Lecture 20

BT 636
Tissue Engineering and Regenerative Medicine
(3-0-0-6)

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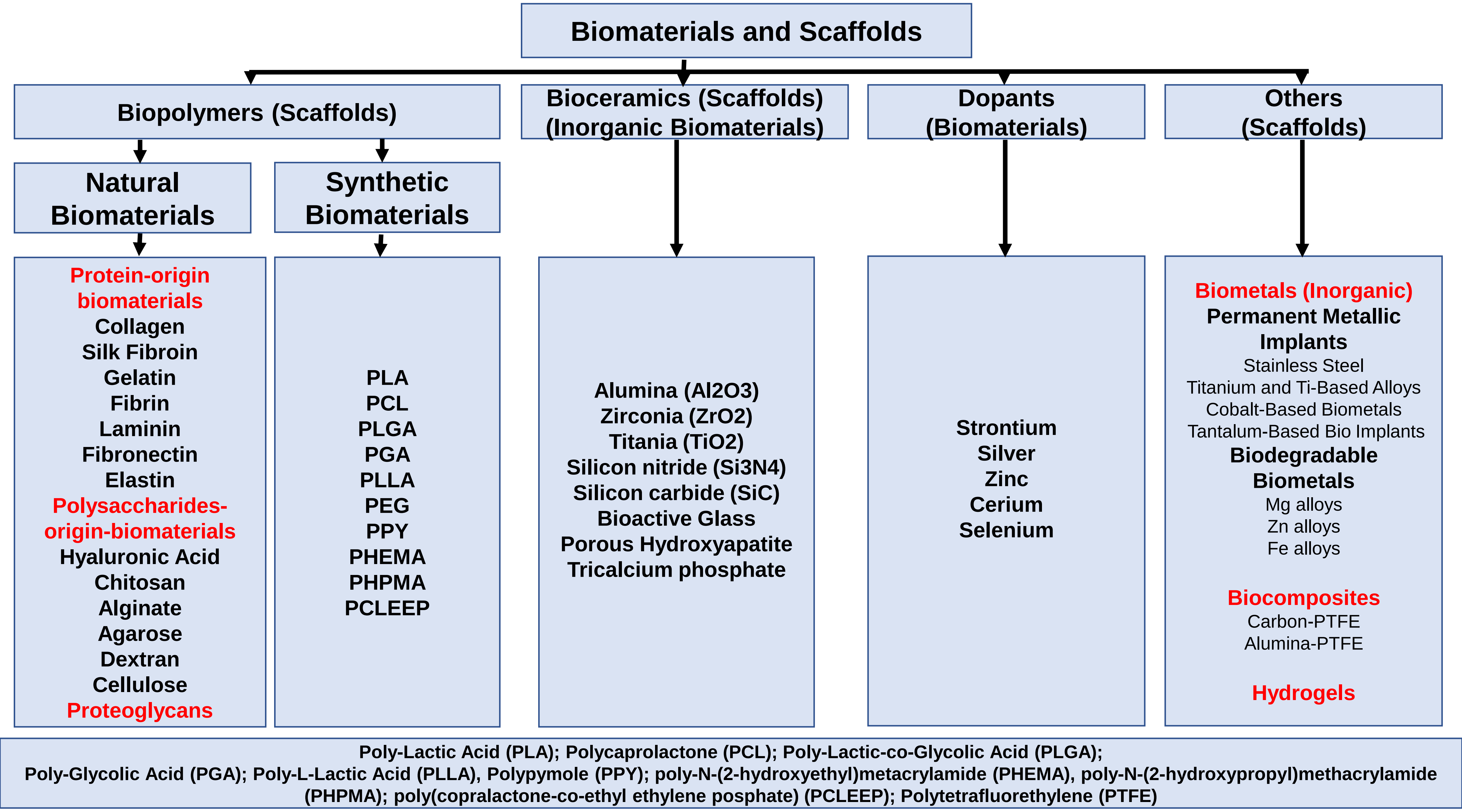

Biomaterials and Scaffolds
Biopolymers (Scaffolds)
Bioceramics (Scaffolds) (Inorganic Biomaterials)
Dopants (Biomaterials)
Others (Scaffolds)
Natural Biomaterials
Synthetic Biomaterials
Protein-origin biomaterials
Collagen
Silk Fibroin
Gelatin
Fibrin
Laminin
Fibronectin
Elastin
Polysaccharides-origin-biomaterials
Hyaluronic Acid
Chitosan
Alginate
Agarose
Dextran
Cellulose
Proteoglycans
PLA
PCL
PLGA
PGA
PLLA
PEG
PPY
PHEMA
PHPMA
PCLEEP
Alumina (Al2O3)
Zirconia (ZrO2)
Titania (TiO2)
Silicon nitride (Si3N4)
Silicon carbide (SiC)
Bioactive Glass
Porous Hydroxyapatite
Tricalcium phosphate
Strontium
Silver
Zinc
Cerium
Selenium
Biometals (Inorganic)
Permanent Metallic Implants
Stainless Steel
Titanium and Ti-Based Alloys
Cobalt-Based Biometals
Tantalum-Based Bio Implants
Biodegradable Biometals
Mg alloys
Zn alloys
Fe alloys
Biocomposites
Carbon-PTFE
Alumina-PTFE
Hydrogels
Poly-Lactic Acid (PLA); Polycaprolactone (PCL); Poly-Lactic-co-Glycolic Acid (PLGA);
Poly-Glycolic Acid (PGA); Poly-L-Lactic Acid (PLLA), Polypymole (PPY); poly-N-(2-hydroxyethyl)metacrylamide (PHEMA), poly-N-(2-hydroxypropyl)methacrylamide (PHPMA); poly(copralactone-co-ethyl ethylene posphate) (PCLEEP); Polytetrafluorethylene (PTFE)

Natural biopolymer(biomaterial)-based scaffolds

- Over the past couple of decades, the natural biopolymers have been gaining popularity as a primary component of medical materials. They are categorized into three different classes based on their structure and properties. Polypeptide- and protein-based, polysaccharide-based, and polynucleotide-based biomaterials.

  - **Polypeptide- and protein-based:** collagen, fibrin, fibrinogen, gelatin, silk, elastin, myosin, keratin, and actin.
  - **Polysaccharide-based:** chitin, chitosan, alginate, hyaluronic acid, cellulose, agarose, dextran, and glycosaminoglycans.
  - **Proteo-glycan-based:**
  - **Polynucleotide-based:** DNA, linear plasmid DNA, and RNA.

