Lecture 21

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

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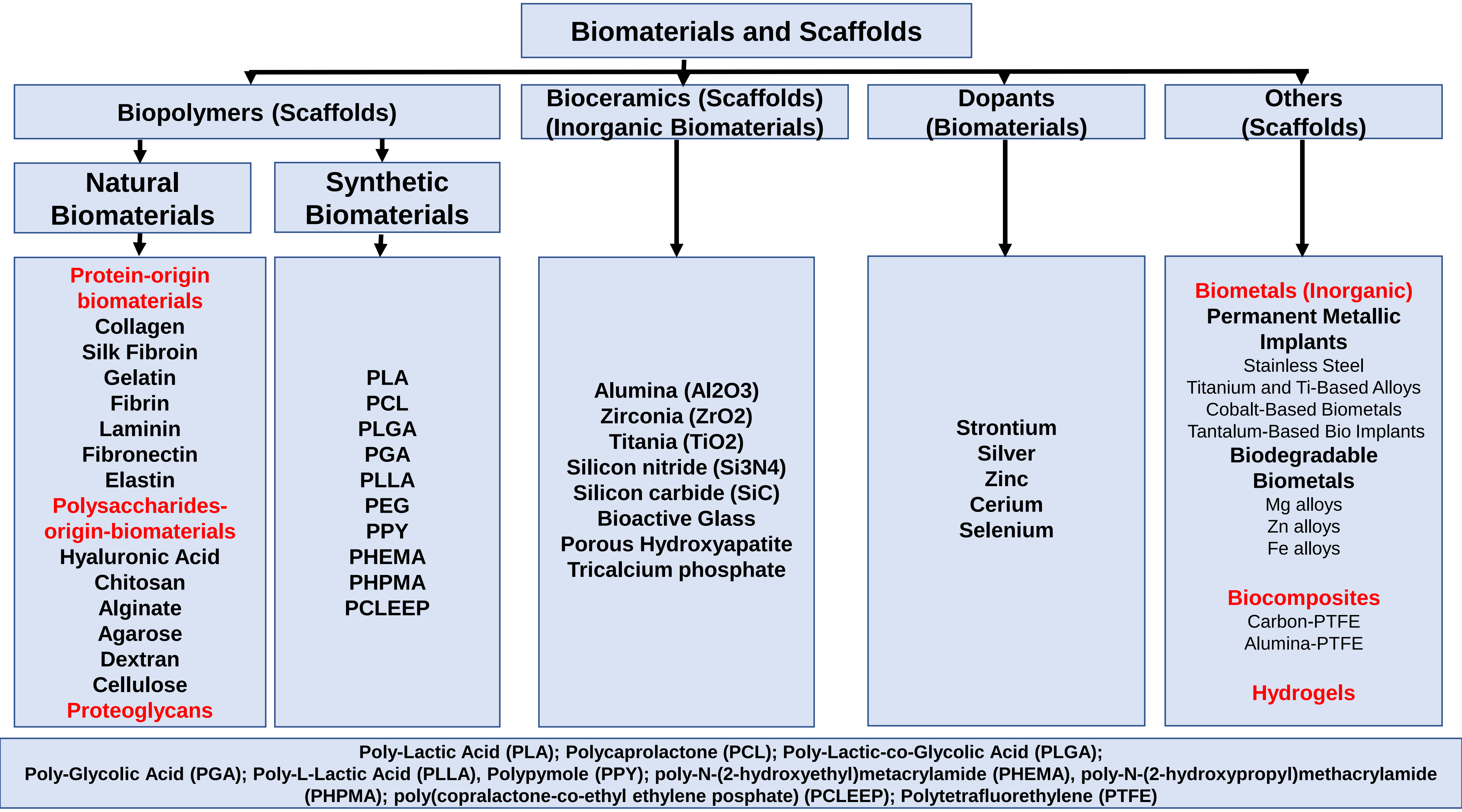
Biomaterials and Scaffolds
Biopolymers (Scaffolds)
Natural Biomaterials
Protein-origin biomaterials
Collagen
Silk Fibroin
Gelatin
Fibrin
Laminin
Fibronectin
Elastin
Polysaccharides-origin-biomaterials
Hyaluronic Acid
Chitosan
Alginate
Agarose
Dextran
Cellulose
Proteoglycans
Synthetic Biomaterials
PLA
PCL
PLGA
PGA
PLLA
PEG
PPY
PHEMA
PHPMA
PCLEEP
Bioceramics (Scaffolds) (Inorganic Biomaterials)
Alumina (Al2O3)
Zirconia (ZrO2)
Titania (TiO2)
Silicon nitride (Si3N4)
Silicon carbide (SiC)
Bioactive Glass
Porous Hydroxyapatite
Tricalcium phosphate
Dopants (Biomaterials)
Strontium
Silver
Zinc
Cerium
Selenium
Others (Scaffolds)
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)

