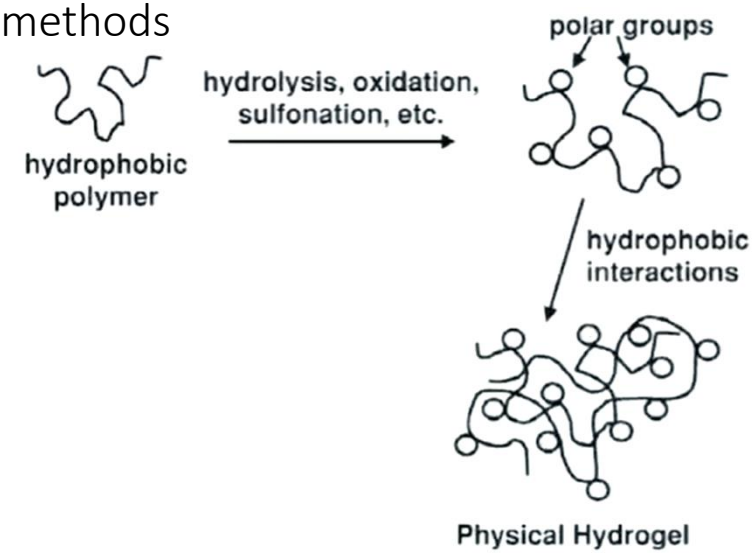
- Mechanical properties (viscoelastic)
- Water content
- Lubricating properties
- Coefficient of friction

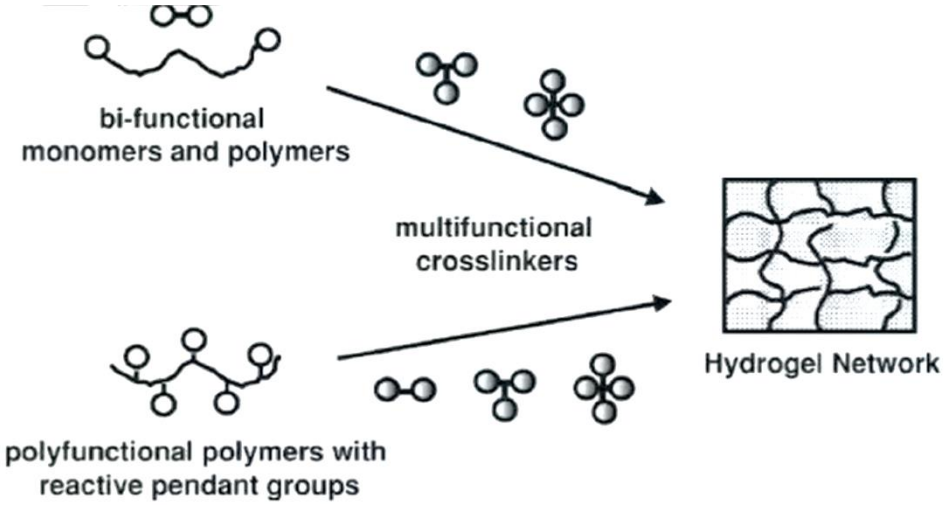
Mostly used for drug delivery and tissue engineering where encapsulated cells interact with hydrogels in a similar way to their native extracellular matrix

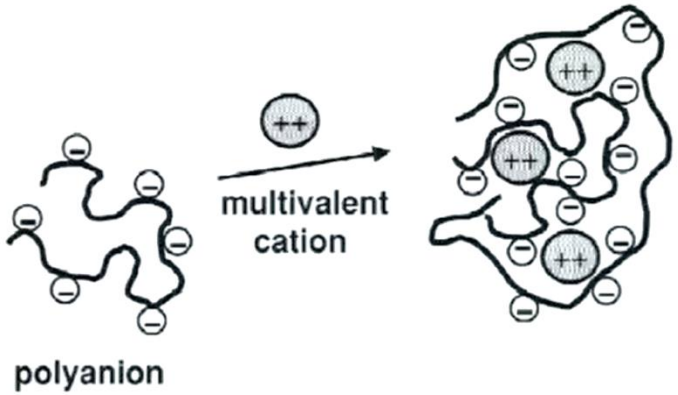
Water swollen structure allows drug release and nutrient transport by diffusion

Two basic types of hydrogels according to preparation methods

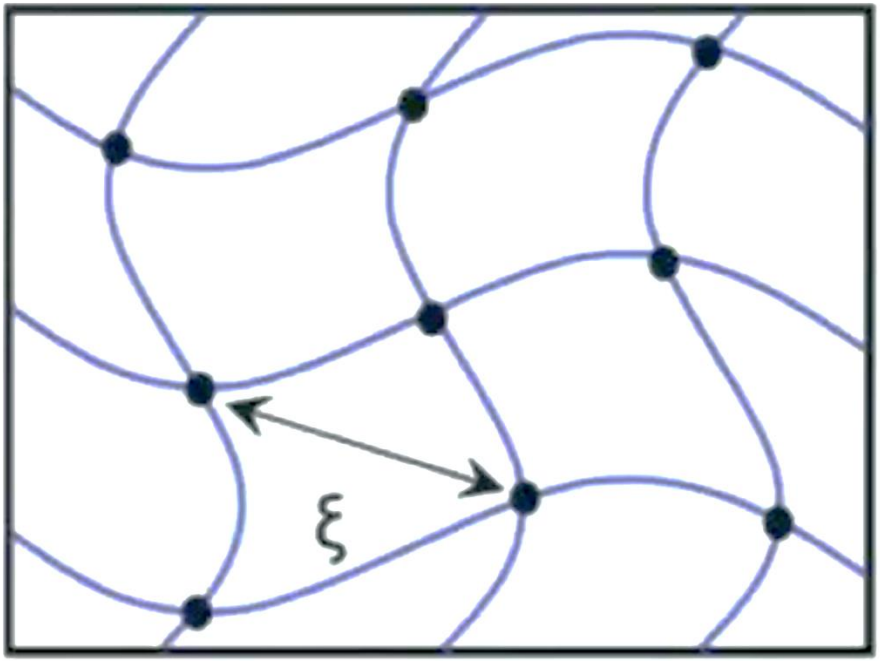
1. Physical bonding
    a. Hydrophobic interactions
    b. Hydrogen bonds
    c. Ionic forces
2. Chemical
    a. Permanent covalent crosslinks