Natural biopolymer(biomaterial)-based scaffolds

- The first biomaterials used, and that continues to be used today, had a natural origin and an identical or very similar nature to the tissue to be restored.
- The motivation of the use of natural biomaterials is that it would generate less rejection by the host cells as they are substances that they can recognize.
- In general, natural polymers promote excellent cell adhesion and growth.
- Not only matrices based on generic mixtures of ECM substances have been used (Bellamkonda et al., 1995) but also scaffolds of purified ECM molecules like collagen (Bozkurt et al., 2007) or other proteins (Shadforth et al., 2012), hyaluronic acid (Austin et al., 2012), and some polysaccharides (Mammadov et al., 2012).
- Scaffolds are defined as three-dimensional porous stable biomaterials designed to have some or all of these characteristics: promote cell-biomaterial interactions, cell adhesion and ECM deposition, allow the exchange of oxygen, nutrients, and factors, be biodegradable, and non-cytotoxic.
- However, although biomaterials of natural origin can be manufactured in a multitude formats such as in situ gellable solutions, scaffolds, fibers or tubular conduits, the truth is that their mechanical properties are poor and many times they biodegrade too quickly to allow adequate support (Tarun et al., 2011).

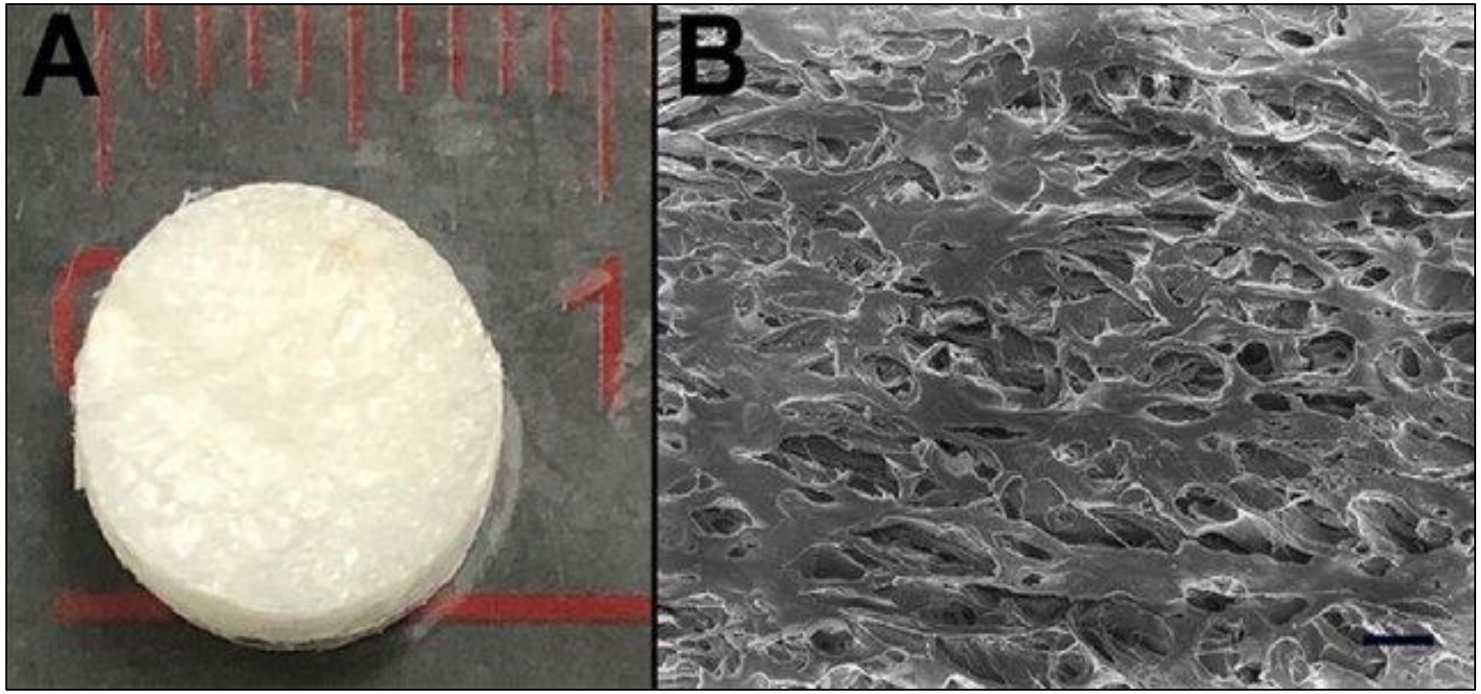
Natural biopolymer(biomaterial)-based scaffolds

- Natural biopolymer biomaterials are composed of long chains, which are usually composed of multiple amino acids, nucleotides, and monosaccharides. These materials are commonly used in various medical applications due to their properties. They can also be used to achieve biomimetic and natural restructuring.
- The various properties of natural polymers such as their bioactivity, 3D geometry, and non-toxic properties are some of the factors that are considered when it comes to their use in medical applications.
- Their various disadvantages include their poor mechanical strength, decreased tunability, and immunogenic reaction. These factors prevent the natural polymers from being used directly in hard tissue regeneration.
- Natural polymers are also useful in the advancement of medical devices, such as scaffolds for the delivery of therapeutic agents. Polymers that are novel and natural are also being used in the development of various medical devices that are designed to enhance the effectiveness of their applications. Due to their properties, natural polymers have been identified as promising materials for the biomedical industry.
- Some of these include collagen, chitosan, and gelatin. Fibrin, proteoglycan, and laminin are also expected to be utilized in the development of medical devices.