Synthetic biopolymer(biomaterial)-based scaffolds

- **Synthetic biopolymer(biomaterial)-based scaffolds:**
- A synthetic polymer is beneficial having various features, such as its ability to **endure multiple forms, tunable properties, and established structures**.
- Biobased materials can be used to restore the function and structure of damaged tissues. They can also be used to treat various diseases and conditions. Due to the combination of various factors such as molecular weight, physical properties, and chemical features, synthetic polymers are easy to synthesize.
- Compared to natural materials, they can be **produced with a minimal effort**. Unfortunately, synthetic biomaterials that natural materials have. They require chemical modifications to improve their properties. Unfortunately, synthetic biomaterials **do not have the cell adhesion sites** that natural materials have.
- Polymers that are commonly used in the production of bio-based materials **exhibit similar mechanical and physical properties to biological tissues**. In terms of type, biodegradable polymers are considered to be the most common type of synthetic materials. The physical and mechanical properties of synthetic materials are expectable and reproducible.
- For example, strength, Young’s modulus, and degradation rate are all common characteristics.
- Degradable synthetic materials are commonly used for several applications, such as in the preparation of bio-based materials. Poly(a-hydroxy esters) along with PCL, PGA, PLA, and their copolymer PLGA and poly(-ethers) including PEO and PEG, PVA, and PU are the most extensively researched degradable synthetic materials, which are bestowed with characteristics like biodegradability and biocompatibility with mechanical strength which no single synthetic polymer can hold all of these properties at the same time.

Synthetic biopolymer(biomaterial)-based scaffolds

- **Synthetic biopolymer(biomaterial)-based scaffolds:**
- Perhaps the lack of predictability and poor processability of natural polymers held them back as biomaterials for tissue engineering.
- In fabricating scaffolds that mimic the complex 3D structure of the living tissues, synthetic polymers became the go-to option.
- This is mainly due to the flexibility by which they are processed, the variety of shapes they can be manipulated into, the ease by which their properties can be modified as well as the lack of immunogenic reactions.
- A key obstacle in using synthetic polymers is their poor biological activities and cellular affinity. Both are attributed to the lack of functional groups, which renders their modification a difficult task.
- Advancements in science introduced new synthetic polymers known as '**functional polymers**' to overcome the limitations of conventional synthetic polymers.
- Functional polymers have unsaturated bonds and/or functional groups in their structure that tailor them for different needs. Aliphatic polyesters such as polyglycolic acid (PGA), polylactide-co-glycolide (PLGA), polylactide (PLA), and poly(#-caprolactone) (PCL) represent the most used synthetic polymers in tissue engineering. Other synthetic polymers include polyhydroxybutyrate (PHB), poly (ester amide) (PEA), polyethylene glycol (PEG), polyurethanes (PU), and polyvinyl alcohol (PVA).

