BIOMATERIALS FOR TISSUE ENGINEERING - POLYMERS

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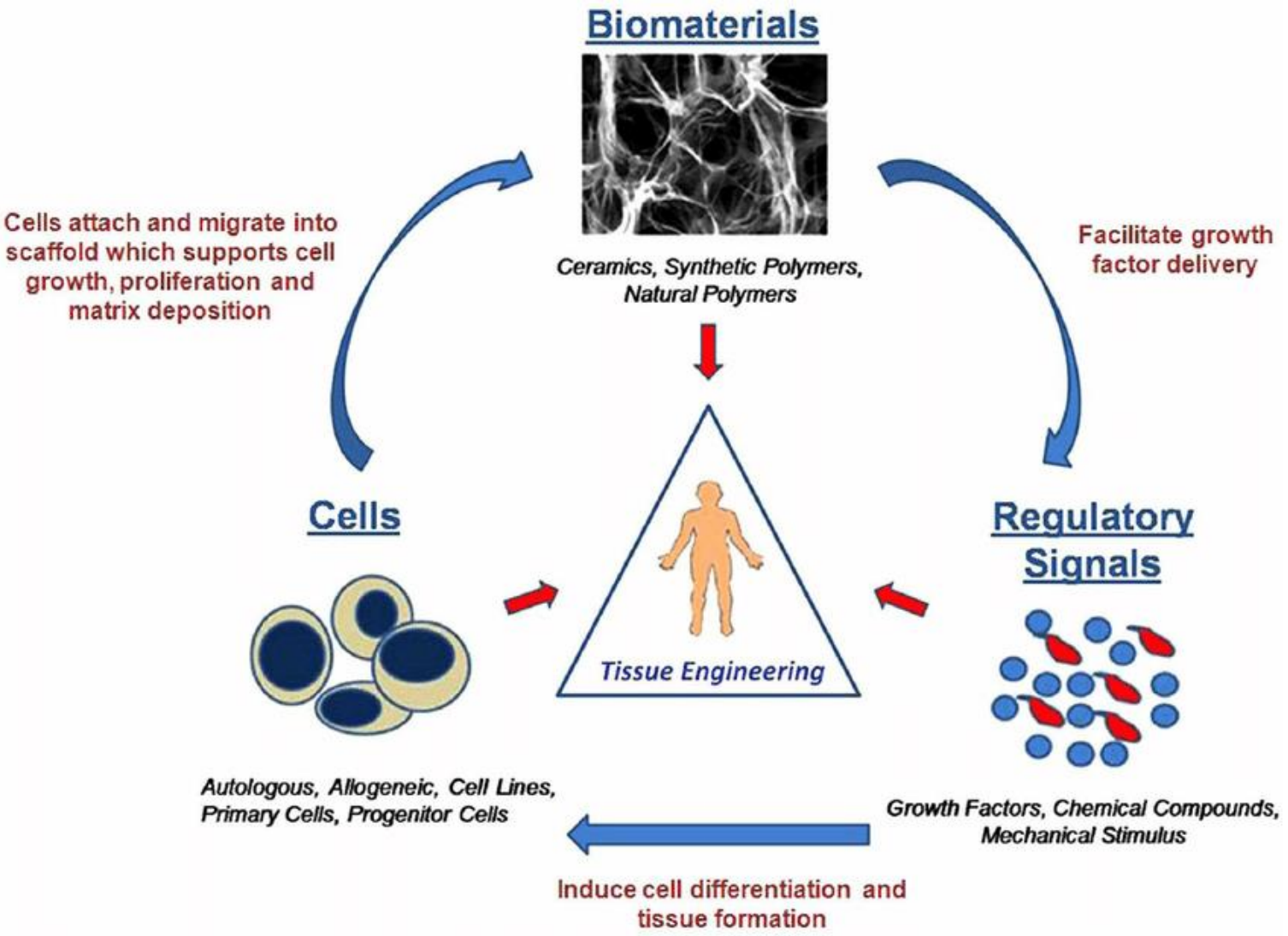
TISSUE ENGINEERING



BIOMATERIALS AND SCAFFOLDS

Biomaterials play a critical role in tissue engineering by acting as synthetic frameworks referred as scaffolds, matrices, or constructs

Scaffolds are defined as three-dimension porous solid biomaterials designed to perform some or all of the following functions:

(i) promote cell-biomaterial interactions, cell adhesion, and ECM deposition,
(ii) permit sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation,
(iii) biodegrade at a controllable rate that approximates the rate of tissue regeneration under the culture conditions of interest, and
(iv) provoke a minimal degree of inflammation or toxicity in vivo

Some importante concepts

Biodegradable: enzymatic degradation of materials. Not clear where products end up.

Bioerodable: undergo surface erosion in the body

Bioresorbable: degradation through hydrolysis, the products are eliminated by the kidneys through normal metabolic processes

Bioabsorbable: erosion through solubilisation

Today we are going to talk about POLYMERS ONLY (NOTE: there are more classes of biomaterials)

POLYMERS AS BIOMATERIALS

Polymers have been widely used as biomaterials for the fabrication of medical device and tissue-engineering scaffolds.

The criteria for selecting the materials as biomaterials are based on their:
- **material chemistry,**
- **molecular weight,**
- **solubility,**
- **shape and structure,**
- **hydrophilicity/hydrophobicity,**
- **lubricity,**
- **surface energy,**
- **water absorption,**
- **degradation,**
- **erosion mechanism.**

Scaffold materials can be synthetic or biologic, degradable or nondegradable, **depending on the intended use.**

Naturally occurring polymers, **synthetic biodegradable**, and **synthetic nonbiodegradable** polymers are the main types of polymers used as biomaterials.

OVERVIEW

- Natural polymers
  - Protein origin
  - Polyssacharadic
  - Polyhydroxyalkanoates
- Synthetic Polymers for solid-state porous scaffolds
  - Synthetic biodegradable polymers
    - Aliphatic polyester
    - Polyanhydrides
    - Polyphosphazenes
    - Polyurethanes
    - Poly(glycerol sebacate)
    - Polymers for hydrogels
  - Functional synthetic biodegradable polymers
    - Conducting polymers
    - Photo-responsive polymers
    - Amino-acid-based polymers
    - Cell-interactive polymers
- Techniques for microfabrication
- How do cells interact with biomaterials?
- Applications – some examples

NATURAL POLYMERS VS SYNTHETIC POLYMERS

- Natural polymers can be considered as the first **biodegradable** biomaterials used clinically. Natural materials owing to the bioactive properties have **better interactions with the cells** which allow them to enhance the cells' performance in biological system. They can present **significant variations** among different batches, though.

- Synthetic polymers are highly useful in biomedical field since their **properties** (e.g., porosity, degradation time, and mechanical characteristics) **can be tailored** for specific applications. Synthetic polymers are often **cheaper** than biologic scaffolds; it can be produced in **large uniform** quantities and have a **long shelf time**.

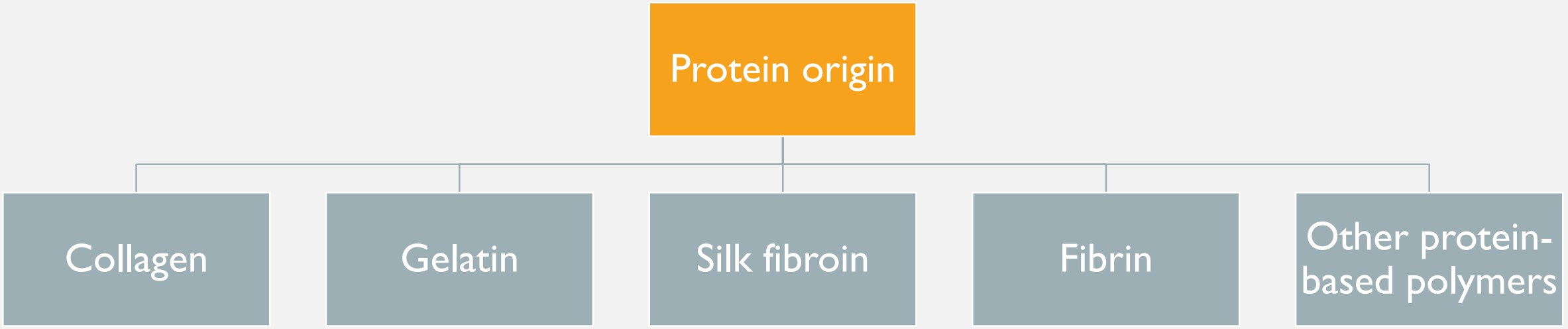
NATURAL POLYMERS

Protein origin

Polyssacharidic

Polyhydroxyalkanoates

NATURAL POLYMERS

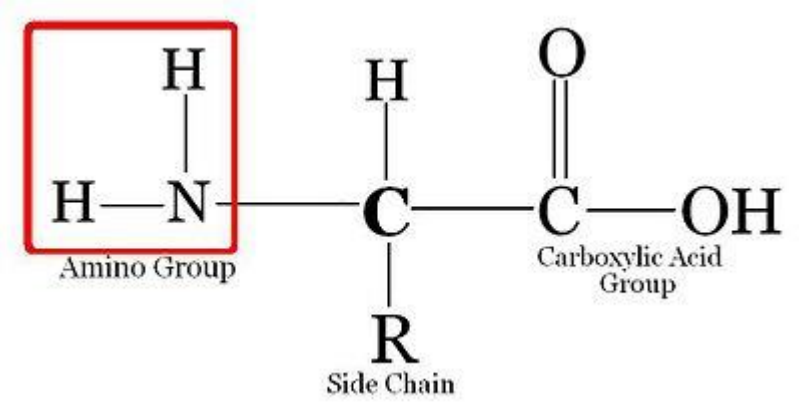


NATURAL POLYMERS

Protein origin

Why proteins?
Where can we find them?
What are they?