Natural biopolymer(biomaterial)-based scaffolds

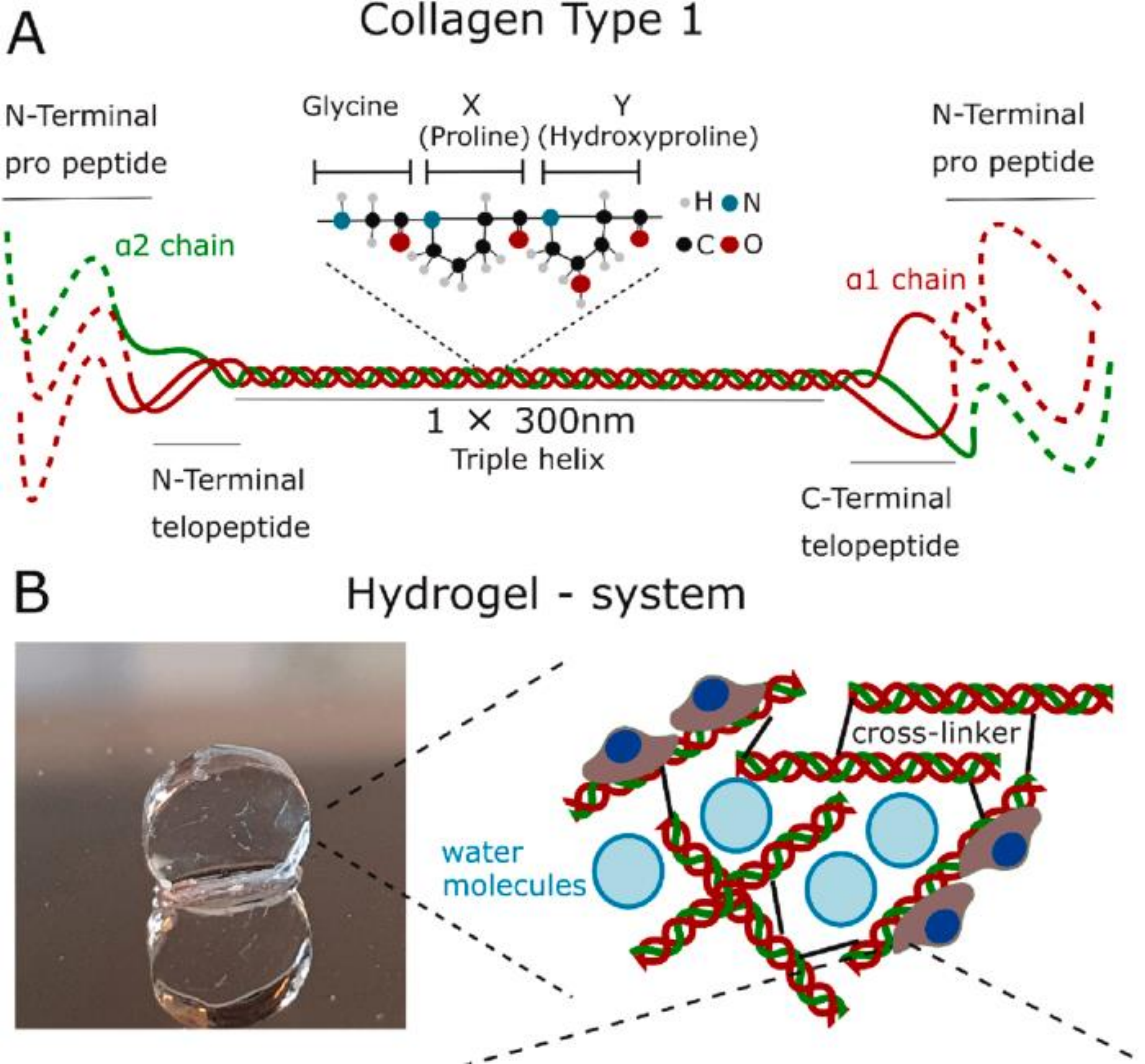
- **Collagen**

- The fibrillary type of collagen, the primary element of the connective tissues, is the most significant of the proteins found in the human body. It supports and structures the entire body, including the bones, tendons, skin, ligaments, nerves, and cartilage.



Prepared collagen scaffold. The pure collagen scaffold was prepared as a round disk (a). SEM images of the morphology of the collagen scaffolds (b). Scale bar = 100 μm

Despite collagen being largely responsible for the tensile properties of native connective tissues, collagen hydrogels have relatively low mechanical properties in the absence of covalent cross-linking. This is particularly problematic when attempting to regenerate stiffer and stronger native tissues such as bone.

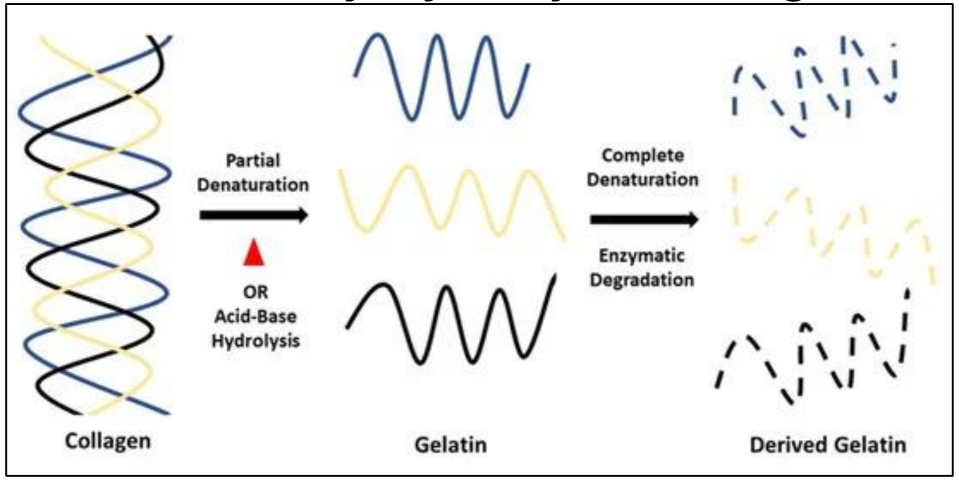


A. Type 1 collagen structure shows the triple helix (made up of two α1 and one α2 chains) and the N- and C-terminal telo- and pro-peptides of a collagen type I molecule.
B. Collagen type I is gelled using various methods to form a hydrogel system. Cross-linking is carried out by taking advantage of collagen's free amine and carboxyl groups. Example of collagen–cell interaction through GFOGER-integrin ligation.

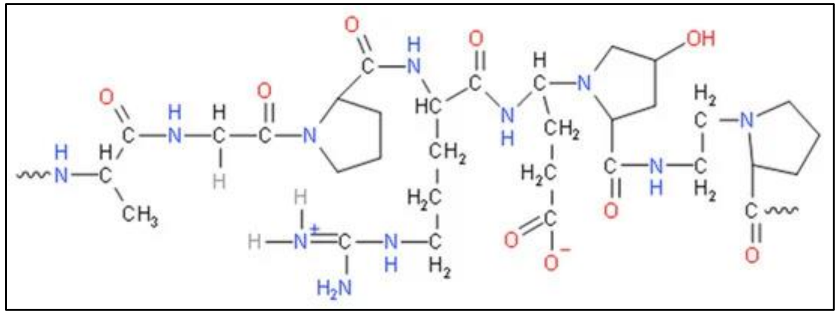
Natural biopolymer(biomaterial)-based scaffolds

