Lecture 19

BT 636
Tissue Engineering and Regenerative Medicine
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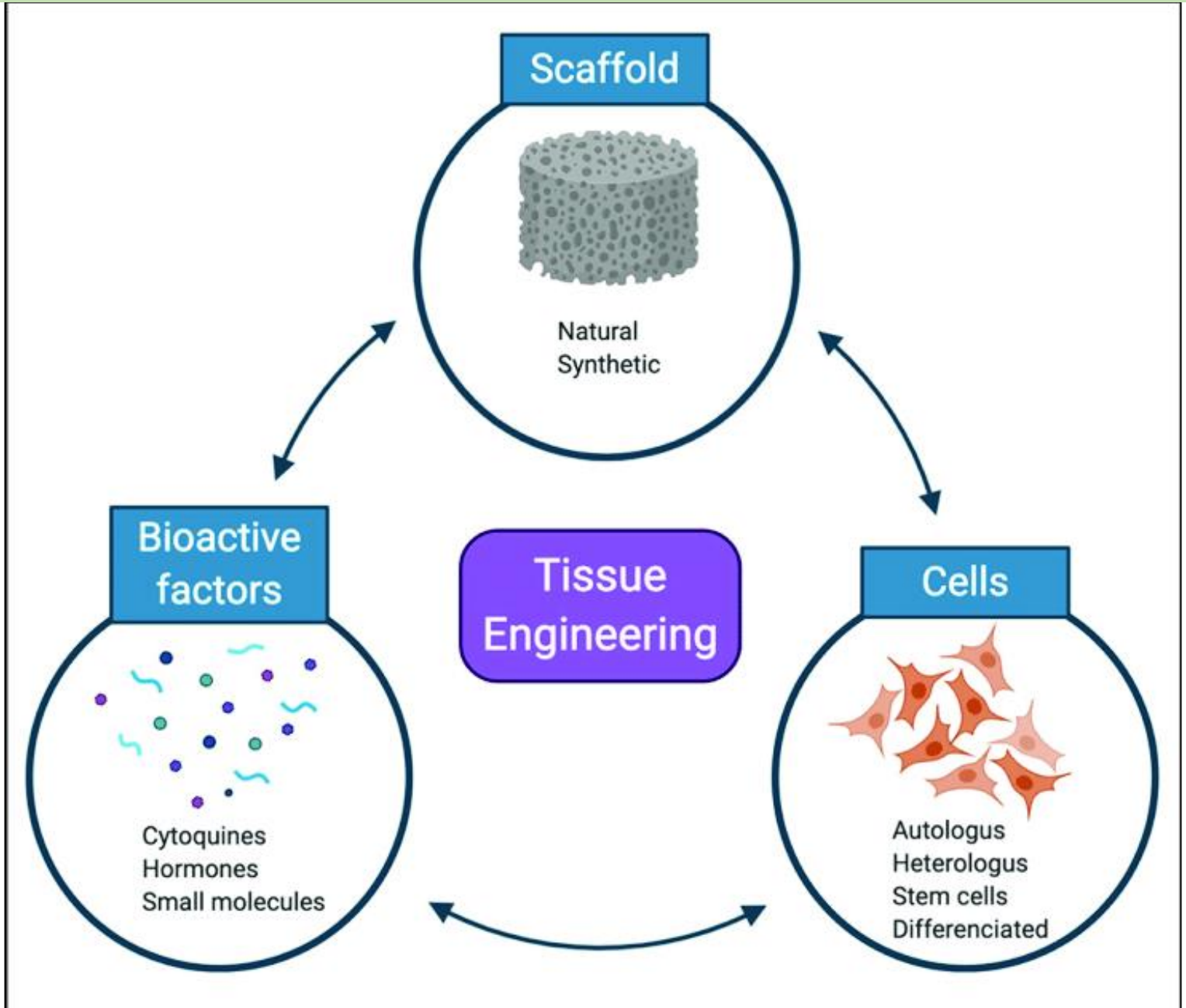