Amino acids are the building blocks of polypeptides and proteins, which consist of a central carbon linked to an amine group, a carboxyl group, a hydrogen atom, and a side chain (R groups). The distribution of the R groups along the protein backbone renders proteins with distinct characteristics.



NATURAL POLYMERS

Protein origin

Collagen

- Collagen is regarded by many as an ideal scaffold or matrix for tissue engineering as it is the **major protein component of the extracellular matrix**, providing support to connective tissues such as **skin, tendons, bones, cartilage, blood vessels, and ligaments**.
- Twenty-seven types of collagens have been identified to date, but **collagen type I** is the most abundant and the most investigated for biomedical applications.
- Collagen is defined by high mechanical strength, good biocompatibility, low antigenicity and ability of being crosslinked, and tailored for its mechanical, degradation and water-uptake properties. It **is mainly isolated from animal tissues** (which raises some concerns about its safety).

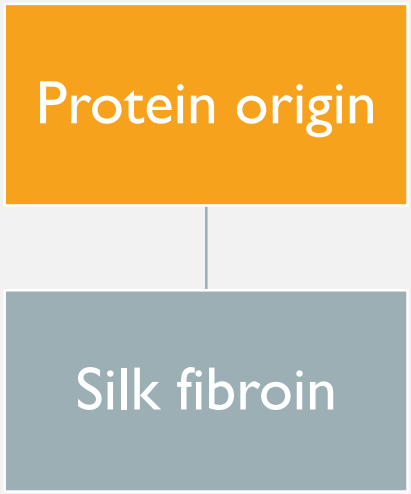
NATURAL POLYMERS

Protein origin

Gelatin

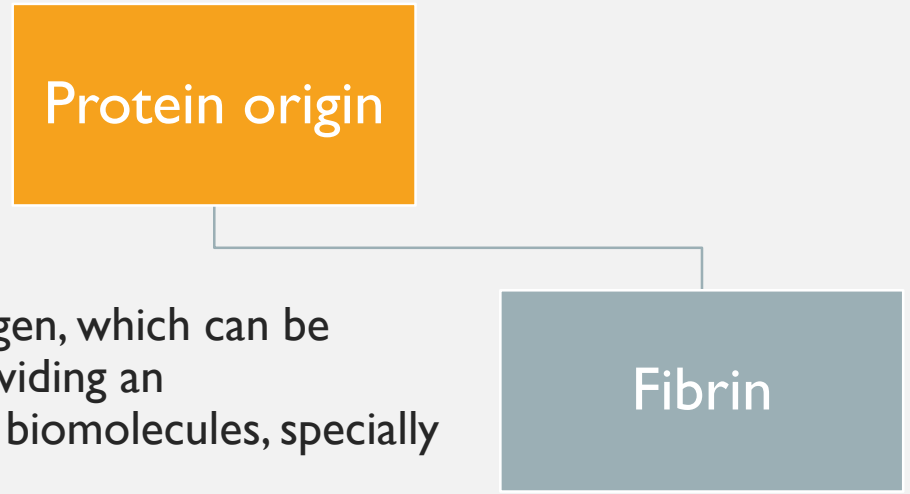
- Gelatin is commonly used for pharmaceutical and medical applications because of its biodegradability and biocompatibility. It is often used as a component in **drug formulations** (used as a drug delivery system usually encapsulating the active compound) or as a **sealant for vascular prostheses**.
- Gelatin is a **denatured protein** obtained by acid and alkaline processing of collagen.
- It has relatively **low antigenicity** because of being denatured in contrast to collagen which is known to have antigenicity due to its animal origin.
- Under specific conditions, such as temperature, solvent or pH, gelatin macromolecules present sufficient flexibility to realize a **variety of conformations.**

NATURAL POLYMERS



- Silk is generally defined as protein polymers that are spun into fibers by some *lepidoptera* larvae such as silkworms, spiders, scorpions, mites and flies.
- Spider silk is an intriguing biomaterial that is **lightweight, extremely strong and elastic**, and exhibits mechanical properties comparable to the best synthetic fibers produced by modern technology.
- Silk fibroin, a mass-producible natural polymer produced by silkworms, commonly used as a textile fiber. In the medical field, silk has long been used for **surgical sutures**

NATURAL POLYMERS



- Fibrin is a protein matrix produced from fibrinogen, which can be **autologously harvested** from the patient, providing an immunocompatible carrier for delivery of active biomolecules, specially cells.
- Polymerized fibrin is a major component of blood clots and plays a vital role in the subsequent **wound healing** response.
- For these reasons, scientists developed a Fibrin glue used in surgery.
- Its rapid degradation can represent a problem for use as a shape-specific scaffold in tissue engineering, therefore optimizing fibrin composition is a fundamental

NATURAL POLYMERS

Protein origin

Other protein-based polymers

- There are other very interesting and attractive protein-origin polymers namely **elastin** and **soybean** that have been applied in some extent in the tissue engineering applications.

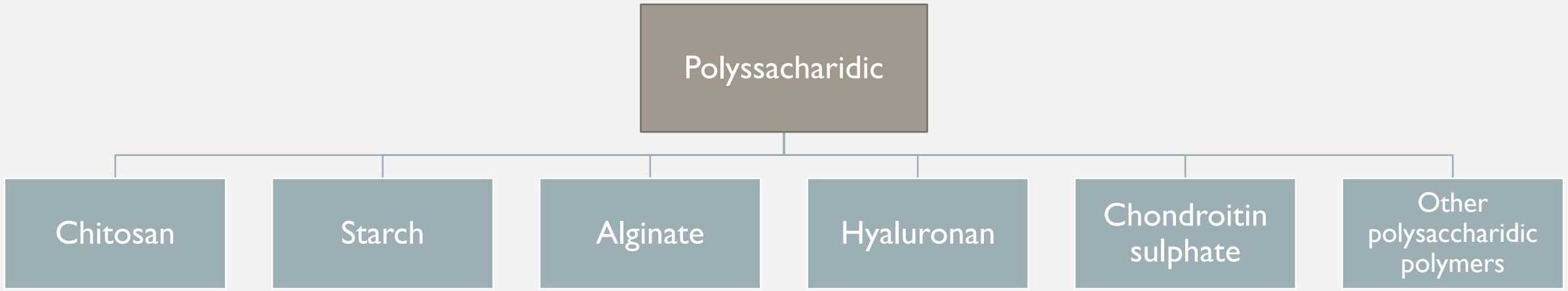
NATURAL POLYMERS

Protein origin

Polyssacharidic

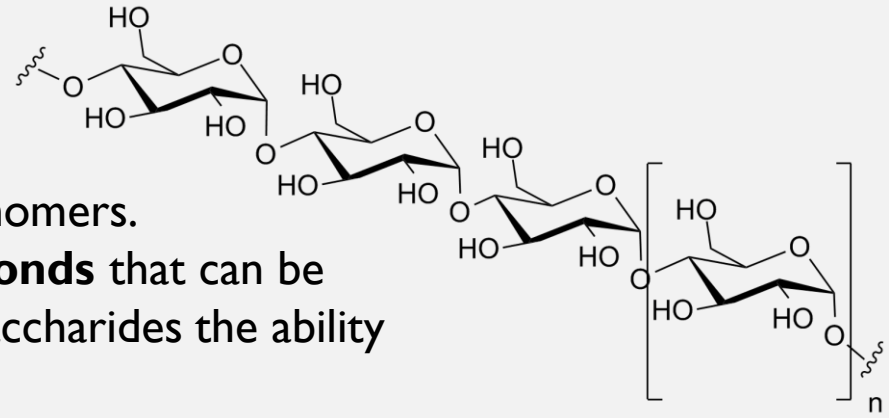
Polyhydroxyalkanoates

NATURAL POLYMERS



NATURAL POLYMERS

Polyssacharidic



Polysaccharides are a class of biopolymers constituted by simple sugar monomers.
The monomers (monosaccharides) are linked together by **O-glycosidic bonds** that can be made to any of the hydroxyl groups of a monosaccharide, conferring polysaccharides the ability to form both linear and branched polymers.