➢ Gelatin

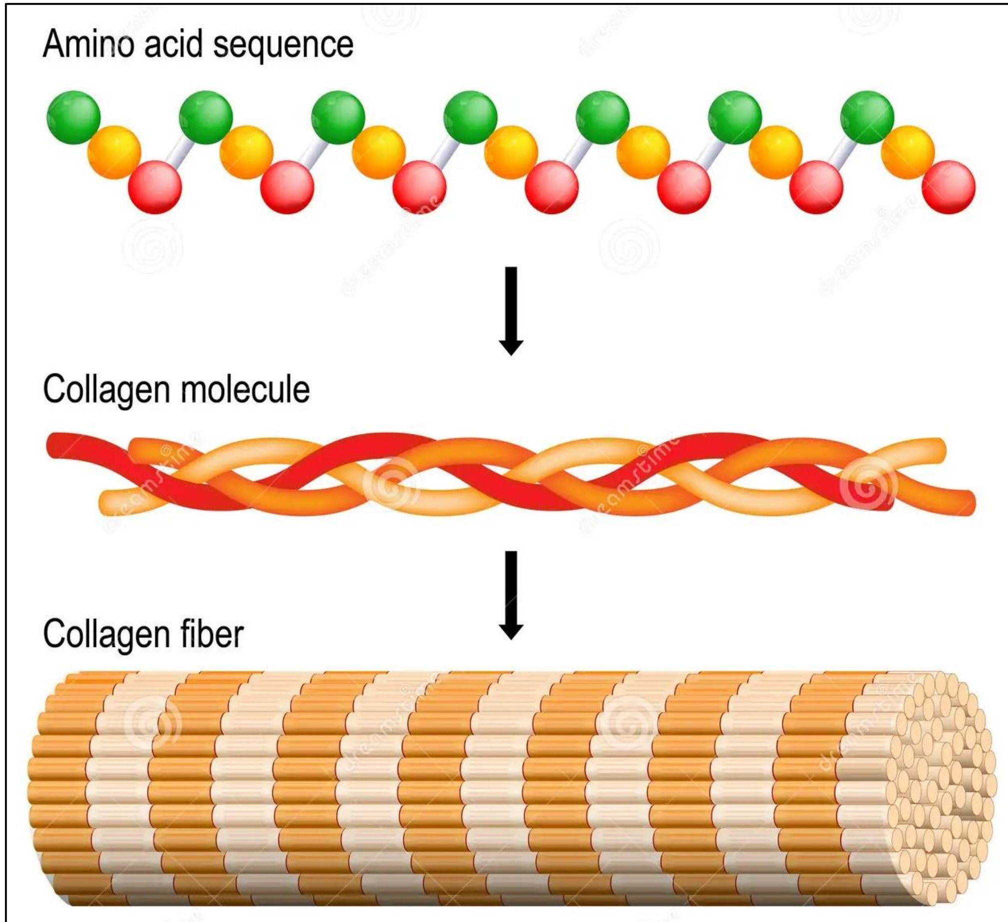
- Gelatin is a denatured protein obtained by hydrolysis of animal collagen. Gelatin is a polymer made up of skin-derived collagen, white connective tissues, and animal bones that have been partially hydrolyzed and generated by employing heat and enzymatic denaturation to irreversibly hydrolyze collagen’s triple helical structure, resulting in random coiled domains.



Process of denaturation of collagen into gelatin.



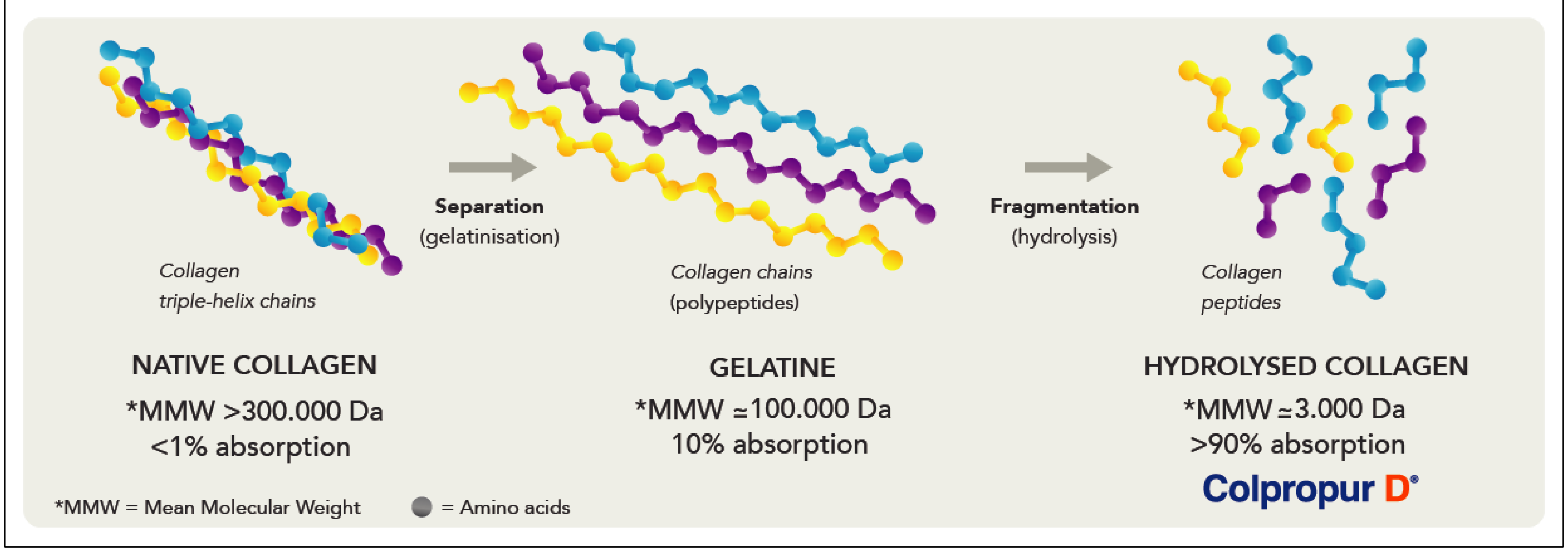
The chemical structure of gelatin.



Natural biopolymer(biomaterial)-based scaffolds

Gelatin

- Gelatin is a hydrolyzed collagen that has been receiving more attention recently in terms of tissue engineering systems. This is due to the fat that although it is biocompatible and biodegradable, in comparison to collagen it is more cost effective and has a lower antigenicity.
- As with many other polymers, gelatin is often chemically modified or combined with other natural or synthetic polymers in order to optimize its characteristics for the specific system.
- The method by which gelatin is obtained from collagen; either through acid or base treatment which leads to the hydrolysis results in either type A or type B gelatin, respectively.
- Each type of gelatin will have different characteristics such as gel strength, isoelectric point and charge. For example, the isoelectric point of type A gelatin is between 8 and 9, where it is between 4.8 and 5.4 for type B.
- Gelatin is soluble in hot water, which gives it improved stability and therefore bioavailability when it is used within the body.
- Researchers are also able to alter the strength of a gelatin systems using various crosslinking methods, ranging from chemical (such as genipin and glutaraldehyde) to physical (such as UV radiation).
- Gelatin systems composed for nanofibers have been successfully investigated for tissue engineering purposes for a number of ocular tissues.