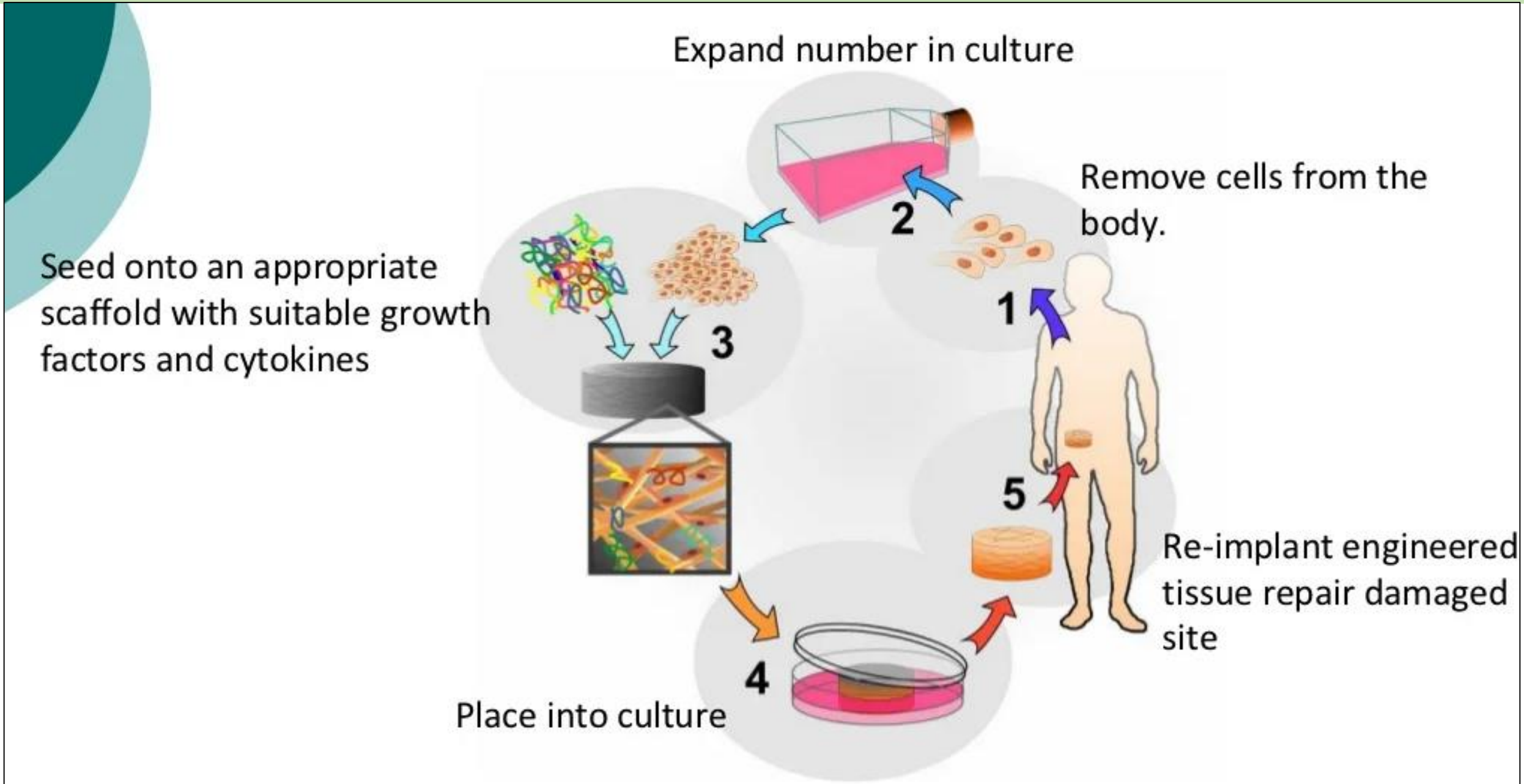
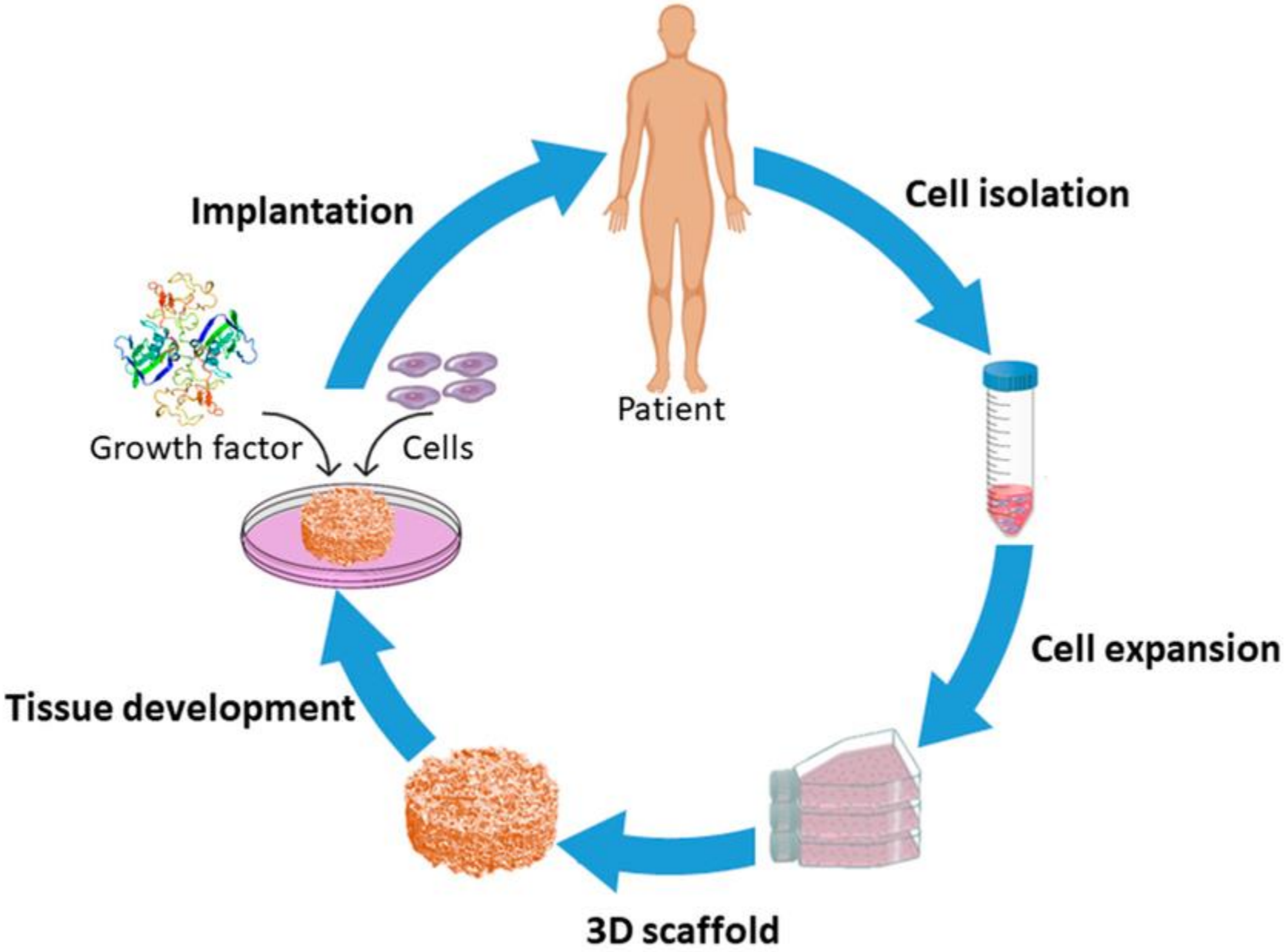
Tissue Engineering