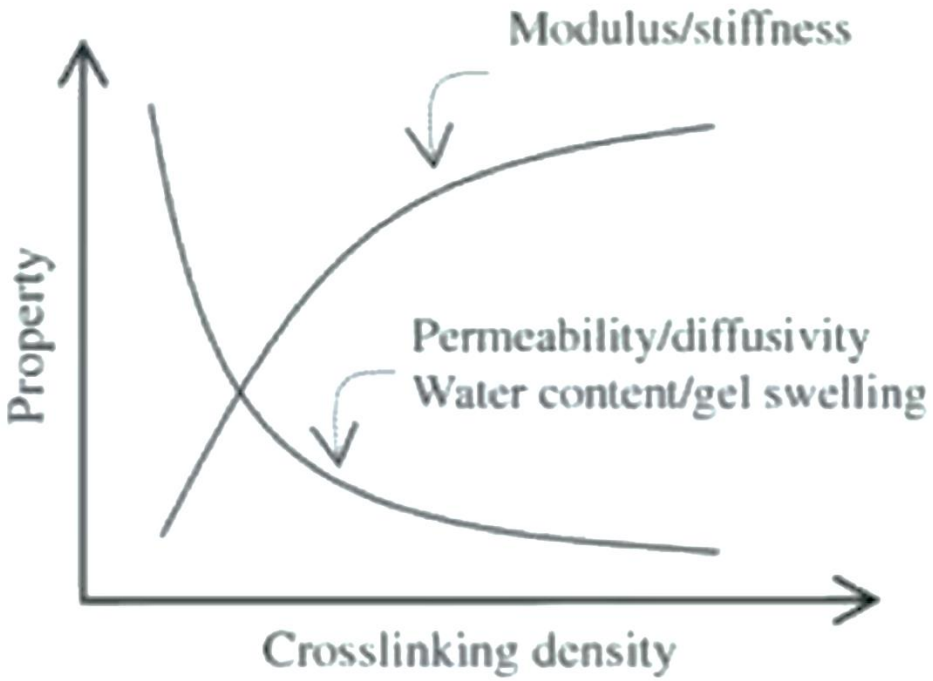






Crosslinking density affect the mesh size and the properties of the hydrogel





Hydrophilicity of the hydrogel inhibits protein adsorption and cell attachment

Applications of biopolymers

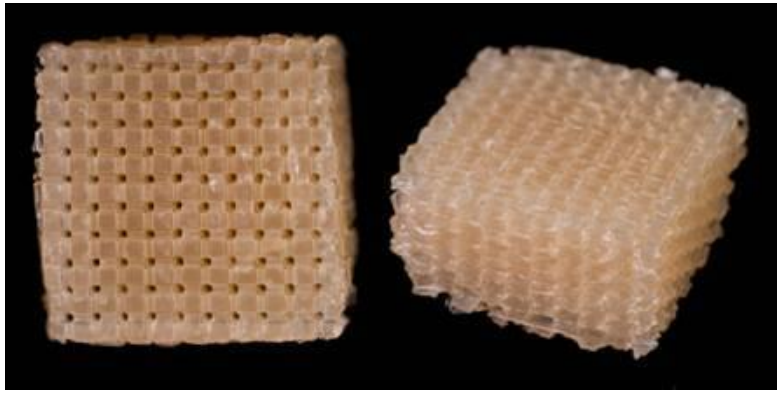
<u>Biodegradables</u>
Scaffolds

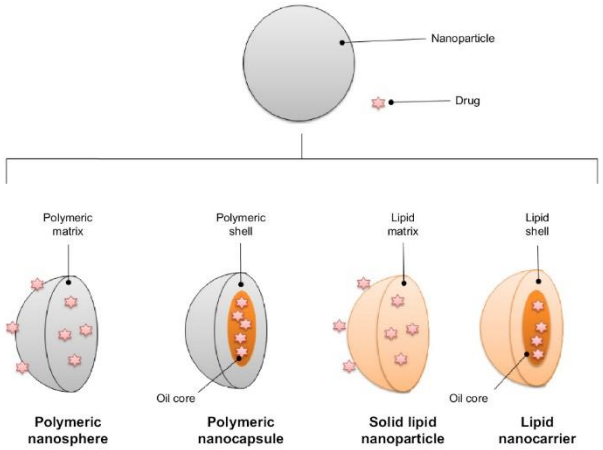
Drug delivery carriers

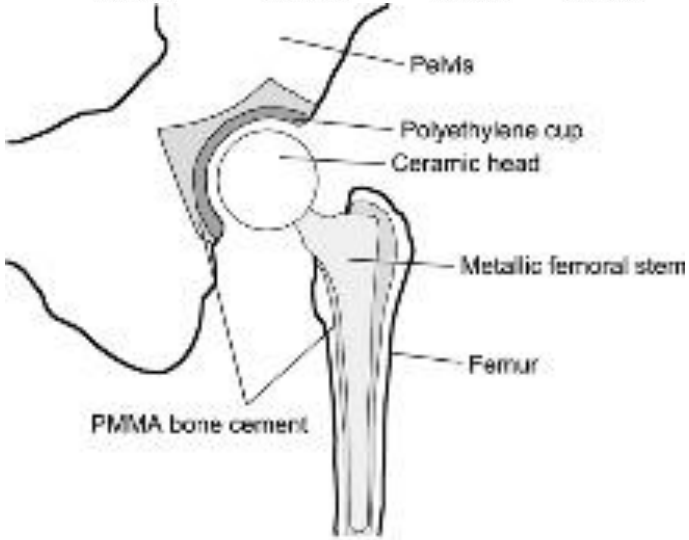
Implants

<u>Permanently inert</u>
Prosthetics

Medical disposable supplies







In a tissue engineering strategy, cells are seeded on a scaffold that acts as a template to guide cell growth and to facilitate the formation of functional new tissues and organs