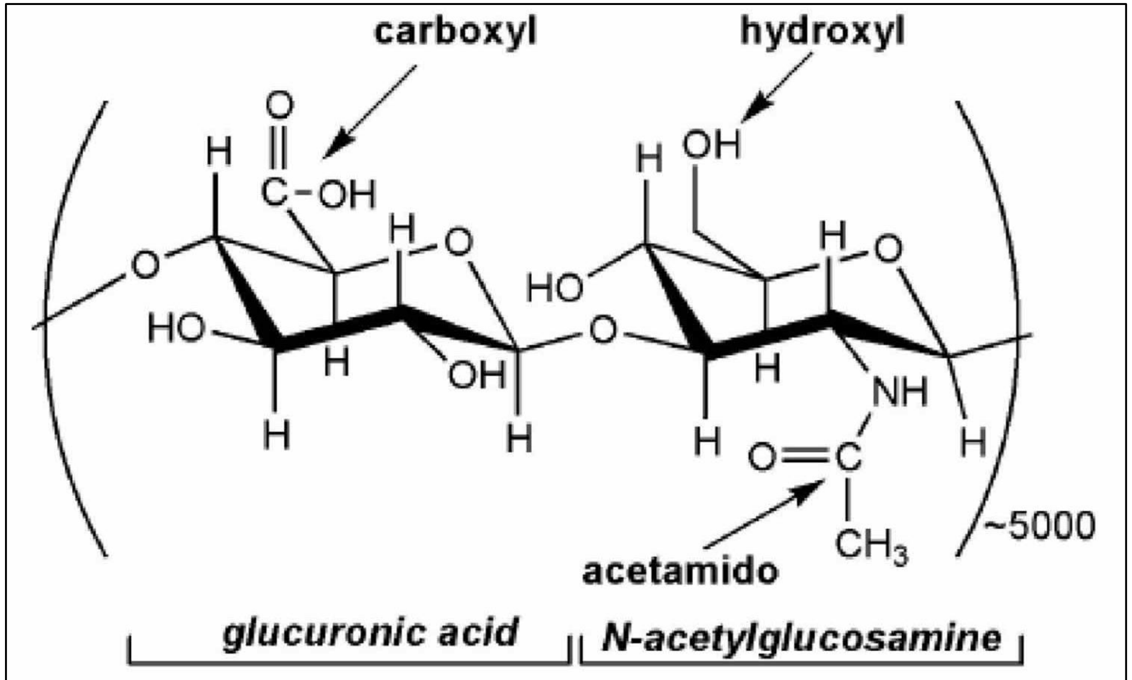

Separation
(gelatinisation)
Fragmentation
(hydrolysis)
Collagen
triple-helix chains
Collagen chains
(polypeptides)
Collagen
peptides
NATIVE COLLAGEN
*MMW >300.000 Da
<1% absorption
GELATINE
*MMW ≃100.000 Da
10% absorption
HYDROLYSED COLLAGEN
*MMW ≃3.000 Da
>90% absorption
Colpropur D®
*MMW = Mean Molecular Weight
= Amino acids

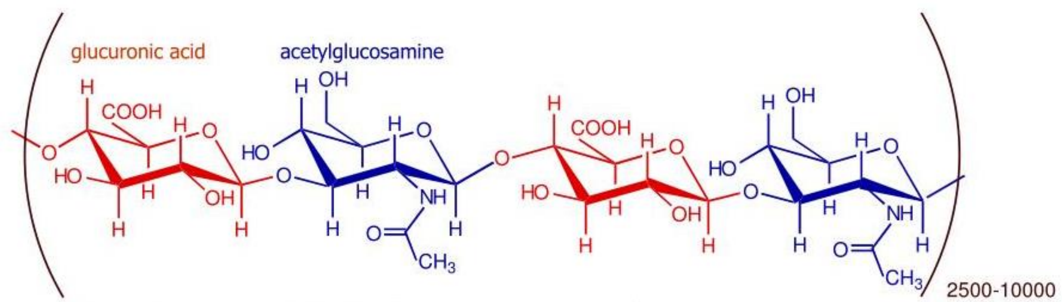

COLLAGEN
HYDROLYSATE
COLLAGEN
JOINT CARE
COLLAGEN DIETARY SUPPLEMENT
Net weight 16 oz (1 LB) 454 grams
UNFLAVORED
GELATIN
COLLAGEN
JOINT
CARE
Gelatin Dietary Supplement
NET WEIGHT 16 OZ. (1 LB.) 454 grams

Property	Collagen	Gelatin
Origin	Animals/humans	Col from bones/skin
Precursor	Fibroblast	Col type I
Physical characteristics	Elastic, tough and versatile structural protein	Smooth and gel like substances
Number of Amino acids	Approximately 1050	Less than 20
Structure of peptide	Triple helix of polypeptide chain	Small peptides
Types	Fibril-forming and nonfibril forming	A and B
Aromatic radicals	Present	Absent
Solubility	NaCI solution/dilute acid	H_2O
Mechanical strength	Poor	Poor
Antigenic response	Possible, in case of crosslinking/integration with antibacterial agent	Impossible, Because of its hydrophilicity nature.
Digestion	Difficult	Easy
Protease	Resistant	Susceptible
Gelling properties	No	Yes
In vitro degradation	Serine protease, pepsin-cleaving enzyme, gelatinease and collagenase	Collagenase
In vivo degradation	Endopeptidase	MMP-2 and MMP-9
Disease transmission	Xenozoonoses if the Col is impure	Not encountered
Usage	Burns, hemostasis, tissue defects, regeneration of nerves, wound dressings, augmentation of soft tissue, artificial dermis skin replacement	Adhesive of soft tissues, artificial skin, regeneration of nerves, wound dressings

Natural biopolymer(biomaterial)-based scaffolds

- **Hyaluronic Acid**
- Hyaluronic acid (HA) is an unbranched glycosaminoglycan.
- HA is present naturally in all vertebrates and is a significant constituent of the ECM in many parts of the human body.
- This material has gained significant interest in the area of tissue engineering, supporting cell growth.
- HA can be used in combination with other natural polymers to promote regeneration, like collagen and chitosan and also with synthetic polymers.





- Natural ECM component
- Easily produced in large quantities
- Non-immunogenic
- Multiple sites available for modification
- Enzymatically degradable

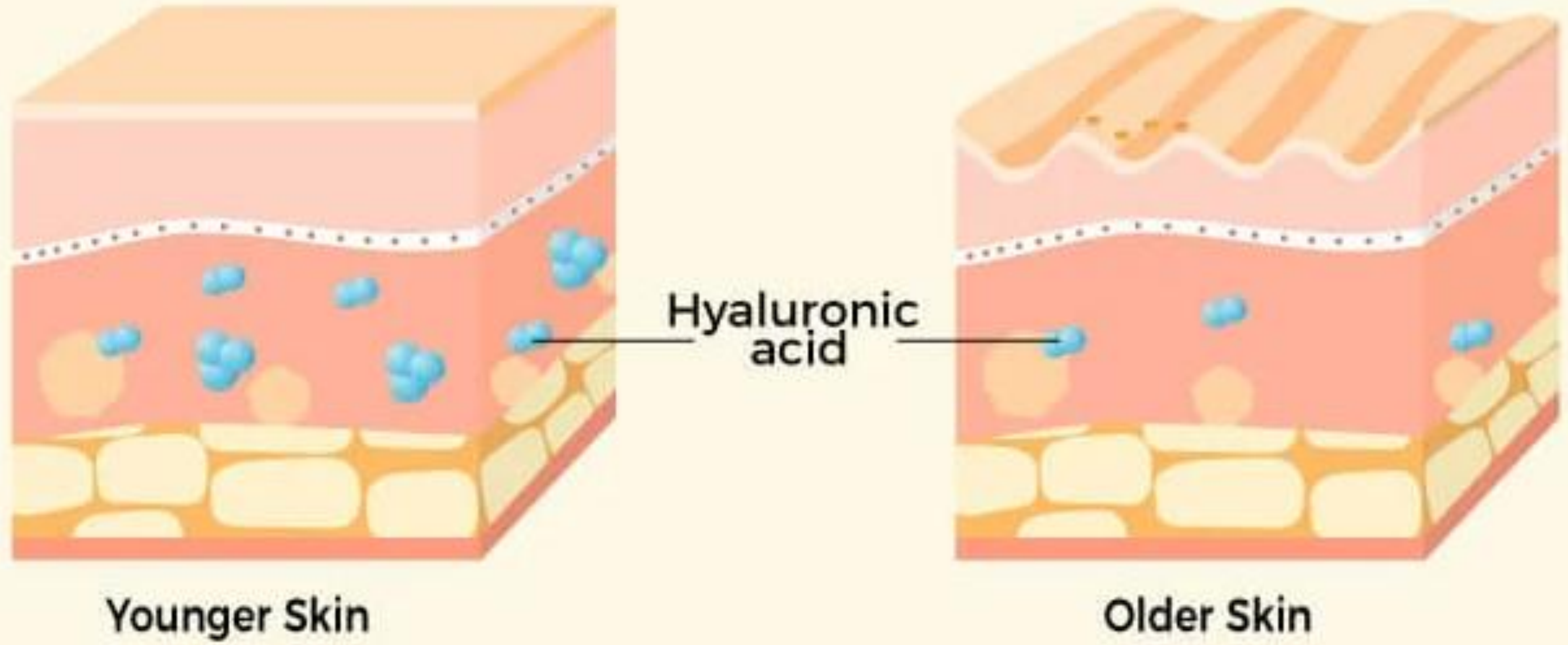
Natural biopolymer(biomaterial)-based scaffolds