These biological polymers can be obtained from different sources: **microbial, animal and vegetal**.

They are **non-toxic, show interaction with living cells** and, with few exceptions, have **low costs** in comparison with others biopolymers such as collagen

NATURAL POLYMERS

Polyssacharidic

Chitosan

- Chitin is a natural polysaccharide found particularly in the shell of crustacean, cuticles of insects and cell walls of fungi.
- Chitosan, the fully or partially deacetylated form of chitin, due to its properties as attracted much attention in the tissue engineering and drug delivery fields. It has been proved to be **biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic and biofunctional**.
- In addition, chitosan molecule has amino and hydroxyl groups which can be **modified chemically** providing a high chemical versatility and is metabolized by certain human enzymes.
- Moreover, chitosan exhibits a **pH-sensitive behavior** as a weak poly-base due to the large quantities of amino groups on its chain. Chitosan dissolves easily at low pH while it is insoluble at higher pH ranges.

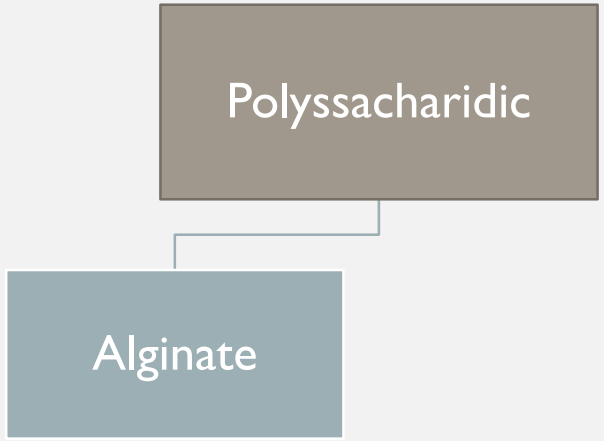
NATURAL POLYMERS

Polyssacharidic

Starch

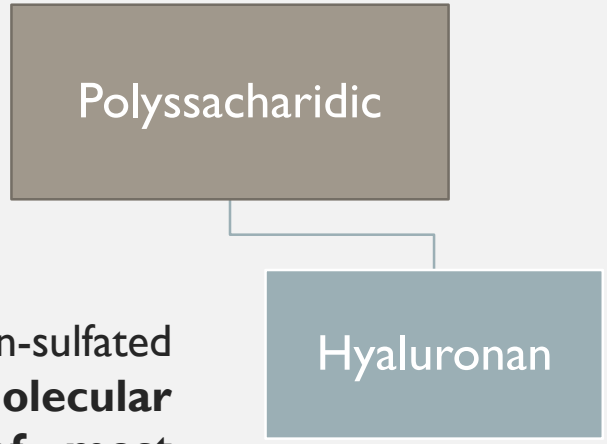
- Starch is one of the most promising natural polymers because of its inherent biodegradability, overwhelming abundance and renewability.
- Starch by itself is extremely **difficult to process** and is brittle when used without the **addition of a plasticizer** (such as water or some alchols).
- Due to its **degradation by amylases**, this constitutes another strategy to tailor the degradation of starch-based materials
- Additionally, blending two or more chemically and physically dissimilar natural polymers has shown potential to overcome these difficulties

NATURAL POLYMERS



- Alginates are **abundant** in nature and are found as structural components of marine brown **algae** and as capsular polysaccharides in some soil **bacteria**.
- Alginates undergo **reversible gelation in aqueous solution under mild conditions** through interaction with divalent-cations such as Ca^{2+} that can cooperatively bind between the G-blocks of adjacent alginate chains creating ionic inter-chain bridges.
- Theoretically, alginate shrinks at low pH and the encapsulated drugs are not released. This pHdependent behavior of alginate is exploited to tailor release profiles and in the development of 'smart' systems. However, at higher pH alginate undergoes a rapid dissolution which may result in burst release of protein drugs.

NATURAL POLYMERS



- Hyaluronan is a naturally occurring non-sulfated glycosaminoglycan and **a major macromolecular component of the intercellular matrix of most connective tissues** such as cartilage, vitreous of the human eye, umbilical cord and synovial fluid.
- Hyaluronan and its associated networks have many physiological roles that include tissue and matrix water regulation, structural and space-filling properties, lubrication, and a number of macromolecular functions.

- Hyaluronan has been widely studied for **drug delivery**, for dermal, nasal, pulmonary, parenteral, liposome-modified, implantable delivery devices and for **gene delivery**. For tissue engineering it has been focused on **cartilage, bone and osteochondral** applications

NATURAL POLYMERS

Polyssacharidic

Chondroitin sulphate

- Chondroitin sulfate, is one of the most physiologically important **glycosaminoglycans.** Glycosaminoglycans (GAGs) are found in the lubricating fluid of the joints and as components of cartilage, synovial fluid, bone, and heart valves.
- **With the exception of hyaluronan**, these polysaccharides are covalently linked to a protein core, thereby **forming proteoglycans**.
- Biocharacteristics of GAGs include the **binding and modulation** of growth factors and cytokines, the inhibition of proteases, and the involvement in adhesion, migration, proliferation and differentiation of cells.
- Due to its GAG nature, chondroitin sulfate is an attractive natural–origin polymer applied essentially in **cartilage tissue** engineering.

NATURAL POLYMERS

Polyssacharidic

Other polysaccharidic polymers

Some of them, the so-called cold set gels which form a gel on cooling the solution like **agarose**, **carrageenans** and **gellan gum** have been studied in some extent the frame of **drug delivery for tissue engineering**

NATURAL POLYMERS

Polyhydroxyalkanoates

In nature, a **special group of polyesters** is produced by a wide variety of microorganisms as an internal carbon and energy storage

Although a great variety of materials of this family can be produced, the use of PHAs in tissue engineering has been mainly restricted to PHBs and poly(hydroxybutyrateco-valerate) (PHBV).

Interesting physical properties of **polyhydroalkanoates** include **nonlinear optical activity** and **piezoelectricity**, i.e. the capacity of a material to suffer electric polarization due to mechanical stress.

On the other hand, **Polyhydroxybutyrates** have already been studied to some extent for tissue engineering applications, mainly **for scaffold materials** in combination with ceramic materials, as a vehicle for **drug delivery**, and also as a material for **cardiac tissue engineering**

NATURAL POLYMERS

Protein origin

Polyssacharidic

Polyhydroxyalkanoates

SYNTHETIC POLYMERS

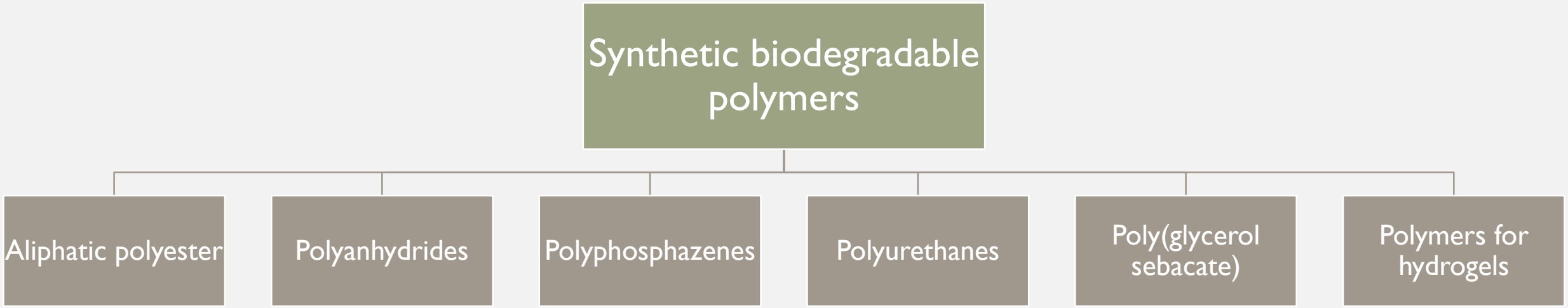
- Many commercially available synthetic polymers show physicochemical and mechanical properties comparable to those of biological tissues.
- Synthetic polymers represent the **largest group of biodegradable polymers**, and they can be produced under controlled conditions.
- They exhibit, in general, **predictable and reproducible** mechanical and physical properties such as tensile strength, elastic modulus, and degradation rate.
- However, their biocompatibility and biodegradability in some cases can be insufficient, limiting their potential use in the clinical side. We can overcome these issues by **blending synthetic and natural polymers** or by using **composite materials** that improve the scaffold properties and thereby allowing controlled degradation and improving the biocompatibility in tissue engineering applications.