Tissue Engineering and Regenerative Medicine



Tissue Engineering and Regenerative Medicine



SCAFFOLDS

- Cells are often implanted or 'seeded' into an artificial structure capable of supporting **three-dimensional** tissue formation. These structures, typically called **scaffolds.**

- **Scaffolds usually serve at least one of the following purposes:**
  - Allow cell attachment and migration
  - Deliver and retain cells and biochemical factors
  - Enable diffusion of vital cell nutrients and expressed products
  - Exert certain mechanical and biological influences to modify the behavior of the cell phase

- To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients.
- Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal.

SCAFFOLDS

- Goal of tissue engineering is to regenerate diseased or damaged tissues in the body.
- Cell loaded are attached to the extracellular matrix (ECM) (generally scaffold)
- Composition of ECM varies from tissue to tissue.
- Typically, it comprises of:
  - Structural proteins (Eg. Collagen, elastin)
  - Adhesive proteins (Eg. Fibronectin, laminin)
  - Proteoglycans (proteins attached to polysachharides complexes in which sugars branch off core protein molecules) … sugars are typically glycosaminoglycans (GAGs). For eg. Chondroitin sulfate, dermatin sulfate, heparin sulfate
- **Cartilage:** Collagen, GAG, hyaluronic acid (proteoglycan)
- **Bone:** Collagen and Hydroxyapaptite
- **Skin:** Collagen, Elastin and proteoglycans
- Cells, in most cases, have to be attached to scaffolds (ECM) or other cells to function (Eg. Proliferate, migrate, differentiate)

MATERIALS

- Biomaterials usually serve as extracellular matrix (ECM), giving both structural and functional support.

- Many different materials (natural and synthetic, biodegradable and permanent) have been investigated. Examples of the materials are collagen and some polyesters.

- New biomaterials have been engineered to have ideal properties and functional customization: injectability, synthetic manufacture, biocompatibility, non-immunogenicity, transparency, nano-scale fibers, low concentration, resorption rates, etc.

- A commonly used synthetic material is PLA - polylactic acid. This is a polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body.

MATERIALS

- Scaffolds may also be constructed from natural materials: in particular different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth.

- Protein materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans (GAGs), have all proved suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains.

- Functionalized groups of scaffolds may be useful in the delivery of small molecules (drugs) to specific tissues.

MATERIALS

- The design of scaffolds with multifunctional properties is attractive for tissue engineering due to their potential to provide and improve the quality of life of people who require surgery or have bone diseases or defects.

Scaffold requirements

Numerous scaffolds produced from a variety of biomaterials and manufactured using a plethora of fabrication techniques have been used in the field in attempts to regenerate different tissues and organs in the body. Regardless of the tissue type, a number of key considerations are important when designing or determining the suitability of a scaffold for use in tissue engineering:

- **(i) Biocompatibility**

The very first criterion of any scaffold for tissue engineering is that it must be biocompatible; cells must adhere, function normally, and migrate onto the surface and eventually through the scaffold and begin to proliferate before laying down new matrix. After implantation, the scaffold or tissue engineered construct must elicit a negligible immune reaction in order to prevent it causing such a severe inflammatory response that it might reduce healing or cause rejection by the body.

- **(ii) Biodegradability**

The objective of tissue engineering is to allow the body's own cells, over time, to eventually replace the implanted scaffold or tissue engineered construct. Scaffolds and constructs, are not intended as permanent implants. The scaffold must therefore be biodegradable so as to allow cells to produce their own extracellular matrix5. The by-products of this degradation should also be non-toxic and able to exit the body without interference with other organs. In order to allow degradation to occur in tandem with tissue formation, an inflammatory response combined with controlled infusion of cells such as macrophages is required. Now that tissue engineering strategies are entering clinical practice more routinely, the field of immunology is a playing a role of increasing prominence in the research area.