- **Hyaluronic Acid**
- Hyaluronic acid (HA), an anionic non-sulfated glycosaminoglycan also referred to as hyaluronan, is ideal for use in tissue engineering systems as it is naturally found within many connective tissues, specifically within the extracellular matrix.
- HA is also known to be found within synovial fluid, the vitreous of the eye, the nervous system and the skin. This highlights its biocompatibility and biodegradability. Other biological properties attributed to HA include anti-inflammation non-immunogenicity.
- The chemical structure of HA, including a variety of functional groups (carboxyl, hydroxyl and amide groups) allow for it to be easily modified to comply with the requirements of a specific tissue. HA is able to interact with a number of different cells in order to affect aggregation, proliferation and migration.
- Although HA has many beneficial properties, it does have some disadvantages such as its rapid degradation and poor mechanical strength. For this reason, many systems which incorporate HA often require chemical modifications or crosslinking in order to overcome these challenges.
- This biopolymer is able to be formulated into a number of different systems including hydrogels and meshes.

Natural biopolymer(biomaterial)-based scaffolds

➢ Hyaluronic Acid

- The most used natural biomaterial is probably the hyaluronic acid, which is a common anti-aging production skin-care products and injectable facial fillers.



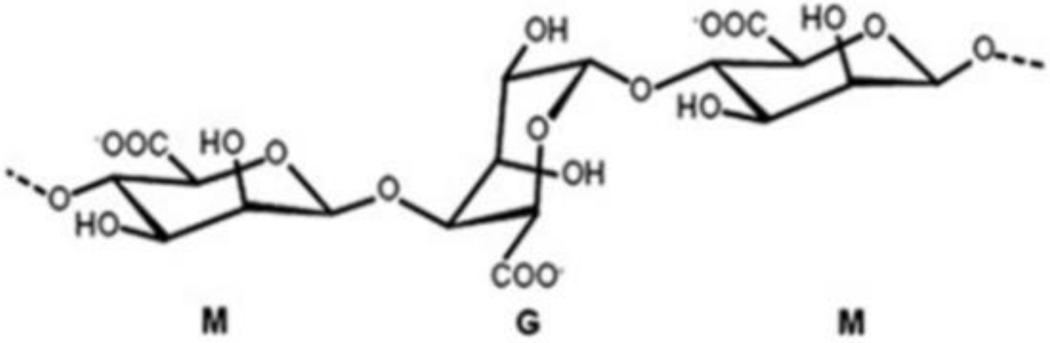
Hyaluronic acid is a naturally occurring substance in the body that helps maintain tissue hydration and structure. It is a glycosaminoglycan with the ability to hold onto water molecules, making it an effective ingredient in skincare products for improving hydration, reducing fine lines and wrinkles, and promoting a smoother complexion.

Natural biopolymer(biomaterial)-based scaffolds

➢ Alginate

- ❑ Alginate is a naturally derived, linear polysaccharide obtained from brown algae and bacteria.
- ❑ Alginate is a biosynthesized anionic biopolymer derived from brown seaweed. Alginate is made up of blocks of (1–4) monomers bound together by b-Dmannuronic acid (M) and a-Lg-uluronic acid (G).
- ❑ A bioactive and biodegradable hydrogel with alginate/gelatin was used as a bio-ink in 3D printing for tissue engineering applications.

Brown algae



Alginate

Natural biopolymer(biomaterial)-based scaffolds

- **Alginate**

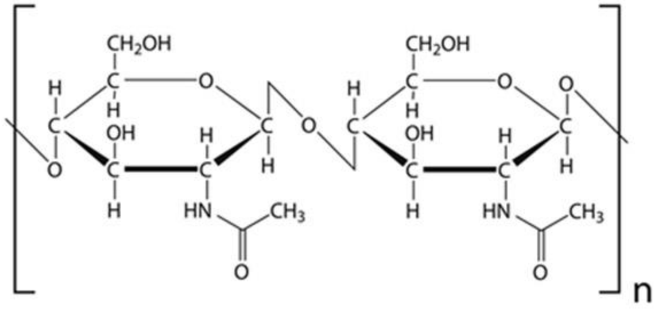
- Alginates, a group of natural polymers derived from algae and bacteria, are a favorable option for use in tissue engineering systems because they have properties which mimic the extracellular matrix within tissues.
- Alginate presents with a highly beneficial biodegradability and biocompatibility profile as well as other useful properties such as mucoadhesion.
- In addition to being negatively charged, alginates possess several beneficial properties; favorable solubility profiles, suitable porosity and shear-thinning capabilities. However, this biopolymer and its derivatives are also known to have poor mechanical strength and biological stability. These shortfalls can be overcome by crosslinking (using either calcium, barium, magnesium or strontium ions) which increases alginates ability to form a gel or by oxidizing the alginate.
- Sodium alginate, along with other natural polymers, have been formulated into a bioink designed to be used for 3D bioprinting in articular cartilage tissue engineering.
- The researchers found that the mechanical strength of the system was improved by the addition of collagen or agarose (when compared to the system with sodium alginate on its own).
- Alginate has often been used in combination with other biomaterials.