Synthetic biopolymer(biomaterial)-based scaffolds

- **Synthetic biopolymer(biomaterial)-based scaffolds:**
- For example, poly-+-caprolactone (PCL), poly-L-lactic acid (PLLA), poly-D,L-lactic-coglycolic acid (PLGA) among others (Sinis et al., 2005; Srinivasan et al., 2015).
- These materials seemed to make up for the shortcomings of natural biomaterials since they can be manufactured with a multitude of techniques and architectures adapted to the type of tissue to be regenerated.
- Furthermore, by choosing the proper polymer or its composition, it is possible to obtain greater control over biodegradability. A wide variety of formats can be obtained, such as aligned scaffolds, fibers or filaments, and tubular structures.
- However, they also have certain limitations, such as reduced bioactivity that increases the risk of rejection after implantation.
- Therefore, the choice of the origin of the biomaterials to be used is especially important, being widespread systems composed of combination of various types of biomaterials.

TABLE 2 | Advantages and disadvantages of synthetic biomaterials.

Material	Advantages	Disadvantages	References
Polycaprolactone (PCL)	Biodegradable, biocompatible, possesses high elasticity, low toxicity, good mechanical properties, and a slow degradation profile.	Cytotoxic effects on using organic solvents.	Flynn et al., 2003; Aurand et al., 2012; Nectow et al., 2012
Poly-L-lactic acid (PLLA)	Biodegradable, ultrafine continuous fibers, high surface-to-volume ratio, high porosity, varied distribution of pore size.	Poor biocompatibility, release of acidic products on degradation, poor process ability, and premature failure of mechanical features during degradation.	Ngo et al., 2003; Lu et al., 2009; Sun et al., 2012; Zhao et al., 2013; Li et al., 2015
Poly-D,L-lactic-co-glycolic acid (PLGA)	Biodegradability, non-toxicity, and film-forming ability.	Plastic deformation and failure on exposure to long-term strain, releases acidic products on degradation.	Xue et al., 2012; Malinovskaya et al., 2017; Mir et al., 2017
Polypyrrole (PPY)	Exhibits rigidity, good biocompatibility and cell adhesion properties, non-toxic, non-allergic, non-mutagenic, and non-haemolytic.	Insoluble, non-biodegradable, and poor process stability.	Lee et al., 2009
Carbon nanotubes (CNTs)	Superior conductivity, remarkable stiffness, high aspect ratio, maintains structural stability of scaffolds, biocompatibility, optimal nanotopography, and induces conductivity.	Cytotoxicity and non-biodegradability.	Malarkey et al., 2009

Synthetic biopolymer(biomaterial)-based scaffolds

- As mentioned earlier, synthetic polymers can be easily modified to alter their properties as needed.

- PHB, despite being difficult to process with its poor mechanical profile, is one of the most used polymers in scaffolds intended for bone tissue engineering. By adding 3% w/w alumina to the PHB-chitosan alloy solution, the produced scaffolds gained a 10-fold increase in the tensile strength compared to the plain alloy.
- In another study, the poor mechanical profile of PHB-gelatin nanofibers was significantly improved following the inclusion of collagen in the mix. PCL has several advantages in tissue engineering, including biocompatibility, ease of processability, and stability under normal conditions. However, due to its hydrophobicity and poor wettability, it exhibits poor cellular adhesion behavior.
- Air plasma treatment of PCL and PCL-hydroxyapatite (HAp) nanofibers improved their wettability and hydrophilicity, as indicated by the massive reduction in water contact angle. This, in turn, resulted in enhanced cell adhesion and proliferation.
- It can be concluded that advances in synthetic polymers and the introduction of functional polymers had them tailored for the construction of different scaffolds with vast physicochemical properties by tuning their structure, further strengthening the potential of synthetic polymers in tissue engineering.