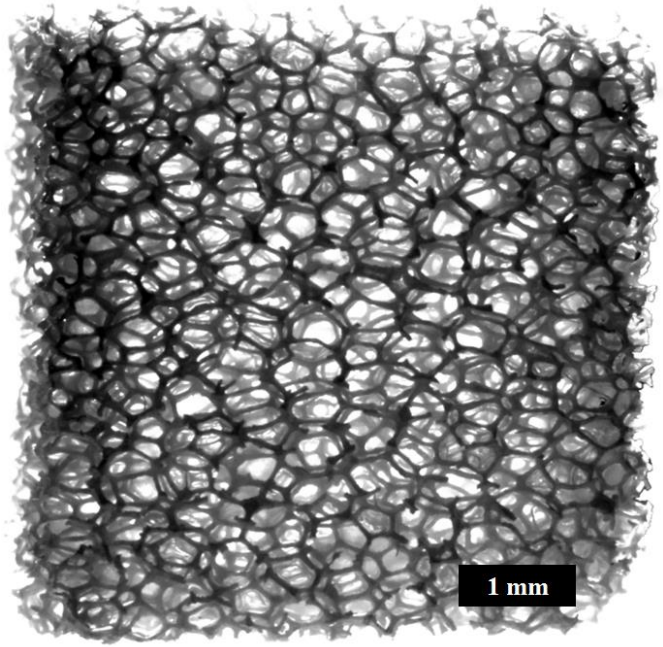
Scaffolds promote new tissue formation by providing an appropriate surface and adequate spaces to foster and direct cellular attachment, migration, proliferation, and desired differentiation to specific cells in three dimensions

The design of a scaffold is critical because it affects the formation and ultimate function of neo tissues

Critical variables in scaffold design and function include

- the bulk material,
- the mechanical properties,
- the 3D architecture,
- the surface morphology and chemistry,
- the scaffold environment during and after

fulfilling the function, which is affected by degradation characteristics



Generally, a tissue engineering scaffold should be:
(1) biocompatible, that is, non-immunogenic and non-toxic to living cells and tissues;
(2) biodegradable or capable of being remodeled in tune with regeneration or repair process;
(3) porous to provide a suitable 3D environment for cell and tissue penetration as well as nutrients and wastes transportation;
(4) surface conductive to facilitate cellular functions;
(5) mechanically stable for surgical handling; and
(6) easy to manufacture and sterilize
In addition, the scaffolds also should possess the ability to carry biological signals such as growth factors, cytokines and to deliver them in a controlled manner.

The above-mentioned strategy works well in cases where the tissue around the defect has inherent potential for regeneration

For the contrasting situation where tissue lacks regenerative ability, tissue regeneration cannot always be expected to occur only by supplying the scaffold
In such cases the scaffold is used in combination with relevant cells, usually also adding growth factors, which have the potential to accelerate tissue regeneration

The search for ideal scaffolding materials and appropriate scaffolding structure that fulfill the above design criteria continues to be important in tissue engineering

A 3D scaffold offers a local micro-environment that mimics natural ECM-cell interaction more closely and where the functional properties of cells can be manipulated and optimized

Such hierarchical porous scaffolds affect many wound healing parameters:

- cell seeding, survival, migration, proliferation, and organization
- cellular gene expression and phenotypic characteristics
- mechanical structure of the scaffold
- initial void space that is available for cells to form new tissues, including new blood vessels, as well as the pathways for mass transport via diffusion and/or convection.

The importance of macroporosity (>100 microns) on neo tissue formation has been demonstrated in many studies

For example, PLGA scaffolds with a higher porosity (>80%) promote more tissue ingrowth and new tissue formation

It is known that scaffolds with open pore structures favor cell and tissue penetration, blood vessel invasion and new bone formation

However increase in interconnected macroporosity may adversely affect mechanical properties of a scaffold. Advanced scaffold design and fabrication techniques are required

Porosity plays an important role in determining the characteristics of a scaffold

Average pore size, pore size distribution, pore volume, pore inter-connectivity, pore shape, pore throat size and pore wall roughness are important parameters to consider while designing a scaffold

A minimum pore size is required for tissue ingrowth and high 3D interconnectivity is necessary for access of nutrients, transport of waste products, better cell spreading and vascularization

It is recognized that pore structure and properties of the scaffolds are dictated by the choice of the manufacturing process

Techniques such as solvent casting, particulate leaching, 3D printing, thermally induced phase separation are among the most used for fabricating 3D structures with variable porosity

Though each of these techniques has the ability to produce scaffolds with different architecture, they also have limitations

A number of techniques have been explored to fabricate biodegradable polymers into 3D porous scaffolds with different porosities, pore architectures, pore orientations, pore sizes, inter-pore connections, and pore wall surface morphologies

Processing	Advantage	Disadvantages
Thermally induced phase separation (TIPS)	High porosities Control of pore structure and pore sizes Interconnected pores	Shrinkage issues Long time to sublime solvents Small-scale production
Solvent casting and particulate leaching	Controlled porosity Faster and relatively inexpensive approach	Pore interconnectivity Limited to thin membranes Solvent residue
Microsphere sintering	Controllable and graded porosity Fabricated in complex shapes	Pore interconnectivity Mechanical properties
Scaffold coating	Rapid and simple approach Applied to all types of composites	Lack of interfacial strength Clogging of pores
Fibrous composites	Superior compressive strength Independent control of porosity and pore sizes	Solvent residue Oriented pore structures

Solvent casting in combination with **particulate leaching** is one of the simplest and more common methods used for scaffold preparation.