SYNTHETIC POLYMERS

Synthetic biodegradable polymers

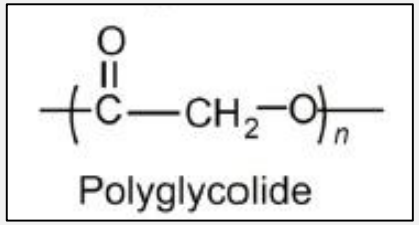
Functional synthetic biodegradable polymers

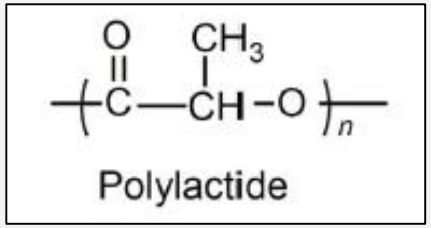
SYNTHETIC POLYMERS



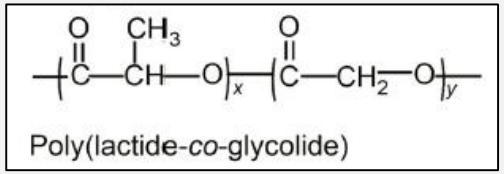
SYNTHETIC POLYMERS

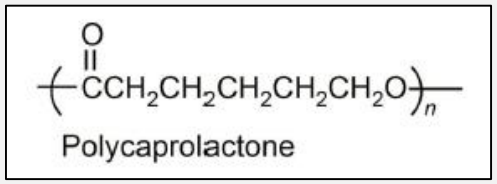
Synthetic biodegradable polymers



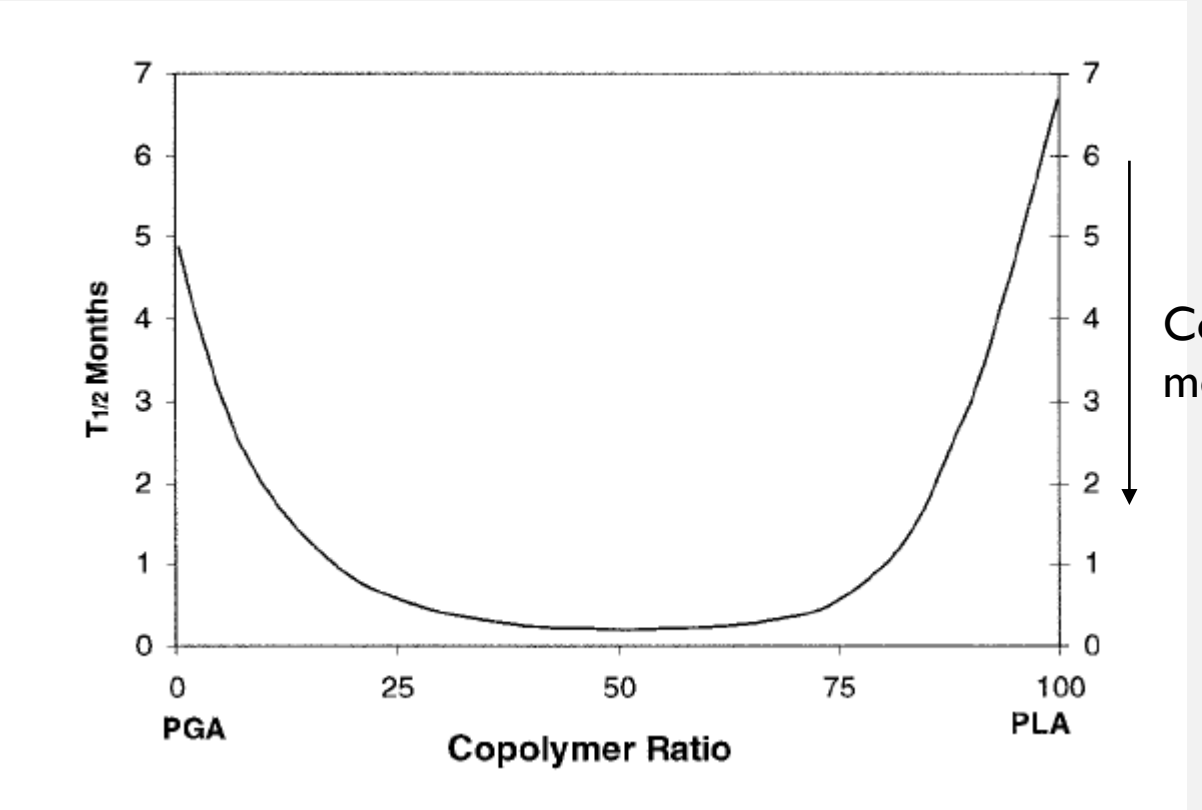


Aliphatic polyester





- These polymers can form stable porous materials that do not dissolve or melt under in vitro tissue culture conditions and can serve as **predesigned 3D scaffolds**
- These polymers usually undergo **degradation through hydrolysis of the ester groups** in their backbones; degradation rates and degradation products can be tuned according to composition, structure, and molecular weight.
- **Polylactide (PLA), polyglycolide (PGA),** and their copolymer **poly(lactide-co-glycolide) (PLGA)** are widely used.
- In addition to their biodegradability and biocompatibility, these polymers are among the few synthetic polymers **approved by the FDA for human clinical applications** such as **surgical sutures and some implantable devices**
- **Poly(ε-caprolactone) (PCL)** degrades at a much slower rate than PLA, PGA, and PLGA, which makes PCL less attractive for general tissue regeneration applications but more attractive for **long-term implants and drug-delivery systems**



The degradation rate of PLGA depends on MW, hydrophilicity and the degree of crystallinity, pH and temperature

TABLE 1. PROPERTIES OF BIODEGRADABLE POLYMERS[27,29,31,32]

Polymer type	*Melting point (°C)*	*Glass trans. temp. (°C)*	*Degration time (months)*[a]	*Density (g/cm^3)*	*Tensile strength (MPa)*	*Elongation, %*	*Modulus (GPa)*
PLGA	Amorphous	45–55	Adjustable	1.27–1.34	41.4–55.2	3–10	1.4–2.8
DL-PLA	Amorphous	55–60	12–16	1.25	27.6–41.4	3–10	1.4–2.8
L-PLA	173–178	60–65	>24	1.24	55.2–82.7	5–10	2.8–4.2
PGA	225–230	35–40	6–12	1.53	>68.9	15–20	>6.9
PCL	58–63	−65	>24	1.11	20.7–34.5	300–500	0.21–0.34

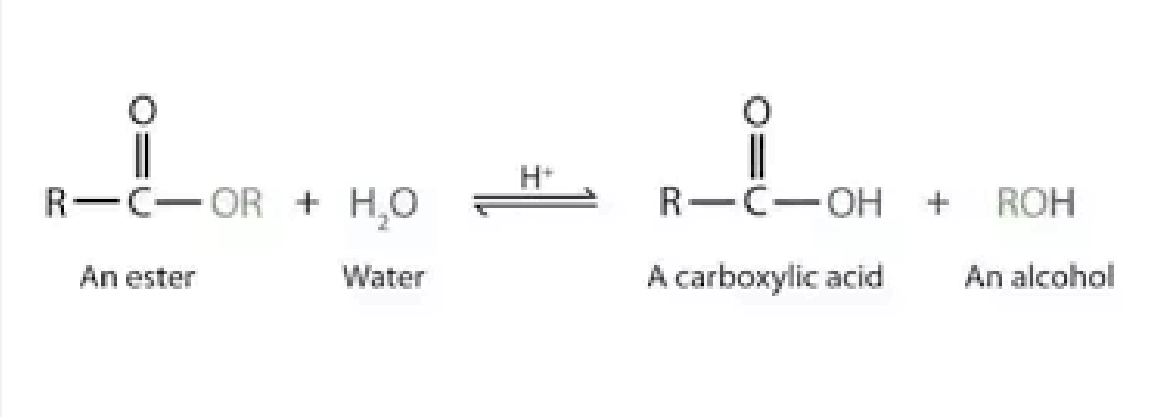
[a]Time to complete mass loss. Time also depends on part geometry.

TABLE 3. MECHANICAL PROPERTIES OF HUMAN TISSUES

	Tensile strength (MPa)	*Compressive strength (MPa)*	*Youngs' modulus (GPa)*	*Fracture toughness (MPa.m1/2)*
Cancellous bone[56]	N/a	4–12	0.02–0.5	N/a
Cortrial bone[56]	60–160	130–180	3–30	2–12
Cartilage[57]	3.7–10.5	N/a	0.7–15.3 (MPa)	N/a
Ligament[58]	13–46	N/a	0.065–0.541	N/a
Tendon[58]	24–112	N/a	0.143–2.31	N/a

Problems with PGA e PLA e PCL:

They are rigid.
Do not possess functional groups to modify and bind proteins.
Can generate too much local acidity. (degrade by hydrolysis).
Question: write a reaction for hydrolysis of PGA (assume 3 monomers)



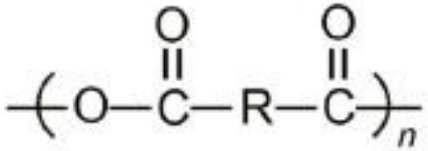
Please note that there is a whole world of polymers out there- but only a handful actually approved for in-vivo use.