Bioceramics and Bioactive Glass-based scaffolds

- **Bioceramics:**
- Bioceramics include a large group of inorganic biomaterials with suitable biocompatibility, excellent mechanical profiles, and high melting points, which render them suitable in orthopedic and dental tissue engineering. However, they are brittle and thus, cannot be used in load-bearing situations.
- To overcome this, they are usually combined with certain polymers.
- Ceramics can be categorized based on their tissue response into; bioinert ceramics such as alumina and zirconia, bioactive ceramics such as glass ceramics, and finally, biodegradable ceramics such as calcium phosphates.
- HAp and beta-tricalcium phosphate (-TCP) are the most used calcium phosphates owing to their excellent osteoconductivity and biocompatibility. While bioinert ceramics cannot create stable bonds with the tissue, both bioactive and biodegradable ceramics allow bond formation, with biodegradable ones having the added benefit of degrading over time as they are being replaced by the newly formed bone tissue. One parameter is of great value when choosing calcium phosphates is the calcium: phosphate ratio (Ca: P). Ceramics with Ca: P < 1 are not biologically favored, while those with Ca: P > 1.67 tend to resorb slowly.
- The merging of nanotechnology with the use of Zn-HAp ceramics for the drug delivery of doxorubicin was proven successful in targeting post-operative cancer tissues. The ceramic implant (Zn-HAp) ensured the targeting part while the incorporated drugloaded nanoparticles enabled the enhanced drug release as well as boosted drug uptake kinetics. Overall, Zn-HAp ceramic showed excellent results against MG-63 osteosarcoma cell lines.
- One way to improve the osteogenic abilities of bioceramics is to include silica ions in their construction. Silica ions improve osteogenesis through activation of multiple gene transduction pathways, which imparts osteoinductive effect to the bioceramic.

Bioceramics and Bioactive Glass-based scaffolds

- **Bioactive Glass:**
- The combination of silica ions with calcium and phosphate to improve silica reabsorption is known as ‘bioactive glass’.
- Bioactive glasses can bind strongly to bone tissue and, upon exposure to physiological conditions, they form a superficial HAp layer on the target site and induce bone tissue regeneration.
- Bioactive glass can be manufactured using either the melt quenching technique or the sol-gel transition method.
- Many bioactive glasses are available with large variations in their mechanical strength. For example, 45S5 Bioglass® is brittle while CEL2 and SCNA were optimized to reach much higher compressive strength values at 5–6 MPa and 15 MPs, respectively.
- Attempts to enhance the mechanical strength and biological properties of bioglass were made by doping several metal ions individually or in combination into the bioglass.
- Metals used include strontium, iron, manganese, etc. These dopants can be selected according to the body minimum requirements of such elements. This can assist in the repair process.

Table 1. Examples of biomaterials used in tissue engineering highlighting key advantages

Biomaterial	Category	Advantages	Disadvantages
PLA	Synthetic Polymer, Polyester	• Easily biodegradable with nontoxic byproducts • Suitable mechanical properties • Biocompatible	• Hydrophobic with poor cell attachment • Lack of thermal stability, degrades above 200 °C
PLGA	Synthetic Polymer, Polyester	• Controllable biodegradability • Biodegradable with faster degradation rate than PLA and PGA	• Poor osteoconductivity • Suboptimal mechanical strength
PEG	Synthetic Polymer, Polyol	• Low immunogenicity and antigenicity • Easily modifiable • Biocompatible, rapidly cleared	• Bioinert • Non-biodegradable
HAp	Ceramic, Biodegradable	• Excellent resemblance to the natural HAp • Osteoconductive activity • Biocompatible and bioresorbable • Suitable carrier for growth factors and osteoblasts	• Brittle • Poor mechanical strength
Zirconia	Ceramic, Bioinert	• High fracture toughness • Biocompatible • Osteoconductive	• Undergoes spontaneous transformation to the monoclinic phase causing surface instability and microcracking

Table 1. Examples of biomaterials used in tissue engineering highlighting key advantages

Biomaterial	Category	Advantages	Disadvantages
Mg	Metal, Biodegradable	• High tensile strength • Lightweight implant • Comparable elastic modulus to that of bones	• Excessive corrosion in biological fluid • Release Mg ions on corrosion causing premature implant failure
Ta	Metal, Non-biodegradable	• Exceptional corrosion resistance • Biocompatible • Enhance osseointegration	• High elastic modulus • High melting point, difficult to process
Collagen	Natural Polymer, Polypeptides	• Rough surface • Low immunogenicity • Low toxicity	• Susceptible to contraction and deformation • Unstable in aqueous surroundings
Gelatin	Natural Polymer, Polypeptides	• Lack of antigenicity • Easily accessible functional groups for surface modification • Its byproducts are nontoxic	• Poor mechanical stability and low elasticity under physiological conditions
Chitosan	Natural Polymer, Polysaccharides	• Anti-inflammatory, Antibacterial activities • Nontoxic • Enhances wound healing and tissue regeneration	• Low mechanical resistance • Unstable with uncontrollable dissolution
Hyaluronic acid	Natural Polymer, Polysaccharides	• Promotes wound healing and fibroblast proliferation • Bacteriostatic activity • Non-immunogenic, nontoxic	• Rapid in vivo degradation • High viscosity