- **(iii) Mechanical properties**

Ideally, the scaffold should have mechanical properties consistent with the anatomical site into which it is to be implanted and, from a practical perspective, it must be strong enough to allow surgical handling during implantation. While this is important in all tissues, it provides some challenges for cardiovascular and orthopedic applications specifically. Producing scaffolds with adequate mechanical properties is one of the great challenges in attempting to engineer bone or cartilage. For these tissues, the implanted scaffold must have sufficient mechanical integrity to function from the time of implantation to the completion of the remodeling process8. A further challenge is that healing rates vary with age; for example, in young individuals, fractures normally heal to the point of weight-bearing in about six weeks, with complete mechanical integrity not returning until approximately one year after fracture, but in the elderly the rate of repair slows down. This too must be taken into account when designing scaffolds for orthopedic applications. However, as the field has evolved, it could be argued that too much focus has been placed on trying to develop scaffolds with mechanical properties similar to bone and cartilage. Many materials have been produced with good mechanical properties but to the detriment of retaining a high porosity and many materials, which have demonstrated potential in vitro have failed when implanted in vivo due to insufficient capacity for vascularization. It is clear that a balance between mechanical properties and porous architecture sufficient to allow cell infiltration and vascularization is key to the success of any scaffold.

Scaffold requirements

- **(iv) Scaffold architecture**

The architecture of scaffolds used for tissue engineering is of critical importance. Scaffolds should have an interconnected pore structure and high porosity to ensure cellular penetration and adequate diffusion of nutrients to cells within the construct and to the extra-cellular matrix formed by these cells. Furthermore, a porous interconnected structure is required to allow diffusion of waste products out of the scaffold, and the products of scaffold degradation should be able to exit the body without interference with other organs and surrounding tissues. The issue of core degradation, arising from lack of vascularization and waste removal from the centre of tissue engineered constructs, is of major concern in the field of tissue engineering9,10. Another key component is the mean pore size of the scaffold. Cells primarily interact with scaffolds via chemical groups (ligands) on the material surface. Scaffolds synthesized from natural extracellular materials (e.g. collagen) naturally possess these ligands in the form of Arg-Gly-Asp (RGD) binding sequences, whereas scaffolds made from synthetic materials may require deliberate incorporation of these ligands through, for example, protein adsorption. The ligand density is influenced by the specific surface area, i.e. the available surface within a pore to which cells can adhere. This depends on the mean pore size in the scaffold. The pores thus need to be large enough to allow cells to migrate into the structure, where they eventually become bound to the ligands within the scaffold, but small enough to establish a sufficiently high specific surface, leading to a minimal ligand density to allow efficient binding of a critical number of cells to the scaffold. Therefore, for any scaffold, a critical range of pore sizes exists, which may vary depending on the cell type used and tissue being engineered.

- **(v) Manufacturing technology**

In order for a particular scaffold or tissue engineered construct to become clinically and commercially viable, it should be cost effective and it should be possible to scale-up from making one at a time in a research laboratory to small batch production. The development of scalable manufacturing processes to good manufacturing practice (GMP) standard is critically important in ensuring successful translation of tissue engineering strategies to the clinic.

Another key factor is determining how a product will be delivered and made available to the clinician. This will determine how either the scaffold or the tissue engineered construct will be stored. Clinicians typically prefer off-the shelf availability without the requirement for extra surgical procedures in order to harvest cells prior to a number of weeks of in vitro culture before implantation. However, for some tissue types, this is not possible and in vitro engineering prior to implantation is required.

Scaffolds – Important characteristics

Table 1. Outline of the properties to be considered when tissue engineering systems for regenerative medicine.

Properties	Design Considerations
Biocompatibility	The compatibility of a scaffold with the cells is of paramount importance. The scaffold should not illicit an immune response when inserted into the body.
Biodegradability	The ability of a scaffold to be biodegraded either through enzymatic or hydrolytic action is advantageous.
Electrical conductivity	Scaffolds which are conductive are able to influence the behavior of cells as a response to the electrical signals present in cell signaling.
Morphology	The morphology of the scaffold is vitality important as it impacts how the cells interact with the scaffold. The porosity of the scaffold ensures that cell infiltration can occur as well as the transfer of nutrients through the system.
Mechanical characteristics	This refers to characteristics such as the stiffness, elasticity and relaxation modulus of the scaffold. These influence cell behavior as well as the ability of the scaffold to mimic the natural microenvironment.
Ease of manufacturing	The cost of manufacturing, ease of the processes and the storage requirements are all factors which must be considered if the scaffold is to be produced on a large scale.