SYNTHETIC POLYMERS

Synthetic biodegradable polymers

Polyanhydrides

- Polyanhydrides are biocompatible and **degradable in vivo into nontoxic diacid byproducts** that can be eliminated from the body as metabolites
- They were initially designed primarily for **drug-delivery applications**, because these polymers are **very hydrophobic** and undergo degradation through surface erosion.

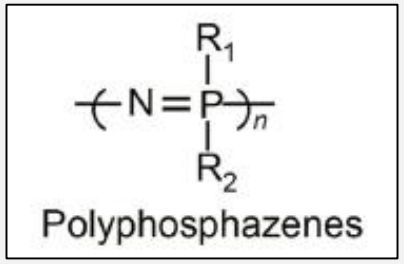


Poly(anhydrides)

SYNTHETIC POLYMERS

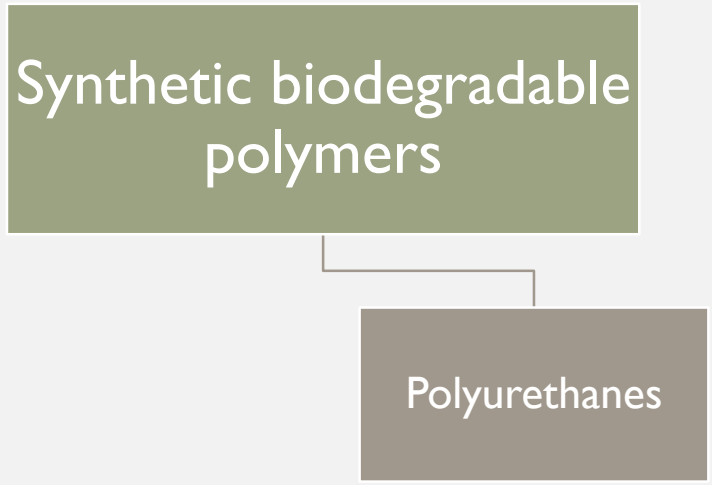
Synthetic biodegradable polymers

Polyphosphazenes



- Polyphosphazenes are usually of high molecular weight, and as linear polymers have an inorganic backbone of alternating phosphorous and nitrogen atoms with two side groups, attached to each phosphorous atom; They can form **elastic structures** and have potential as scaffolds for soft-tissue regeneration.

SYNTHETIC POLYMERS



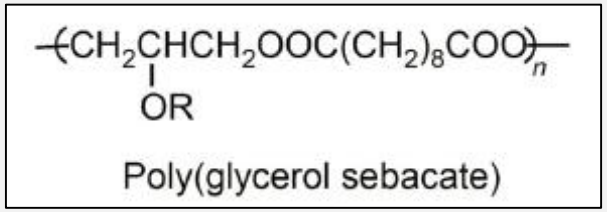
- Polyurethanes (PUs), which remain one of the most popular groups of biomaterials, are used for a broad range of biomedical applications. They are popular because of their segmented-block structural character, which endows them with a broad range of versatility in terms of **tunable mechanical properties,** physical properties, biological properties, blood and tissue compatibility, and more recently their biodegradability.
- PUs have been traditionally used as biostable and inert materials in **heart valves, vascular grafts, catheters, and prostheses**

SYNTHETIC POLYMERS

Synthetic biodegradable polymers

Poly(glycerol sebacate)

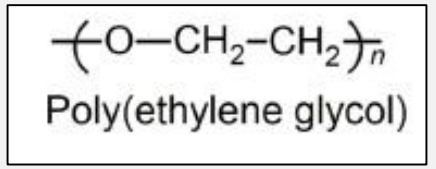
- The starting materials for the synthesis of PGS are glycerol and sebacic acid.
- PGS, which is relatively inexpensive to produce, exhibits **thermoset elastomeric** properties. In addition, PGS can be tuned to achieve mechanical properties and degradation rates targeted to a specific application by controlling curing time, curing temperature, reactant concentrations, and, in acrylated PGS, the degree of acrylation
- PGS has mainly been targeted for **soft-tissue engineering**, such as **cardiac muscle, blood, nerve, cartilage, and retina**, owning to its elastomeric nature



Poly(glycerol sebacate)

SYNTHETIC POLYMERS

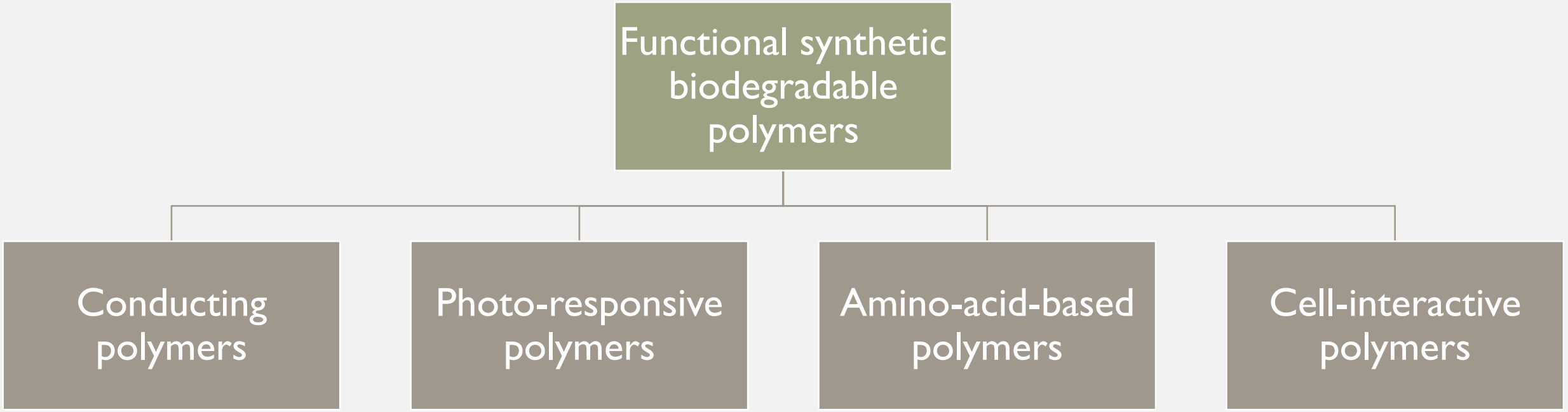
Synthetic biodegradable polymers



Polymers for hydrogels

- Hydrogels are crosslinked hydrophilic polymers that can absorb large amounts of water without dissolution.
- Hydrogels are promising candidates for certain tissue engineering applications owing to their **structural similarity to soft tissue**, allowance of minimally invasive procedures, and excellent biocompatibility.
- Poly (ethylene glycol) (PEG) is the most extensively studied for tissue-engineering research. PEG has been used as a model polymer to study the effect of adhesion peptides because it has been shown to **prevent protein and cell adhesion**. It is also used for its Stealth effect.
- However, PEG hydrogels are not degradable (to obtain a degradable material we have to combine with other molecules).

SYNTHETIC POLYMERS



Functional synthetic biodegradable polymers have been developed as scaffolding materials for tissue regeneration, since their functional groups interact in an unique way with the surrounding environment.

SYNTHETIC POLYMERS

Functional synthetic biodegradable polymers

Conducting polymers

- CPs were found to be able to modulate cellular activities such as cell adhesion, proliferation, and differentiation **via electrical stimuli**.
- Many of these studies were carried out on **nerve, bone, muscle, and cardiac cells, as well as mesenchymal stem cells**, because these tissues or cells are quite sensitive to electrical stimulation
- Therefore, polymer blends and composites that are composed of conducting polymers such as **polypyrrole (PPy)** and **polyaniline (PANi)**, have been intensilvey investigated.
- Conducting materials that are elastic allow more realistic mimicry of the mechanical properties of ECM, such as skin cells, skeletal muscle, and blood vessels (such as composite of PANi with poly(L-lactide-co-ε-caprolactone))

SYNTHETIC POLYMERS

Functional synthetic biodegradable polymers

Photo-responsive polymers

- Design and synthesis of photosensitive polymers has drawn great interest in recent years. The most frequently studied **photochromic groups** are azide groups, cinnamoyl groups, and spiropyran, coumarin, and 2-nitrobenzyl groups

- Photosensitive properties can be to trigger **conformational change** of polypeptides.
- Reversible photoresponsiveness can also be used to **change the wettability** by surface modification
- Light-responsive polymers are applicable in delivery systems during tissue regeneration, for example, by using this photoresponsive interaction as a **molecular switch, the controlled release** of proteins can be performed.