Table 4. *Cont.*

Types	Examples	Advantages	Disadvantages	Major Properties in Wound Healing
Synthetic biomaterials	PCL [93,94]	Biocompatible with relatively slow degradation time	Poor cell attachment due to hydrophobicity	Show desirable electroactivity, biocompatibility, free radical scavenging capacity and antibacterial activity; promoted collagen deposition and granulation tissue thickness during the process of wound healing
	PEG [9,78,95]	Reasonable control over structural and compositional properties	Lacks interactive cell character	Demonstrate biocompatible property, protein resistance, non-immunogenicity, non-toxicity, and good water solubility required for chronic wound healing
	PGA [9,78,96]	Highly biocompatible and biodegradable	Rapid mechanical strength loss	Exhibit reasonable wetting time, preferable surface morphology, low moisture uptake and prolonged swelling behavior
	PHA [97–99]	Low acidity and bioactivity, nontoxic degradation, biocompatibility, and non-carcinogenicity	Poor mechanical properties, high production cost, limited functionalities, incompatibility with conventional thermal processing techniques	Structural porosity and wettability similar to natural ECM, effectively promoting cellular migration, attachment, and proliferation
	PLA [96,100]	Easy modification with other biomaterials and bioactive compounds	Poor cell interaction, low elongation, and hydrophobicity	Exhibit high mechanical properties, reasonable wetting time, preferable surface morphology, low moisture uptake, prolonged swelling behavior and strong antibacterial properties against *Staphylococcus aureus* and *Escherichia coli*
	PLGA [9,78,101]	Biocompatible and biodegradable with a wide range of erosion time	Generates adverse inflammatory reaction upon degradation	Exhibit cytocompatibility and facilitate cell adhesion, spreading and proliferation, release anti-inflammatory factors required for wound healing accelerate collagen deposition and re-epithelialization

polymers MDPI

Review

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

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

Clay minerals scaffolds

- Clay minerals refer to a class of materials belonging to the phyllosilicates and composed mainly of sheets of tetrahedral silicates and octahedral hydroxides blocks, ranging in size from nano to micro range and exhibiting permanent surface charge. Those silicate sheets are fixed together via many sorts of interactions, and, accordingly, clay minerals can be classified into several classes. In the case where the clay is composed of one tetrahedral sheet and one octahedral sheet, we refer to classes such as kaolinites and serpentine, while if the sheets are one octahedral and two tetrahedral, we have groups such as smectites, chlorite, vermiculite, bentonite, and hectorite.
- The combination of clay minerals and nanotechnology is visualized in layered double hydroxides nanoparticles (LDHs). LDHs are two-dimensional (2D) hydrotalcites with particle sizes up to 100 nm with an overall positive charge under acidic pH. LDHs have many advantages that attracted much research over the past few years. These include biocompatibility, biodegradability, low toxicity, high drug loading capacity, controlling drug release, anionic exchange properties, and antibacterial activity. In a study performed by Li et al., the antibacterial activity of penicillin G was prolonged when prepared as penicillin G-loaded Zn-Al LDHs.
- Another example is halloysite nanotubes (HNTs) fabricated from the halloysite of the kaolinite group. Porous nanocomposite scaffolds composed of agarose, gelatin, chitosan, and HNTs were prepared via freeze-drying and doped with allogenic mesenchymal stem cells (MSCs). When tested for the repair of a dog bone defect, the added HNTs were found to impart osteoinductive effect to the prepared scaffolds.