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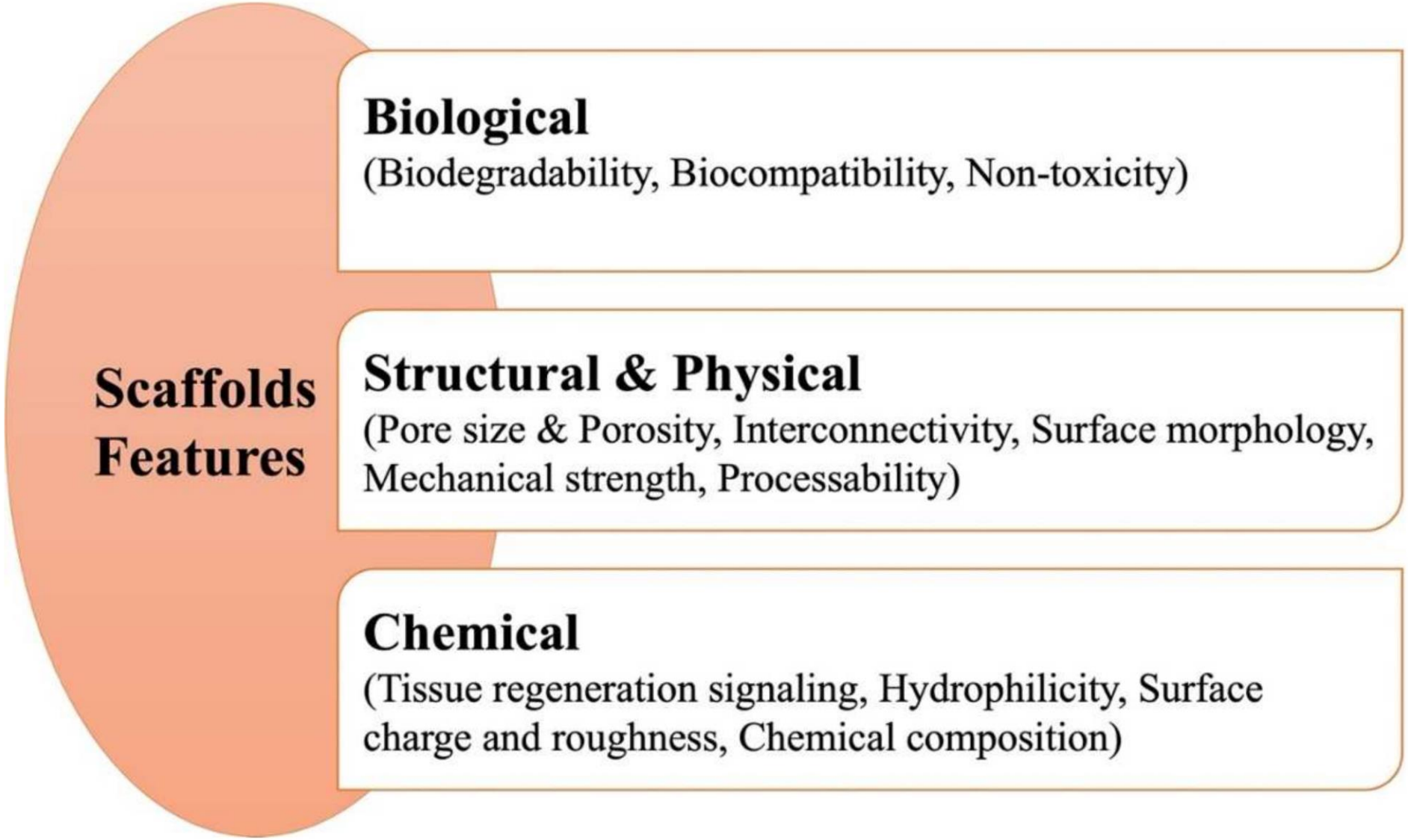

Figure 2. Features to be considered for optimal scaffold design and fabrication. Cus
scaffold features is conceptualized according to the target tissue and the required aim