Natural biopolymer(biomaterial)-based scaffolds

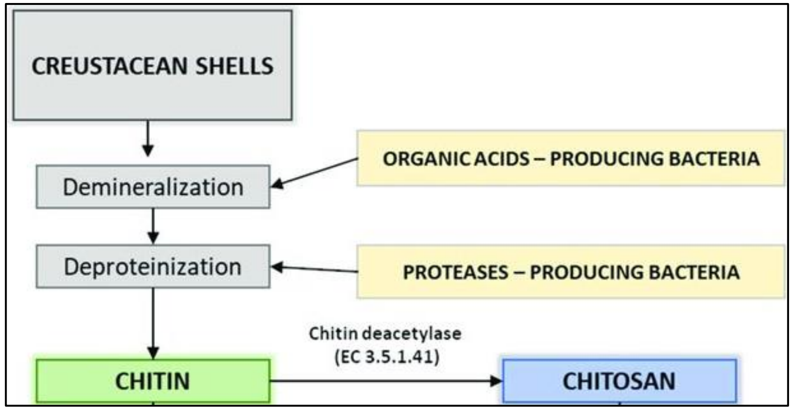
➢ Chitosan

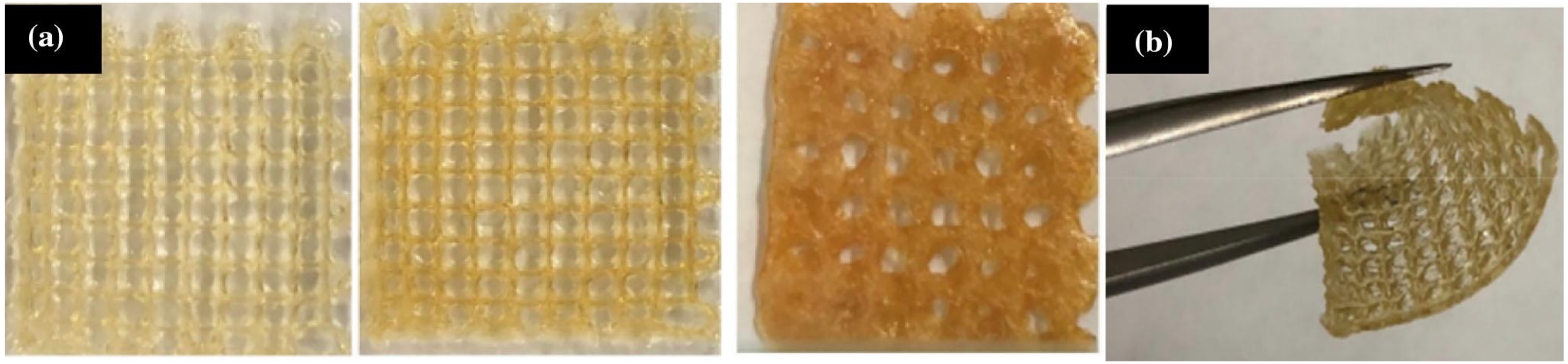
- ❑ Chitosan is a linear polysaccharide derived by the chemical deacetylation of chitin, structural polysaccharide naturally found in crustaceans and shellfish.
- ❑ Chitosan has several intriguing features, such as the gel-forming ability, increased adsorption capacity, superior biodegradability, excellent biocompatibility, and non-cytotoxicity, as well as improved biological qualities like antifungal, antibacterial, and antitumour activity.

Shrimp, crab and squid



Chitin





A 3D-printed chitosan scaffolds at 8%, 10% and 12% (w/v).

B Flexible chitosan scaffold.

Natural biopolymer(biomaterial)-based scaffolds

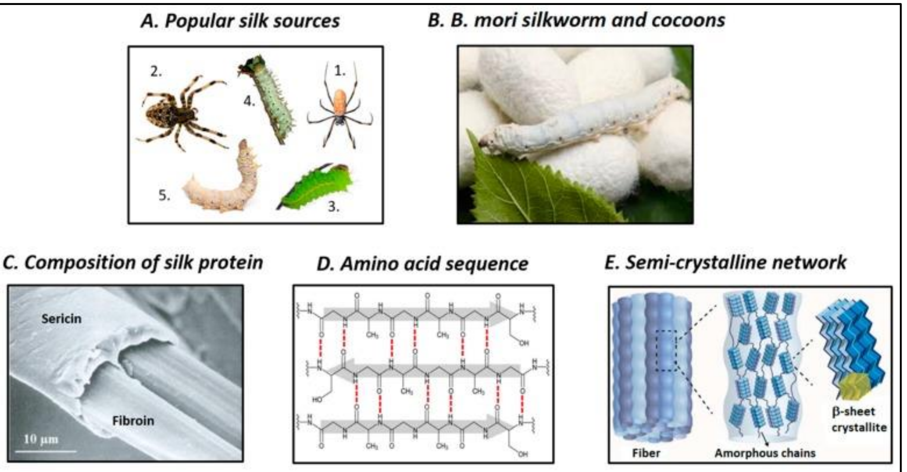
- **Chitosan**

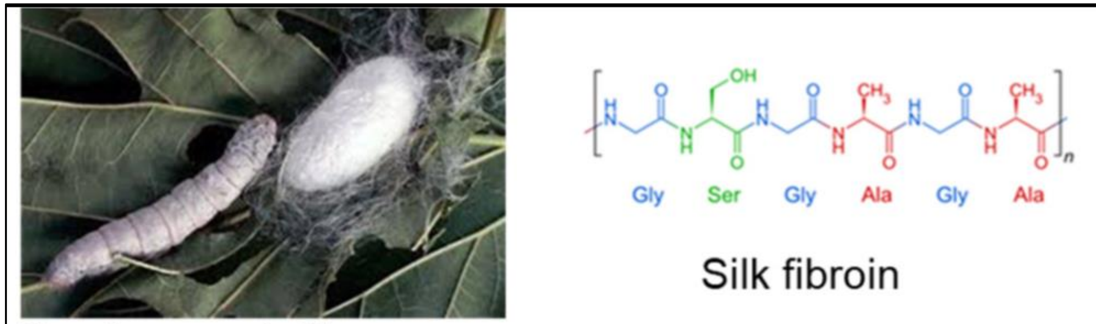
- Chitosan, a derivative of chitin, is one of the most abundantly available biomaterials for application in a variety of fields, including drug delivery and tissue engineering. It is able to lends itself to so many applications because it is biocompatible and biodegradable, offers mechanical strength to a formulation and is a cost-effective material.
- This natural polymer is an amino polysaccharide which is derived from chitin by de-acetylation.
- Factors such as the degree of de-acetylation and the molecular weight have an impact on chitosan’s physicochemical properties.
- In addition to being biocompatible and biodegradable, it has antimicrobial, antioxidant and haemostatic properties.
- Chitosan is favourable for application in tissue engineering as it provides a mechanical structure which closely mimics the extra-cellular matrix. Researchers are also able to modify both the pore size within the scaffold as well as the rate at which it degrades, allowing for adequate tissue integration within the scaffold. It is for these reasons that chitosan, both on its own and in conjunction with other biomaterials, has been formulated for the engineering of a variety of different tissues.