Solvent casting involves the dissolution of the polymer in an organic solvent, mixing with porogen particles and casting the solution into a predefined 3D mould. The solvent is subsequently allowed to evaporate

The main advantage of this technique is the ease of manufacturing and ability to incorporate drugs and chemicals within the scaffold

There are many disadvantages including restriction to only simple shapes
e.g. flat sheets and tubes, can be formed
Secondly, pore interconnectivity is very low and usually unsuitable for tissue engineering applications

Failures of cell/tissue ingrowth often resulted from insufficient inter-pore connection where cell colonization was limited to the very peripheral and superficial layers (e.g. about 240 microns from the surface of a 1.9mm thick scaffold)

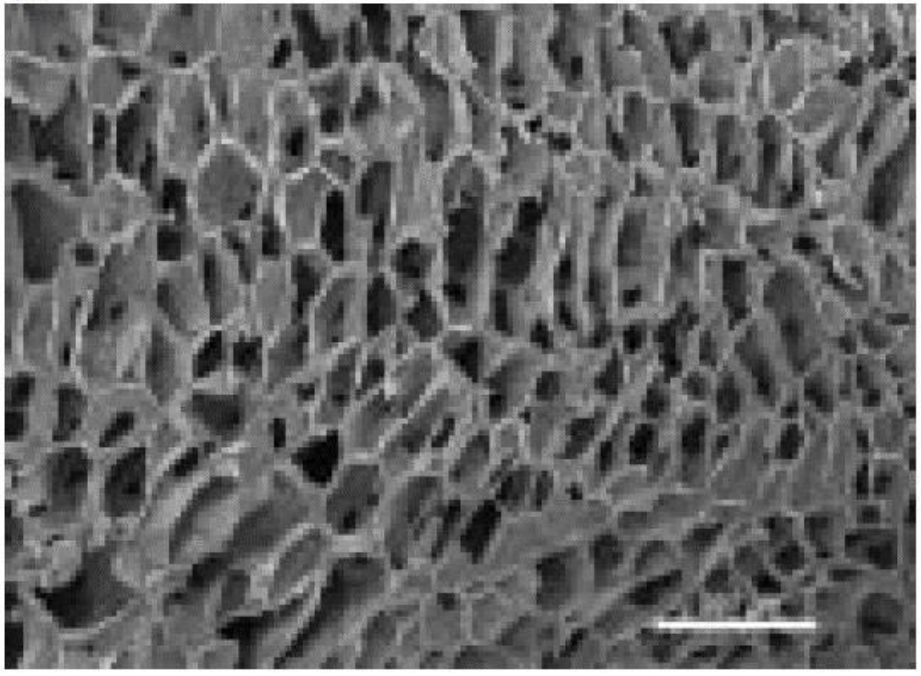
Also there are chances of residual solvent to remain trapped which would reduce the activity of bioinductive molecules (e.g. protein), if incorporated

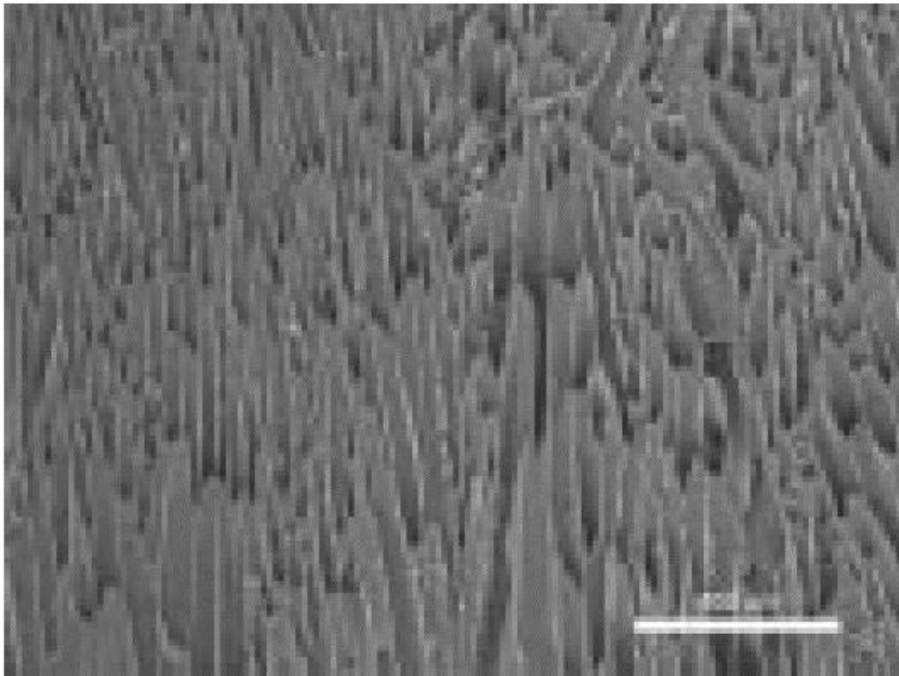
Thermally induced phase separation

A thermally induced solid-liquid phase separation is achieved by lowering the temperature of a homogeneous polymer solution to induce solvent crystallization
Subsequent removal of solvent crystals results in porous polymer scaffolds

The characteristics of pores are varied with polymer material, concentration, solvent, phase separation temperature and thermal transfer direction.

Manipulation of thermal transfer direction controls the direction of solvent crystal growth during the phase separation, which results in a scaffold with anisotropic microtubular structure

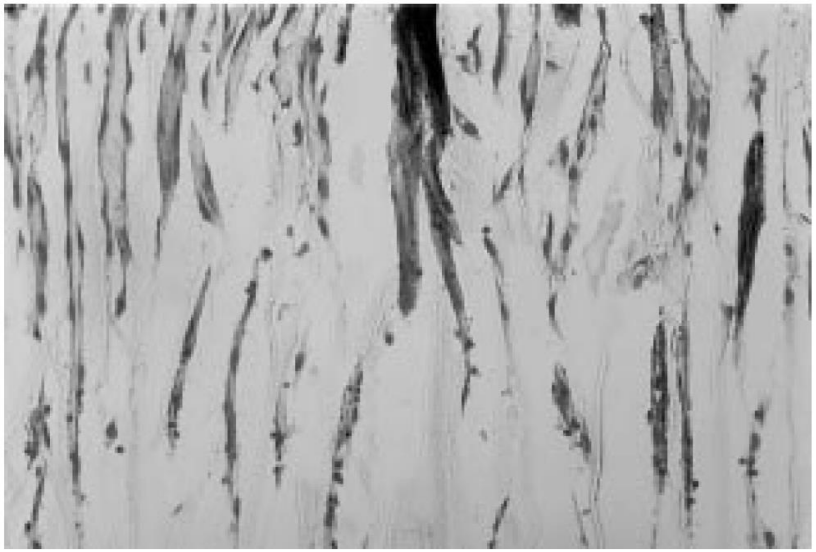




Scaffolds prepared by thermally induced phase separation offer both higher porosity (up to 98%) and improved mechanical properties over scaffolds produced by traditional salt-leaching technique