Dopants used in tissue engineering

- Biocompatible materials are vital for the expansion of tissue substitutes, which are needed for the treatment of diseases.
- To be effective, biocompatible materials should have similar properties to native tissues.
- They should also be able to imitate the biological, chemical, and physical properties of these tissues.
- Porosity in the material is crucial for the transmission of nutrients and gas to cells. It is also used in the attachment and proliferation of cells.
- Inorganic dopants and nanoscale additives are commonly used to improve the characteristics of biocompatible materials for tissue engineering.
- Among the various dopants available, the halloysite nanotube is regarded as the most promising candidate due to its functional and biocompatibility properties.
- Some of the few dopants were explained below.

Dopants used in tissue engineering

- **Strontium (Sr)**
- Strontium is a bioactive trace element, existing in human skeletons which aids in new bone formation and has antiresorptive effect. The Sr ions enhances the osteoblast activity and inhibits the osteoclast function with an increase in bone-healing process.
- When incorporated within biomaterials, these Sr^{2+} substitute the Ca^{2+} ions due to their similar ionic radii changing the porosity and mechanical properties of the materials.
- **Silver (Ag)**
- Silver has attracted considerable attention of researchers as the Ag^{2+} ions show oligodynamic effect with a resistance to microbial activity, thereby preventing implant-based infections. Its antibacterial efficacy is most potent among all other metal ions, which is caused by disrupting the cell membranes of the bacteria and inhibiting enzymatic activities.
- **Zinc (Zn)**
- Zinc as a dopant promotes antibacterial potency while improving the bioactivity of a bioceramic and playing a vital role in bone formation and their mineralization. Zn^{2+} ions induce angiogenesis by generating reactive oxygen species (ROS) and promotes cell differentiation and proliferation of osteoblast cells.
- **Cerium (Ce)**
- Cerium was chosen for its antibacterial and antioxidant characteristics, as well as its beneficial effects on cell differentiation and mineralization.
- **Selenium**
- In a variety of medicinal applications, selenium-doped nanostructures have been proposed as a promising way to improve the antibacterial activity of calcium phosphate (CaP) materials.

Hydrogels

- A hydrogel refers to a system which is made of a network of hydrophilic polymeric materials that are able to interact with water without dissolving.

- These systems are useful in many biomedical applications, including drug delivery and tissue regeneration, for a number of reasons; the primary one being that they are largely biocompatible.

- Hydrogels are able to mimic most soft tissues in the human body. Due to the high level of hydrophilicity of hydrogels, they are able to very closely mimic the structural properties of the extracellular matrix in such a way that they are able to create an ideal environment for new cell growth. These cells are then in turn able to secrete new extracellular matrix.

Hydrogels

- **Hydrogels**
  - **Injectable Hydrogels**
  - **3D Printed Hydrogels**
  - **Porous Hydrogels**

- **Injectable hydrogels** are highly attractive, especially as fillers of soft and hard tissues, promoting a good physical integration into the defect site and possibly avoiding open surgeries with hard recovery of the patients. The high water content of these hydrogels make them adjustable and easy to manipulate for the delivery of cells and growth factors. Injectable hydrogels are able administered to the damaged site with minimally invasive techniques.

- **3D printed hydrogels** are produced through computer-assisted technologies, allowing fabrication of engineered tissues or matrices with superior control over their shape and reproducibility, with controlled physical and mechanical properties, and different layers and gradients, allowing generation of more complex tissue-like 3D architectures.

- **Porous hydrogels:** As mentioned previously, the biocompatibility and structural similarities of hydrogels to the native ECM make them desirable for engineering different complex tissues. However, the big challenge remains to obtain precise control of certain hydrogel properties, such as porosity and mechanical properties. For certain tissues, especially those of the musculoskeletal system, a substantial amount of scaffold porosity is necessary to allow cell infiltration for ECM formation and secretion throughout the engineered tissues. Increased porosity and pore size can benefit the structure interconnectivity and the di usion of nutrients and oxygen, especially in the absence of a pre-vascularized system, which is part of the microarchitectural composition of some tissues, such as cartilage, meniscus and intervertebral disc (IVD).

Thank you for your attention