SYNTHETIC POLYMERS

Functional synthetic biodegradable polymers

Amino-acid-based polymers

- Amino-acid-based biomaterials have been known to undergo naturally controlled degradation processes. **Synthetic polypeptides** have emerged as a type of attractive functional biomaterial because of their unique physical, chemical, and biological properties; they are promising candidates for sutures, **haemostatic agents, and scaffolds for tissue engineering.**
- Early studies on the biomedical use of synthetic polypeptides were related to **poly(L-lysine) and poly(L-aspartic acid)** that were easily prepared, and water-soluble homopolypeptides.

SYNTHETIC POLYMERS

Functional synthetic biodegradable polymers

- Enzymatic degradability is one way to render materials degradable. **The structure of the degradable sequences** should match the active site of respective **enzyme**(s). Oligopeptide sequences are frequently used as degradable cross-linkers in hydrogels.
- This approach mimics the enzymatic biodegradability of collagen and other natural ECM components.
- These materials are usually block copolymers of PEG and oligopeptides. The block copolymers terminated with reactive groups are **crosslinked to form hydrogel networks**, and can be **specifically degraded** by cell-secreted MMPs (such as collagenase).

Cell-interactive polymers

FABRICATION TECHNIQUES IN TISSUE ENGINEERING – AN OVERVIEW

Table 1: Scaffolds' fabrication techniques in tissue engineering applications.

Method	Polymers	Unique factors	Application
Biodegradable porous scaffold fabrication			
Solvent casting/salt leaching method [35–37]	Absorbable polymer (PLLA, PLGA, collagen, etc.)	Biodegradable controlled porous scaffolds	Bone and cartilage tissue engineering
Ice particle leaching method [38–40]	PLLA & PLGA	Control of pore structure and production of thicker scaffolds	Porous 3D scaffolds for bone tissue engineering
Gas foaming/salt leaching method [41–43]	PLLA, PLGA & PDLLA	Controlled porosity and pore structure sponge	Drug delivery and tissue engineering
Microsphere fabrication			
Solvent evaporation technique [44–46]	PLGA, PLAGA	High-density cell culture, due to the extended surface area	Bone repair
Particle aggregated scaffold [47–49]	Chitosan, HAP	High mechanical stability	Bone, cartilage, or osteochondral tissue engineering
Freeze drying method [50–52]	PLGA, PLLA, PGA, PLGA/PPF, Collagen, and Chitosan	3D porous sponge structure, durable and flexible	Tissue engineering scaffolds
Thermally induced phase separation [53, 54]	PEG, PLLA	Highly porous scaffold for cellular transplantation	Complicated shapes for tissue engineering applications
Injectable gel scaffold fabrication			
Ceramic-based injectable scaffolds [55–57]	CP ceramics, HAp, TCP, BCP, and BG	Porosity and bioresorbability	Cartilage tissue engineering
Hydrogel-basedinjectable scaffolds [58–60]	Hydrophilic/hydrophobic diblock and triblock copolymer combinations of PLA, PGA, PLGA, and PEG. Copolymers of PEO and PPO and polyoxamer. alginates, collagen, chitosan, HA, and fibroin	Biomimetically, exhibit biocompatibility and cause minimal inflammatory responses, thrombosis, and tissue damage	Cartilage, bone tissue engineering, and drug delivery

FABRICATION TECHNIQUES IN TISSUE ENGINEERING – AN OVERVIEW (Continuation)

Hydrogel scaffold fabrication			
Micromolding [61–63]	Alginate, PMMA, HA, PEG	Microgels, biologically degradable, mechanical and physical Complexity	Insulin delivery, gene therapy, bioreactor, and immunoisolation
Photolithography [64–66]	Chitosan, fibronectin, HA, PEG, PNIAAm, PAA, PMMA, PAam, and PDMAEM	Microwells, microarrays, controlled size and shape	Microdevices, biosensors, growth factors, matrix components, forces, and cell-cell interactions
Microfluidics [67–69]	PGS, PEG, calcium alginate, silicon and PDMS	Microbeads, microrods, valves, and pumps	Sensing, cell separation, cell-based microreactors, and controlled microreactors,
Emulsification [70–72]	Gelatin, HA, and collagen	Microgels, microsensors, cell-based diagnostics	Sustainable and controllable drug delivery therapies
Acellular scaffold fabrication			
Decellularisation process [73–75]	Biological tissues	Retain anatomical structure, native ECM, and similar biomechanical properties	Tissue engineering
Keratin scaffold fabrication			
Self-assembled process [76–78]	Keratin	Biocompatibility	Drug delivery, wound healing, soft tissue augmentation, synthetic skin, coatings for implants, and scaffolds for tissue engineering

FABRICATION TECHNIQUES IN TISSUE ENGINEERING – AN OVERVIEW (Continuation)

Method	Polymers	Unique factors	Application
Fibrous scaffold fabrication			
Nanofiber electrospinning process [79–81]	PGA, PLA, PLGA, PCL copolymers, collagen, elastin, and so forth	High surface area, biomechanical, and biocompatibility	Drug delivery, wound healing, soft tissue synthetic skin, and scaffolds for tissue engineering
Microfiber wet-spinning process [82–84]	PLGA, PLA, chitosan, and PCL	Biocompatible fibres with good mechanical properties	Solar sails, reinforcement, vascular grafts, nonwetting textile surfaces, and scaffolds for tissue
Nonwoven fibre by melt-blown process [85–87]	Polyesters, PGA, and PDO	Submicron fiber size, highly porous scaffold	Filtration, membrane separation, protective military clothing, biosensors, wound dressings, and scaffolds for tissue engineering
Functional scaffold fabrication			
Growth factor's release process [88–90]	Collagen, gelatin, alginate, chitosan, fibrin, PLGA, PLA, and PEG	Membranes, hydrogels, foams, microsphere, and particles	Angiogenesis, bone regeneration, and wound healing
Ceramic scaffold fabrication			
Sponge replication method [91–93]	PU sponge, PVA, TCP, BCP or calcium sulfate	Interconnected porous ceramic scaffolds	Bone tissue engineering
Simple calcium phosphate coating method [94–96]	Coating on: metals, glasses, inorganic ceramics and organic polymers (PLGA, PS, PP, silicone, and PTFE), collagens, fibres of silk, and hairs	Improve biocompatibility or enhance the bioreactivity	Orthopedic application

FABRICATION TECHNIQUES IN TISSUE ENGINEERING – AN OVERVIEW (Continuation)

Automation and direct organ fabrication			
Inkjet printing process [97–100]	Sodium alginate	To build complex tissues composed of multiple cell types (Hydrogel scaffold)	Biosensor development, microdeposition of active proteins on cellulose, biochips and acellular polymeric scaffolds
Melt-based rapid prototyping process [101, 102]	Biodegradeable polymers or blends	Complex 3D solid object, good mechanical strength	Honey comb structure scaffold, hard-tissue scaffolds
Computer-aided design (CAD) data manipulation techniques [103–105]		Design and fabrication of patient-specific scaffolds and automated scaffold assembly algorithm	Develop a program algorithm that can be used to design scaffold internal architectures
Organ printing [106, 107]	Tubular collagen gel	Layer by layer deposition of cells or matrix	To print complex 3D organs with computer-controlled,
Scaffold sterilization			
Ethylene oxide gas (EOG) [108–110]		For degradable polymers and porous scaffolds, high penetration ability, and compatibility	Absolute freedom from biological contamination in scaffolds
Gamma-radiation sterilization [111–113]		Proven process is safe, reliable, and highly effective at treating single-use medical devices	Surgical disposables: surgical sutures, bandages, dressings, gauge pads, implants
Electron beam radiation [114–116]		Compatibility, low penetration, in line sterilization of thin products	Commercially successful technology for sterilizing a variety of disposable medical devices with a wide range of densities
Dry-heat sterilization [117, 118]		Efficacy, speed, process simplicity, and lack of toxic residues	Heat is absorbed by the exterior surface of scaffold and then passed inward to the next layer
Steam sterilization [119, 120]		Removal of all contamination, and scaffold can be reused	Porous scaffold for living cell immobilization

HOW DO CELLS INTERACT WITH BIOMATERIALS?