The oriented microtubular scaffolds have shown anisotropic mechanical properties similar to some fibrillar and tubular tissues, and has been demonstrated to facilitate cell organization into oriented tissues

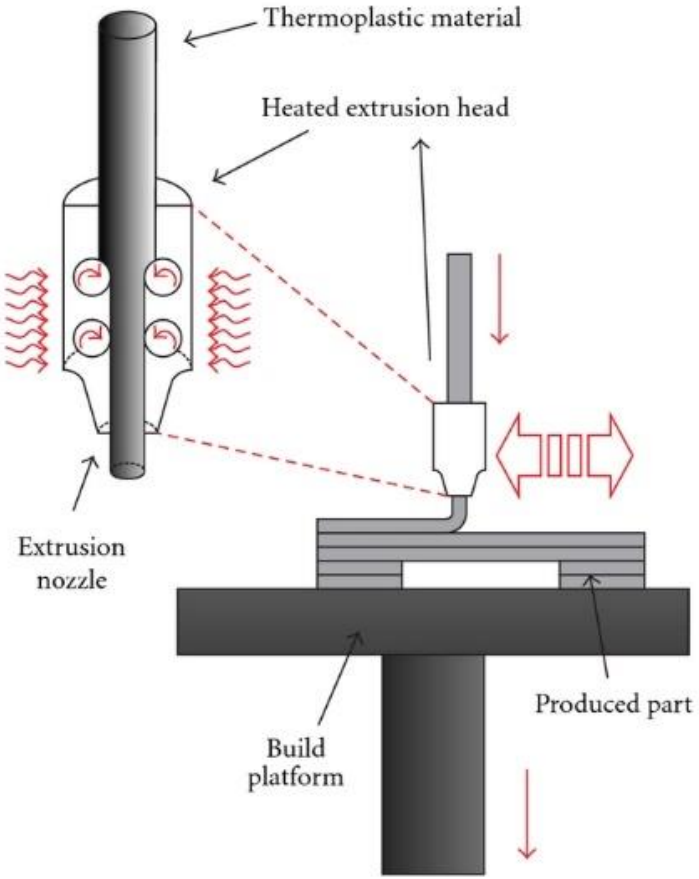
Such a parallel array of microtubules facilitates the organization and regeneration of certain tissues (nerve, muscle, tendon, ligament, dentin} which naturally have oriented tubular or fibrous bundle architectures



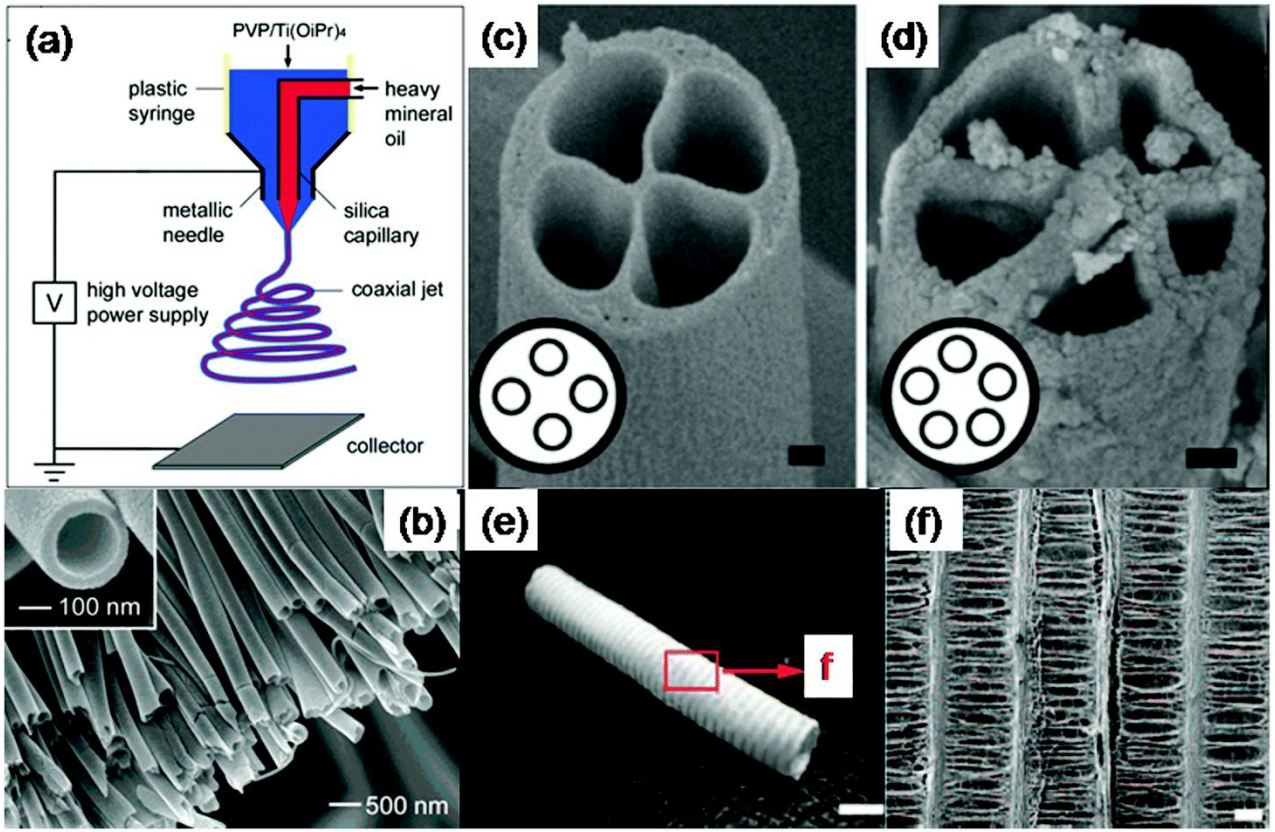
2.2 MC3T3-E1 cell growth on the oriented microtubular PLLA scaffold (4

3D printing refers to computer-aided manufacture (CAD/CAM) methodologies to fabricate polymer scaffolds with well-defined architecture