Natural biopolymer(biomaterial)-based scaffolds

Silk Fibroin

- Fibroin is an insoluble protein present in silk produced by numerous insects, such as the larvae of Bombyx mori, and other moths.
- Silk fibroin is considered a β-keratin related to proteins that form hair, skin, nails and connective tissues.
- The silk worm produces fibroin with three chains, the light, heavy, and the glycoprotein P25.
- The heavy fibroin protein consists of layers of antiparallel beta sheets. Its primary structure mainly consists of the recurrent amino acid sequence (Gly-Ser-Gly-Ala-Gly-Ala)n. The high glycine (and, to a lesser extent, alanine) content allows for tight packing of the sheets, which contributes to silk's rigid structure and tensile strength.
- The raw silk consists of two parallel fibroin fibers held together with a layer of sericin on their surfaces. Upon degumming the raw silk to remove the sericin, the obtained fibroin fibers appear shiny and feel soft to the touch, and are highly sought after in the textile industry. Moreover, the fibroin fibers are endowed with a combination of attractive strength, toughness, biocompatibility/biodegradability and thermal stability, representing one of the most impressive natural protein fibers, with properties that surpass those of many synthetic and natural fibers, and therefore, makes it a material with applications in several areas, including biomedicine and textile manufacture.





Bombyx mori silk cocoon

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6960760/

Natural biopolymer(biomaterial)-based scaffolds

- **Dextran**

- One of the sources used to obtain biopolymers is bacteria. Dextran, for example, is formulated from sucrose by lactic-acid bacterial species such as Leuconostoc mesenteroides.
- It has properties which lends it to use in pharmaceutical applications; it is highly water soluble, biocompatible, biodegradable and does not illicit an immune response when placed into the body. This makes dextran favourable when considering materials for tissue engineering scaffolds.

Natural biopolymer(biomaterial)-based scaffolds

TABLE 1 | Advantages and disadvantages of natural biomaterials.

Material	Advantages	Disadvantages	References
Collagen	Versatility, low antigenicity, inflammatory and cytotoxic response, biocompatibility, good water uptake capabilities, availability of several isolation methods, ability to tailor mechanical and cross-linking properties.	Weak mechanical and structural stability upon uptake of water.	Archibald et al., 1995; Kiyotani et al., 1996; Cao et al., 2011; Kehoe et al., 2012; Sherman et al., 2015; Gonzalez-Perez et al., 2017; Yao et al., 2018b
Gelatin	Biocompatibility, biodegradability, low antigenicity, good cell recognition.	Poor mechanical properties.	Gupta et al., 2009; Alvarez-Perez et al., 2010; Kriebel et al., 2017; Naseri-Nosar et al., 2017
Hyaluronic acid	Good biocompatibility, high water content, safe degraded products, limited immunogenicity, viscoelastic properties and ability to influence wound healing, metastasis etc.	Non-adherence of cells and water solubility.	Horn et al., 2007; Zhang et al., 2008; Suri and Schmidt, 2010; Wang et al., 2011; Liang et al., 2013; Thomas et al., 2017; Li et al., 2018
Alginate	High biocompatibility, high biodegradability, non-antigenicity, and chelating property.	Unstable mechanical properties and lack of the specific cell-recognition signals.	Suzuki et al., 1999; Hashimoto et al., 2002; Ansari et al., 2017; Liu et al., 2017; Wang et al., 2017b; Sitoci-Ficici et al., 2018
Chitosan	Biocompatibility, biodegradability, non-toxicity, inhibition of growth of fungi, yeast, and bacteria, and non-immunogenicity.	Some forms of chitosan may be toxic.	Li et al., 2006, 2017a,b; Valmikinathan et al., 2012; Wang et al., 2017c

Natural biopolymer(biomaterial)-based scaffolds

Table 4. Examples of natural and synthetic biomaterials used in skin tissue engineering along with their pros and cons.

Types	Examples	Advantages	Disadvantages	Major Properties in Wound Healing
Natural biomaterials	Alginate [83,84]	Can retain its shape due to low viscosity and zero shear viscosity	Inert material and only suitable for in vitro assays, requires crosslinking due to low bioactivity	Porous, good absorption, biocompatible and biodegradable nature promote wound healing resulting in less scarring, minimal bacterial infection, and the creation of a moist wound environment
	Cellulose [85,86]	Flexibility in shape, easy processing, good mechanical strength, and biodegradability	Lack of solubility in water and many organic solvents	Hydrophilic nature, purity, ability to maintain appropriate moisture balance and flexibility form a tight barrier between the wound and the environment, preventing bacterial infections
	Chitosan [78,87]	Possess antibacterial, antifungal, mucoadhesive and analgesic property	Poorly soluble in aqueous solutions except for acidic medium	Interact with negatively charged molecules (protein, fatty acid, bile acid, polysaccharide, phospholipids); chelate metal ions (iron, copper, magnesium); stimulate hemostasis and accelerate tissue regeneration
	Collagen [9,78,88]	Suitable mechanical property and biocompatibility	Susceptible to crosslinking and any sterilization procedure	Triple helix conformation of collagen type 1 favour cell adhesion and migration; pore sizes for the 5 and 8 mg/mL collagen type I scaffolds ranged between 126–188 µm promote connective tissue regeneration
	Elastin [78,89]	High elasticity	Poor mechanical strength and availability	Half-life > 70 years and the monomer can reversibly stretch up to eight times its resting length; fibre alignment positively affects cell phenotype, adhesion, and proliferation
	Fibrin [78,90]	Good protein binding ability that promotes vascularization	Limited control over its structural and mechanical properties	Fibrin network serves as a provisional template for promoting cell migration and proliferation; releases cytokines and growth factors attracting inflammatory cells at the wound bed; activates re-epithelialization, angiogenesis, connective tissue formation and contraction
	Gelatin [2,82,91]	Low antigenicity and higher solubility in solvents	Lack high mechanical resistance	Porous gelatin matrices absorb wound exudates, maintain a moist environment essential for wound healing
	Silk fibroin [83,92]	Biocompatible with strong mechanical properties	High brittleness	Porous template supports cell proliferation, differentiation, and ECM production

polymers MDPI

Review

Synergistic Effect of Biomaterial and Stem Cell for Skin Tissue Engineering in Cutaneous Wound Healing: A Concise Review

Shaima Maliha Riha, Manira Maarof and Mh Busra Fauzi *

Polymers **2021**, *13*, 1546. https://doi.org/10.3390/polym13101546

Natural biopolymer(biomaterial)-based scaffolds

❑ Natural biopolymer(biomaterial)-based scaffolds

- Depending on the required property, polymer choice differs.

- For instance, fibrin shows minimal immunogenic reactions and is completely biodegradable; however, its poor mechanical strength limits its application in hard tissues.
- Alternatively, silk fibroin (SF) exhibits excellent mechanical properties, but having a slow rate of degradation presents a major concern in scaffold fabrication.
- In an attempt to overcome this, hydrogels using methacrylated SF, which possessed a vastly enhanced degradation rate as compared to non-modified SF.
- Switching to hyaluronic acid, its in vivo metabolites have angiogenic characteristics, which could come in handy in tissue engineering, particularly when biodegradability, biocompatibility, and non-immunogenicity are considered. However, its high viscosity and high-water retention render its processability troublesome.

Thank you for your attention