It depends on:

Charge

Surface energy

Topology

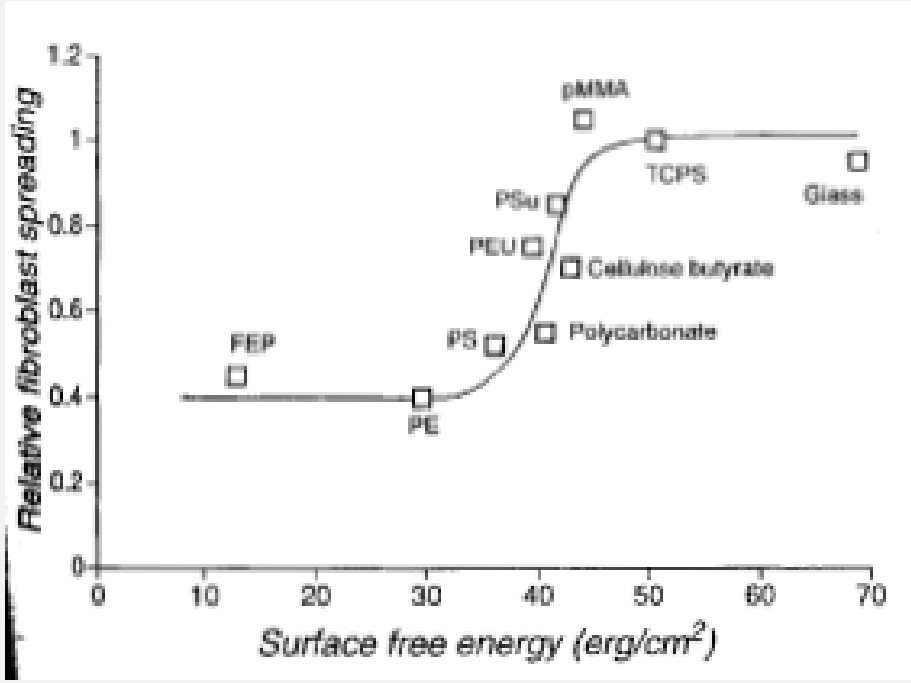
Chemistry

Contact Angle

HOW DO CELLS INTERACT WITH BIOMATERIALS?

The surface energy across an interface or the surface tension at the interface is a measure of the energy required to form a unit area of new surface at the interface. So Joules/m^2 or N/m.

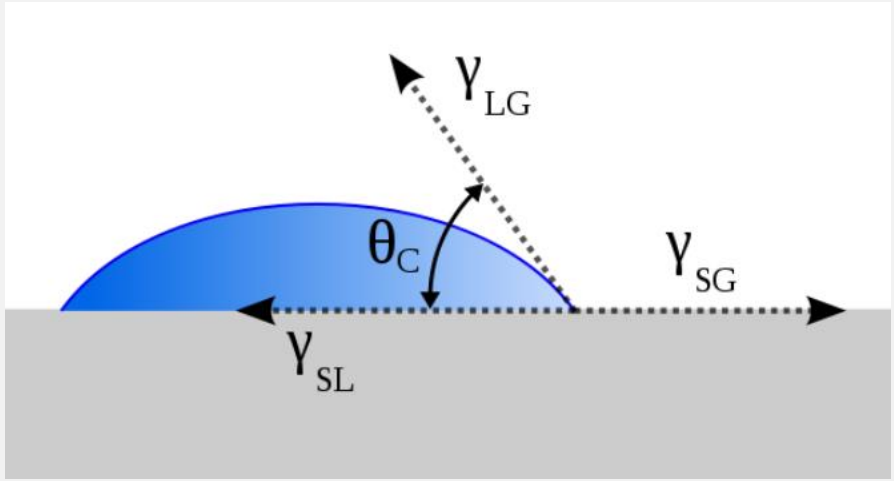
Usually a polymer has to be modified before cells can adhere

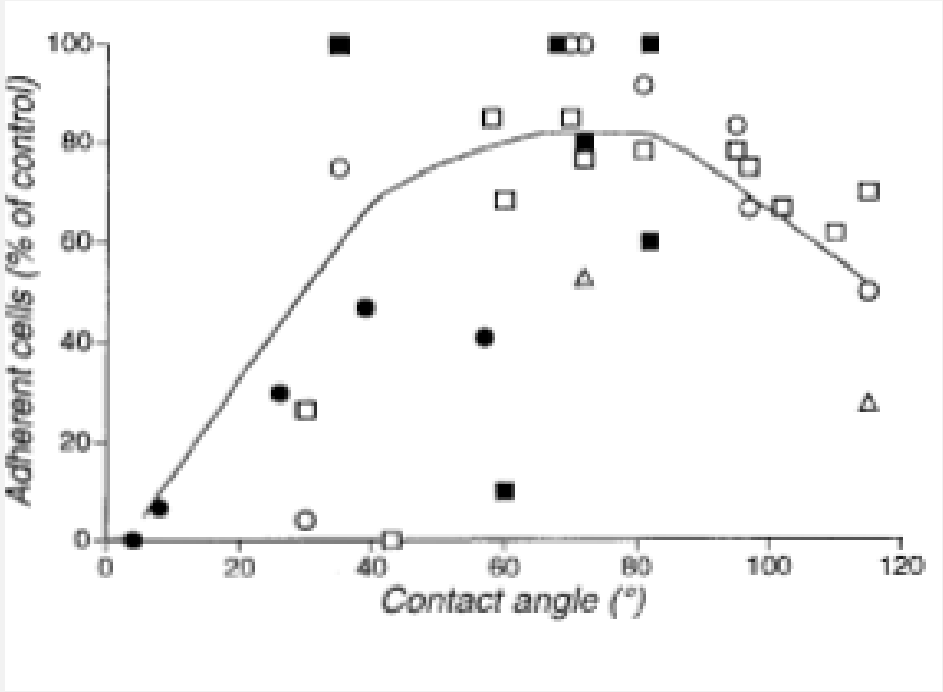


The highest surface energy is that of water: 72 mN/m (only one liquid is higher, which?)

HOW DO CELLS INTERACT WITH BIOMATERIALS?

γ sg= surface interfacial energy solid/gas
γ lg= surface interfacial energy liquid/gas
γsl= surface interfacial energy liquid/solid

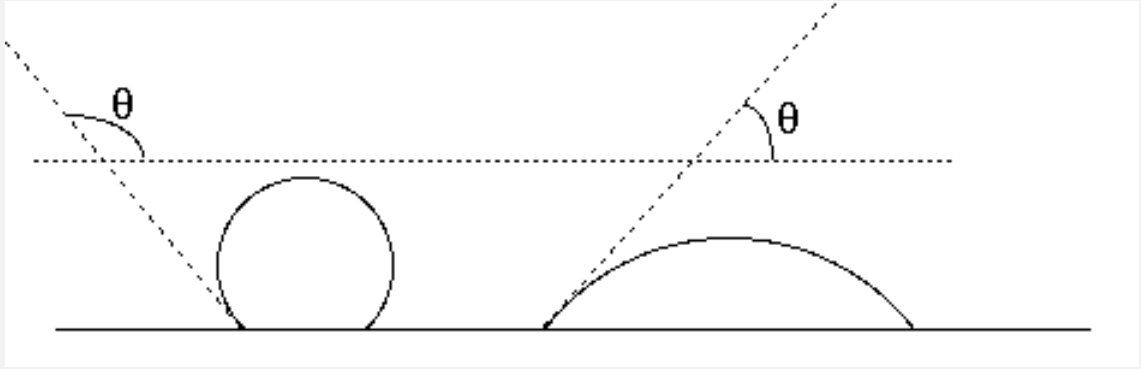




Young's equation is a balance of forces to get θ or γsl

$$\gamma_{SG} - \gamma_{SL} - \gamma_{LG} \cos\theta_C = 0$$

HOW DO CELLS INTERACT WITH BIOMATERIALS?



A midway contact angle is best: too high (hydrophobic), water is repelled, cell outer layer will also be repelled as it is hydrated.

Too low: water is absorbed to the exclusion of everything else, proteins will not adhere, (slip off), so neither cells.

The hydrophilic molecule (presented before) used to repel cells and proteins is PEG (polyethylene glycol), biocompatible.

The hydrophobic molecules are usually alkane silanes , alkane thiols, or perfluorinated carbons.