Local composition, macrostructure and microstructure can be specified and controlled at high resolution in the interior of the components



Electrospinning is an efficient technique that have the capability of producing nanofibers

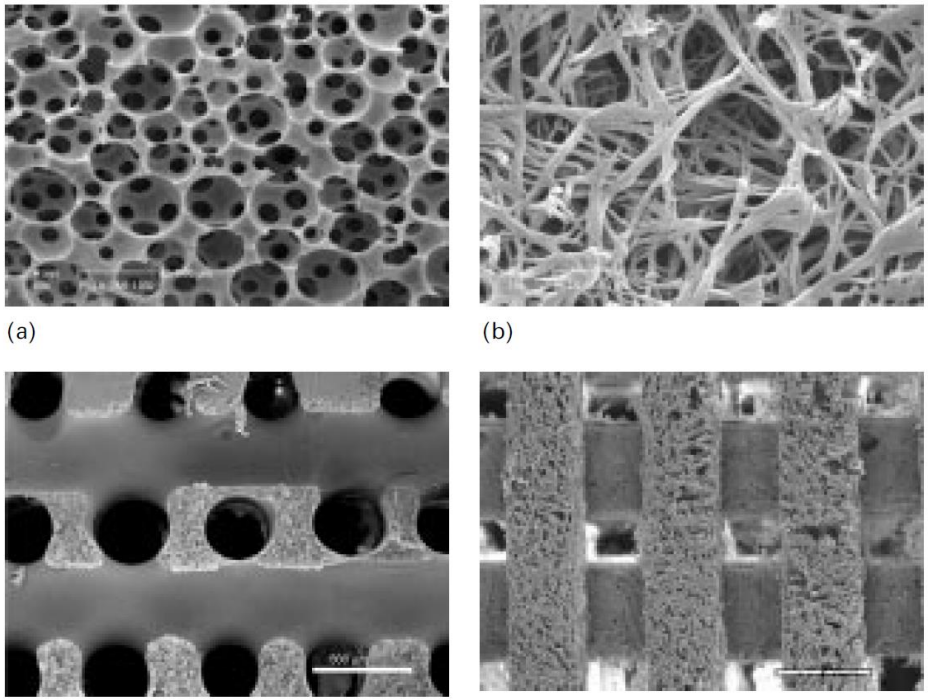


Alternatively phase separation, self assembly and solid freeform fabrication are used to produce nanofibrous scaffolds

Collagen is the major ECM component of many tissues
The nanoscaled collagen fibrillar structure has been recognized to enhance cell-matrix interaction

To mimic collagen fiber bundles in nano size (50-500 nm) nanofibrous features have been introduced into synthetic biodegradable polymer scaffolds

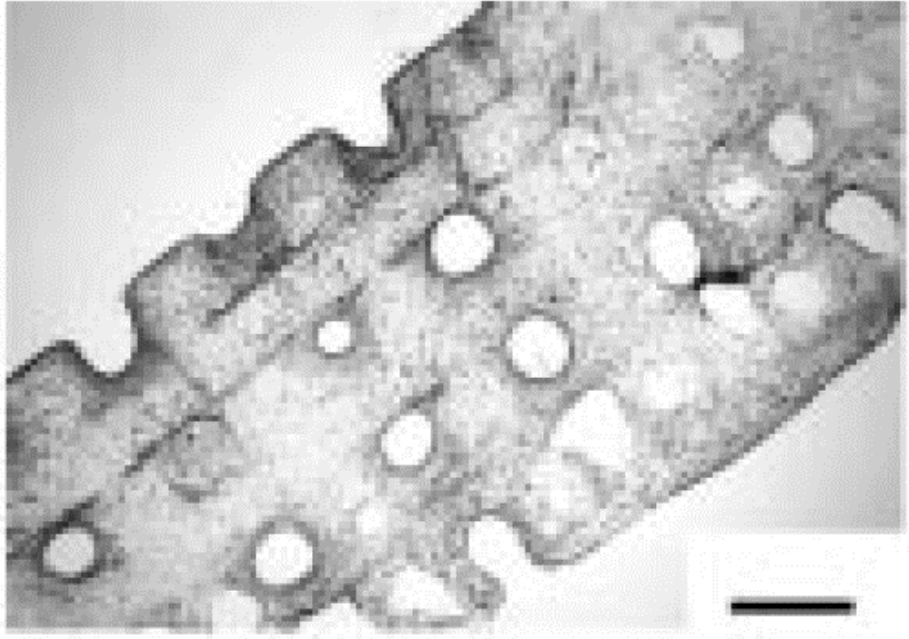
The nanofibrous scaffold has high surface area of about 100m^2/g, which is more than 100 times higher than that of a non-fibrous (solid-walled) scaffold with the same macroporosity and pore structures

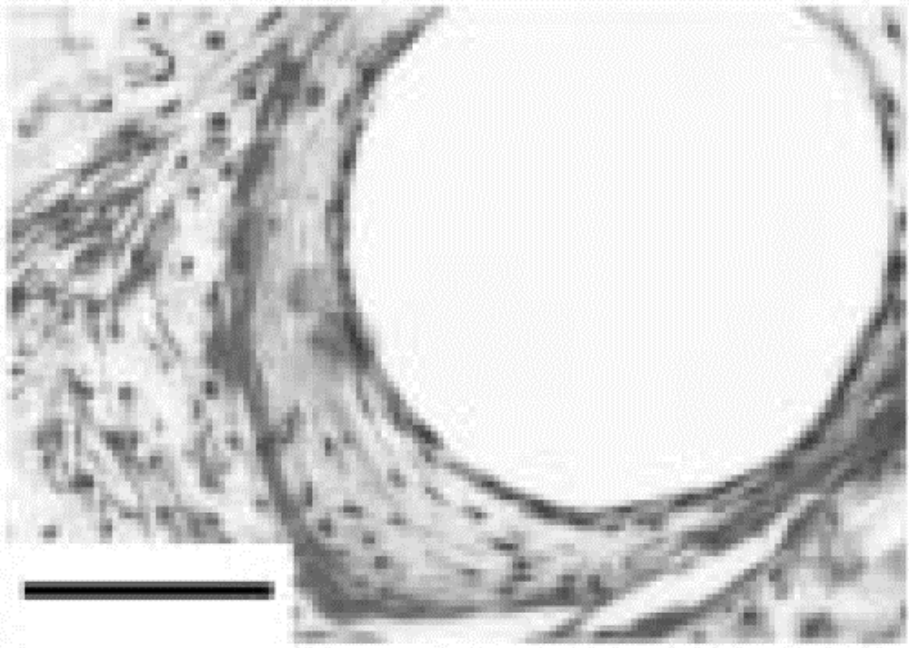


2.3 SEM micrographs of 3D nanofibrous PLLA scaffolds: (a, b) prepared from sugar sphere template leaching and phase separation; (c) prepared from sugar fiber template leaching and phase separation; (d) prepared from solid freeform

The macroporous and nanofibrous scaffolds enhance protein adsorption and positive cellular response as compared to solid-walled scaffolds without nano-fibrous structures

The high surface area, the micro-porosity between nanofibers (several microns), and selective adsorption of ECM proteins in nanofibrous scaffold may all contribute significantly to the enhanced tissue regeneration.





Micro / nano encapsulation

Polymeric particulate carriers (micro- and nano-spheres) are effective to release substances in a controlled manner and to protect unstable biologically active molecules from denature and degradation

Among the natural or synthetic polymers used for particulate carrier fabrication, PLLA and poly(lactic-co-glycolic acid) (PLGA) were found to be remarkable for their application in drug delivery due to their excellent biocompatibility and biodegradability

Most importantly, the released proteins were able to maintain a high level of biological activity with desired prolonged duration

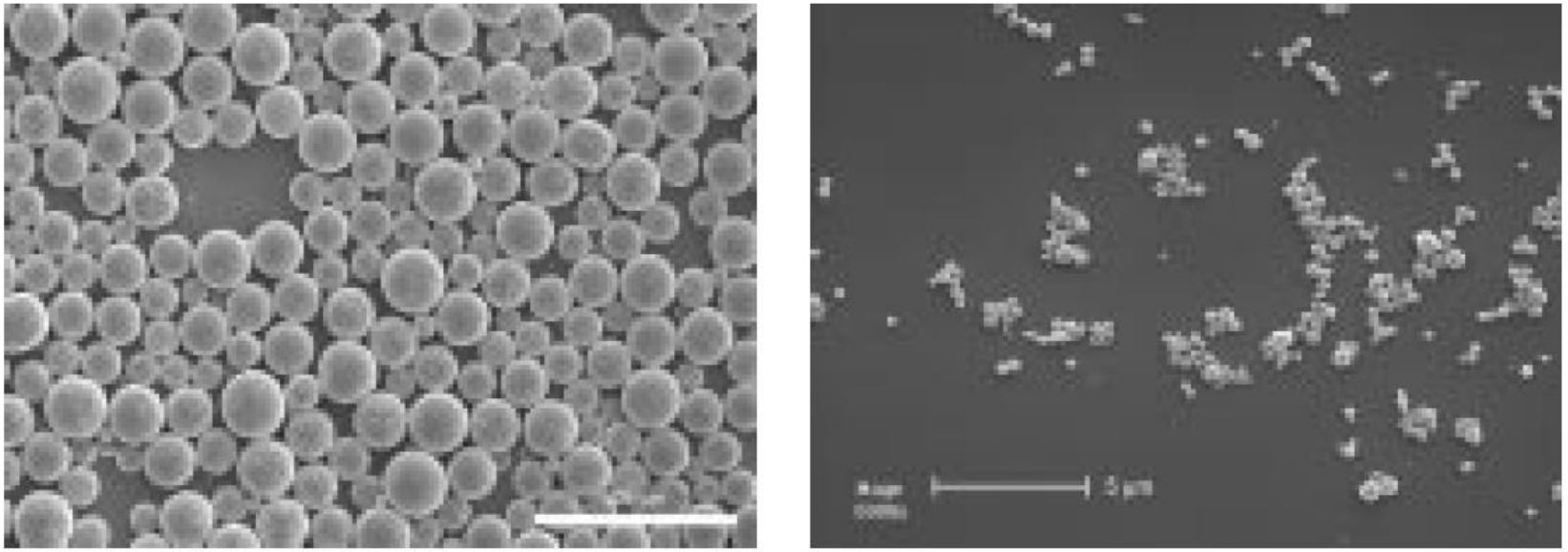
The release of proteins is controlled in the first stage by diffusion and in the second stage by the degradation of polymer micro- or nanospheres.

Sustained protein release over days to months can be achieved by varying the molecular weight and the ratio of LA/GA in PLGA copolymers

Specific growth factors and cytokines are required to regulate tissue regeneration

Hormones and growth factors are common examples to controlled released proteins

Local delivery of bioactive factors by microsphere/nanosphere encapsulation technique to induce cellular responses is a promising therapeutic approach



2.7 SEM micrographs of PLGA50-74K microspheres (a) and PLGA50-6.5K nanospheres (b). (a) From Wei *et al.* (2004), Copyright © 2004 by Elsevier;

Spheres are commonly prepared using a water-oil-water emulsion method

Factors encapsulated in micro or nanospheres can be incorporated into porous polymer scaffolds

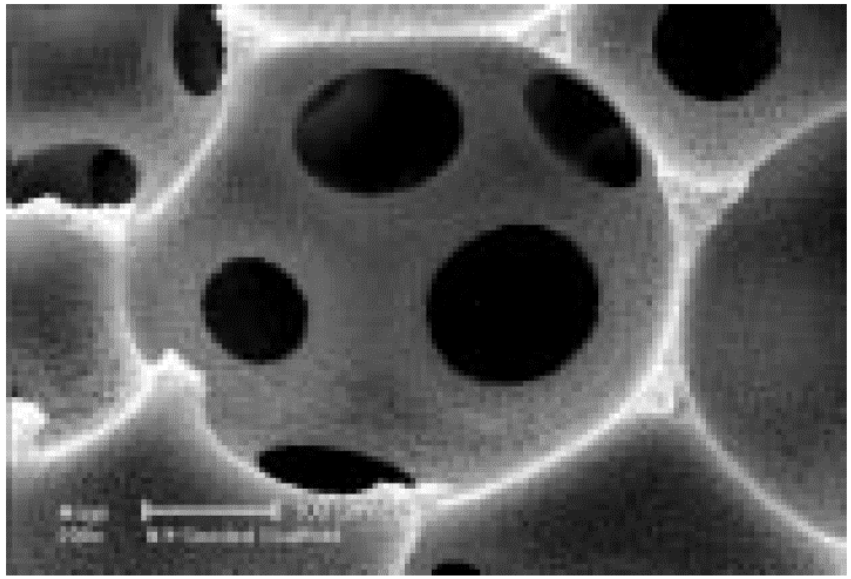
Nanosphere-immobilization technique protects growth factors from denaturation as compared with simple adsorption of growth factors onto a scaffold