SOME APPLICATIONS - EXAMPLES

- Organ-specific regeneration using biodegradable materials
  - Skin
  - Nerves
  - Blood vessels
  - Heart
  - Cornea
  - **Bone**
  - **Cartilage**

SKIN

- Numerous biomaterials have been explotited as skin implants, ranging from **naturally** occurring collagen gels/sponges, alginates, polypeptides, Glycosaminoglycans, hyaluronan and fibronectin to **synthetic** materials, e.g. polyvinyl chloride, Polylactate/glycolatefabrics (PLGA).
- Dermagraft® (Organogenesis, Canton, USA) is a synthetic product which either uses **polygalactic or polyglycolic acid** meshes combined with **neonatal** **fibroblast** to enhance wound healing as temporary skin substitutes. It was followed by the development of OrCel™ (Ortec International US Inc., New York, USA) in 2001. Its use is only for **chronic wounds** such as diabetic foot ulcers (DFUs), pressure ulcers and vascular ulcers (including venous ulcers and arterial ulcers)

SKIN

- Biobrane® (Smith and Nephew, London, UK) – nonbiodegradable **nylon mesh with a silastic coating bonded to collagen type I derived peptides**. Due to its lower cost, ease of storage, application and fix, and reliable when used according to guidelines and being efficacious in treating partial thickness burns are the main reasons of its popularity in usage.
- Recently, cell therapy using **Adipose stem cells (ASCs)** has been shown to be a good potential alternative technique because it is less invasive than reconstructive surgery and the cells can be directly placed onto target areas in cutaneous lesions. Sufficient numbers of ASCs can easily be harvested by liposuction and fat tissue

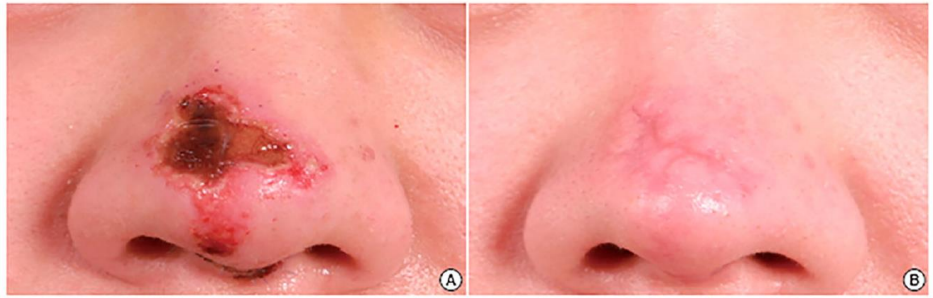


Figure 7. ASC-based cell therapy for a case of acute skin necrosis in the nose dorsum due to inadvertent arterial injection of HA filler: (A) Before stem cell therapy (seven days after HA filler injection); (B) Three weeks after stem cell therapy. (Reprinted as an open access for unrestricted non-commerical use) [126].

NERVES

- Compared to other types of trauma, nerve injuries are particularly complicated as mature **neurons do not replicate**. **Artificial nerve guidance conduits** have been in development for many years, which bridge the gap between the nerve stumps and aid nerve regeneration. The guide may be implanted empty, or it may be filled with growth factors, cells or fibres.
- **Micro-braiding** is a novel technique for the fabrication of polymeric nerve guide conduits composed of **biodegradable PLGA fibres**. The microbraided nerve guide conduit with a fibre architecture has shown promotion of axonal regeneration, with no inflammatory response or swelling. It degraded from the implantation site after serving its purpose.
- Among **natural polymers**, collagen, chitosan and alginate have been used for constructing nerve guidance channel
- An emerging area of nerve guide manufacture using synthetic materials includes the use of additive layer manufacturing. The very first **3D printed nerve guide was produced from poly(ethylene glycol) (PEG)** by ultraviolet(UV) light-induced photocuring using stereolithography.
- Recently, research has been focused on the synthesis of **conductive polymers** to fulfil basic biocompatibility and biodegradability properties by **combining conducting and degradable units**. A series of electroactive and biodegradable polymeric materials were prepared by blending **PLLA** and poly(glycol tetra-aniline) (**PGTA**).
- A novel electrically conductive biodegradable **polyphosphazene** polymer containing **aniline pentamer** and **glycine ethyl ester** as side chains was obtained by a nucleophilic substitution reaction
- McKeon et al. studied several polyaniline and poly(D,L-lactide) (**PANi/PDLA**) mixtures at different weight percentages and were successfully electrospun from 1,1,1,3,3,3-hexafluoroisopropanol solutions

Add a conductive component

BLOOD VESSELS

- There is a substantial patient demand for vascular bypass grafts due to atherosclerosis and related cardiovascular diseases.
- An ideal artificial graft should be **mechanically compatible** with the natural arteries and surrounding tissue and should also **mimic the ECM morphology**; it should have a nanoscale topography (5–500 nm) with **high porosity** and adequate pore sizes (5–500 μm) to **enhance cell** attachment and proliferation for the regeneration of the natural tissues.
- Strategies to create a suitable material for a vascular graft have focused on three areas of research: (1) coatings and surface chemical modifications of synthetic materials; (2) biodegradable scaffolds and (3) biopolymers
- Shin'oka et al. reported the use of PCL-based scaffolds to engineer venous blood vessels. The **PCL/PLA copolymer** was reinforced with **woven PGA** and seeded with **autologous smooth muscle and endothelial cells** harvested from a peripheral vein. After 10 days, the construct was implanted as a pulmonary bypass graft into a 4-year-old child.
- A fibrin-based vascular graft was developed by Swartz et al., who incorporated bovine **SMCs** and **endothelial cells into the fibrin gel**. The grafts were implanted in the jugular veins of lambs and remained patent for 15 weeks (it was a solution to collagen gels that had poor mechanical properties – relatively stiff)
- Electrospinning to create nanofibrous scaffolds composed of **collagen-blended degradable PLLA-co-PCL** was demonstrated. Results indicated that the blended nano-fibres supported endothelial cell attachment and spreading, and preserved the endothelial cell phenotype.

HEART

- To date, the exact relationship between the components of engineered biomaterials, the immune system and tissue regeneration has yet to be fully understood. One potential application of polymeric scaffolds is the development of **efficient degradable heart patches**. These heart patches can provide an optimal platform for cellular growth over a period of time.
- A **three-dimensional** **fibrin gel** construct was reported by Ye et al., where different concentrations of apportioning (a **protease inhibitor**) promoted controlled degradation of the **autologous scaffold** seeded with fibroblasts.
- Improvement: **fibrin gel** was prepared by a nonwoven polyglycolic acid (**PGA**) **fibre mesh coated** with polycaprolactone (**PCL**). Human saphenous vein cells were seeded onto the fibrin gel and a more mature ECM was produced in a short time span (days) with a decrease in the loss of soluble collagen.
- Another study investigated the use of chitosan to increase the compression modulus of collagen-based injectable hydrogel matrices. It has been reported that endothelial cells formed significantly more vascular-like structures on the **collagen–chitosan matrix-hydrogels** improved the ventricular wall stability and showed an ability to reduce heart dilatation upon myocardial infarction.
- Several recent reports have shown the use of **alginate hydrogels** in the **delivery** of sequential growth factor (vascular endothelial growth factor) VEGF-A(165) and Platelet-Derived Growth Factor-BB (PDGF-BB) in an MI model.
- PLLA-co-PCL **(PLCL) electrospun nano-fibres** were **encapsulated with VEGF** using two types of **protective agents** (BSA and dextran) through emulsion electrospinning.
- To date, several studies have focused on elastomeric biodegradable poly(glycerol sebacate) **PGS:gelatin** **nano-fibrous scaffolds** and poly(glycerol sebacate) **PGS/fibrinogen** **core/shell fibres**. These biomaterials exhibited well-defined anisotropy, mimicking the left ventricular myocardium architecture that can be used as constructs for myocardial regeneration and repair.

CORNEA

- Pathological conditions associated with cornea are reported as the major cause of vision impairment. Anatomically, the transparent corneal layer serves to focus light as it enters the eye.
- **Hydrogels-based** corneal implants from concentrated recombinant human **type I and type III collagen** have promoted stable regeneration of corneal tissue.
- A simple corneal substitute was developed from **carbodiimides and N-hydroxysuccinimide crosslinked collagen** and was found to be suitable for transplantation. This was employed in centres having a shortage of corneas available for implants.
- **Fibrillar collagen sponges** were used as a substrate for culturing human **kerotocyte,** epithelial and endothelial cells. This synergy promoted a wound healing in the eye.
- Biodegradable **chitosan–PEG hydrogel films** (CPHFs) and **chitosan-PCL blends** with excellent biocompatibility are enviable candidates as substrates for the regeneration and transplantation of CECs (corneal endothelial cells).
- The transplantation of **fibroblast precursors** on **gelatine hydrogel** into the corneal stroma may be a possible treatment for corneal stromal regeneration.
- Dual layer scaffolds were prepared from Silkworm (Bombyxmori) **silk fibroin** (SF) for corneolimbal reconstruction of diseased or damaged ocular surface.
- **Collagen and phospholipids** are being used to construct corneal implants that can be further **specifically functionalised using printing and laser profile techniques.**

MANY OTHER ORGANS/TISSUES

- …and so much more is yet to come!
- Future perspectives/challenges:
  - **Neovascularisation** is a highly desirable process for almost all of tissue-engineered products to survive.
  - There is an immediate need for the development of **angiogenic biodegradable materials** for tissue engineering. Currently, a number of strategies including the use of growth factors, stem cells and biomolecules (e.g. heparin) are being investigated
  - There has been significant interest to develop **smart functional materials** exhibiting conductive, magnetic and optical properties

ANY QUESTIONS?