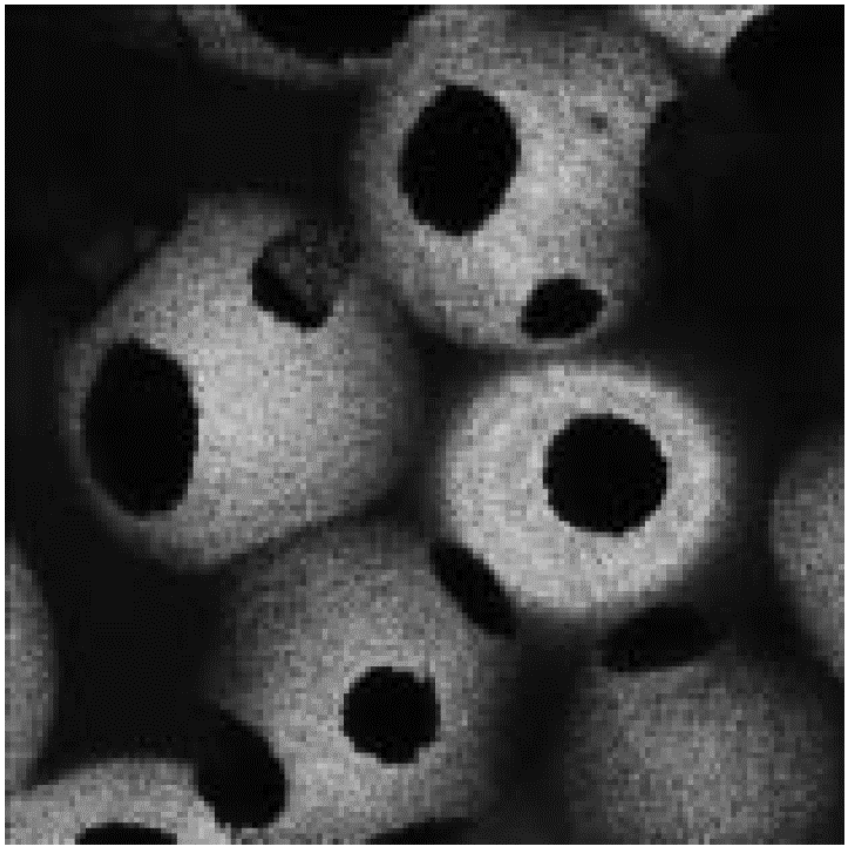
Adsorption results in complete degradation of many growth factors during a very short release time of 3 days

In addition, the burst release is very high and control over release kinetics is very limited when a simple adsorption method is used

Immobilization onto a scaffold significantly reduces the initial burst release of the growth factors

Various release profiles were achieved through the use of nanospheres with different degradation rates





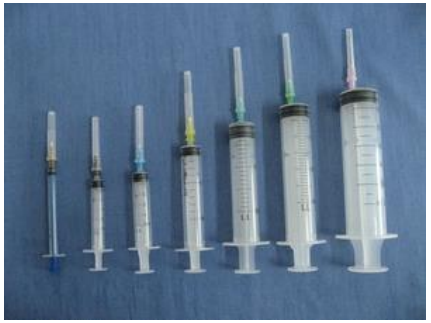
Medical disposable supplies

Polyvinylchloride (PVC)

- Amorphous & rigid polymer, high melt viscosity
- Made flexible and soft by the addition of plasticizers
- Products: blood and solution bag, surgical packaging

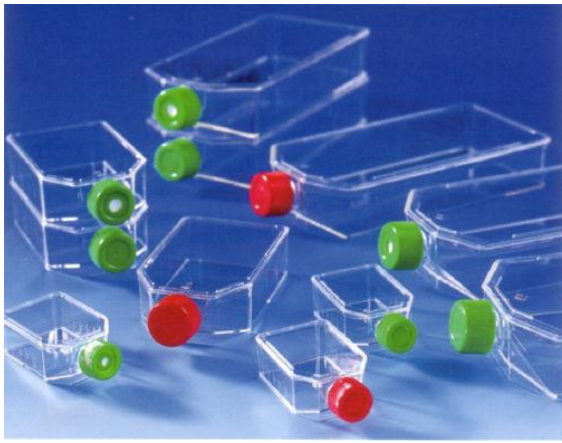
Polypropylene (PP)

- High tensile strength
- Excellent stress-cracking resistant
- Applications: Disposable syringes, blood oxygenerator membrane, artificial vascular grafts



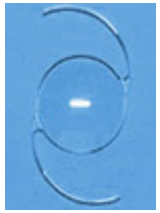
Polystyrene (PS)

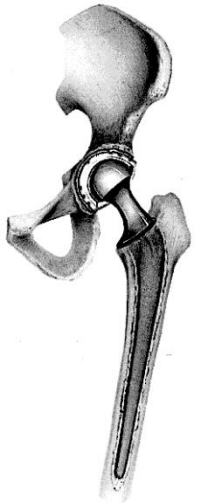
- Good transparency,
- Ease of fabrication,
- Thermal stability,
- Relatively high modulus
- Used in tissue culture flasks, vacuum canisters, filterware



Implants

Polymethyl methacrylate (PMMA)
Various forms and properties
Excellent optical properties
Products: Implantable ocular lenses, bone cement





Prosthetics

Polysulfone
High tensile stress , compression and friction resistance
Resist to acid, base and salt solutions