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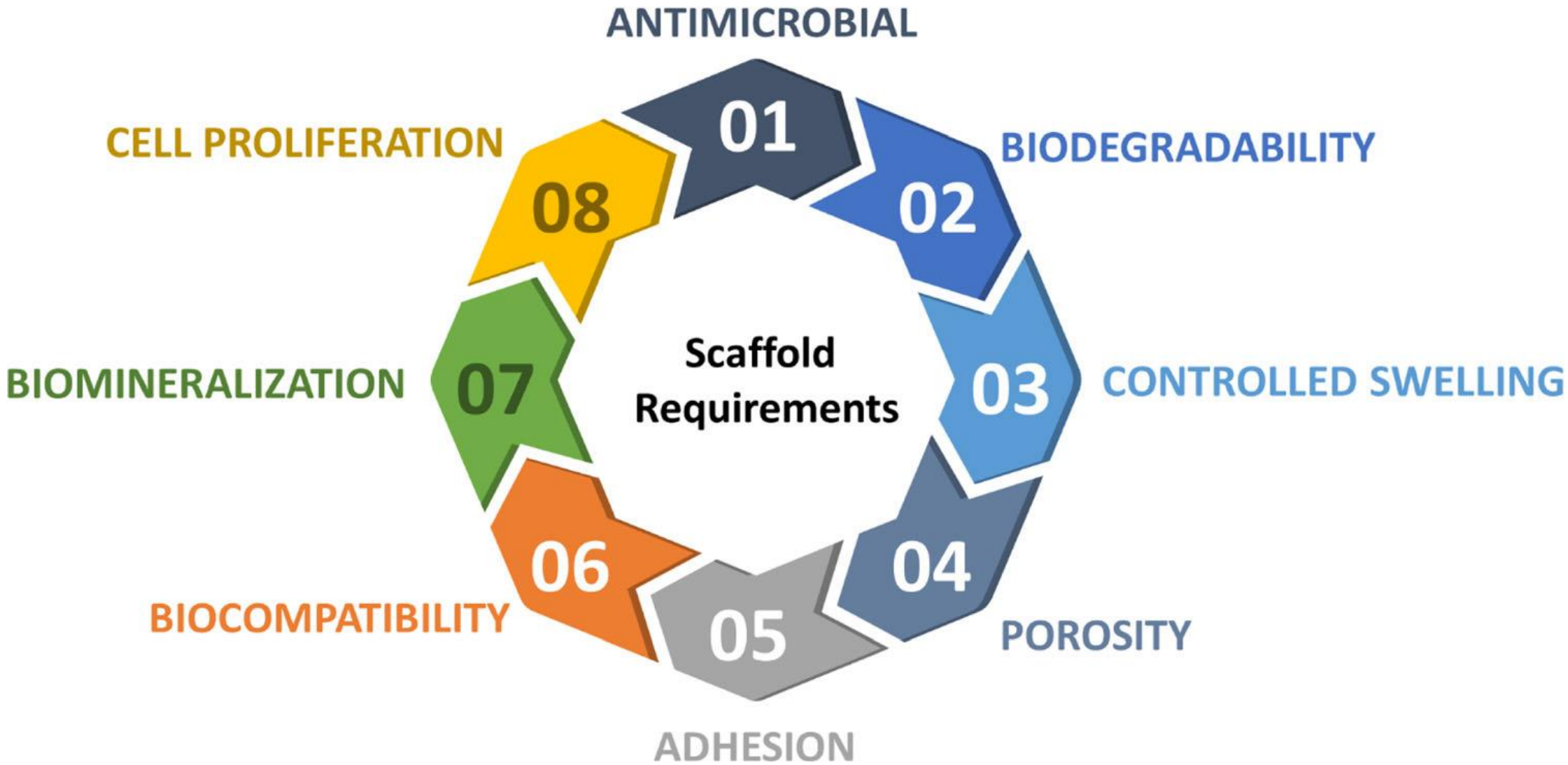
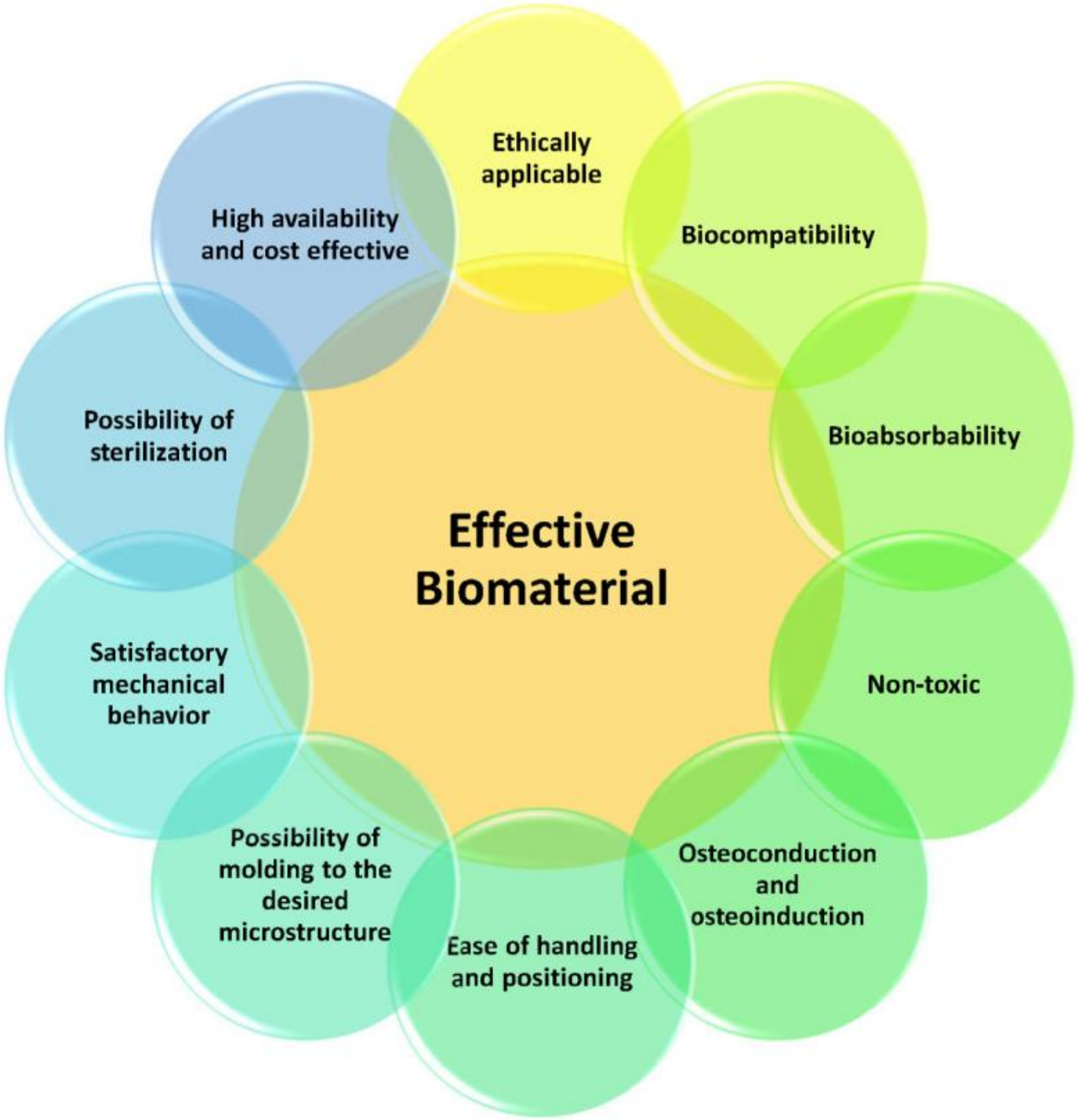


Advancing strategies towards the development of tissue engineering scaffolds: a review. August 2023 Journal of Materials Science 58(32):1-52; DOI: 10.1007/s10853-023-08798-5



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Ceramic Materials for Biomedical Applications: An Overview on Properties and Fabrication Processes

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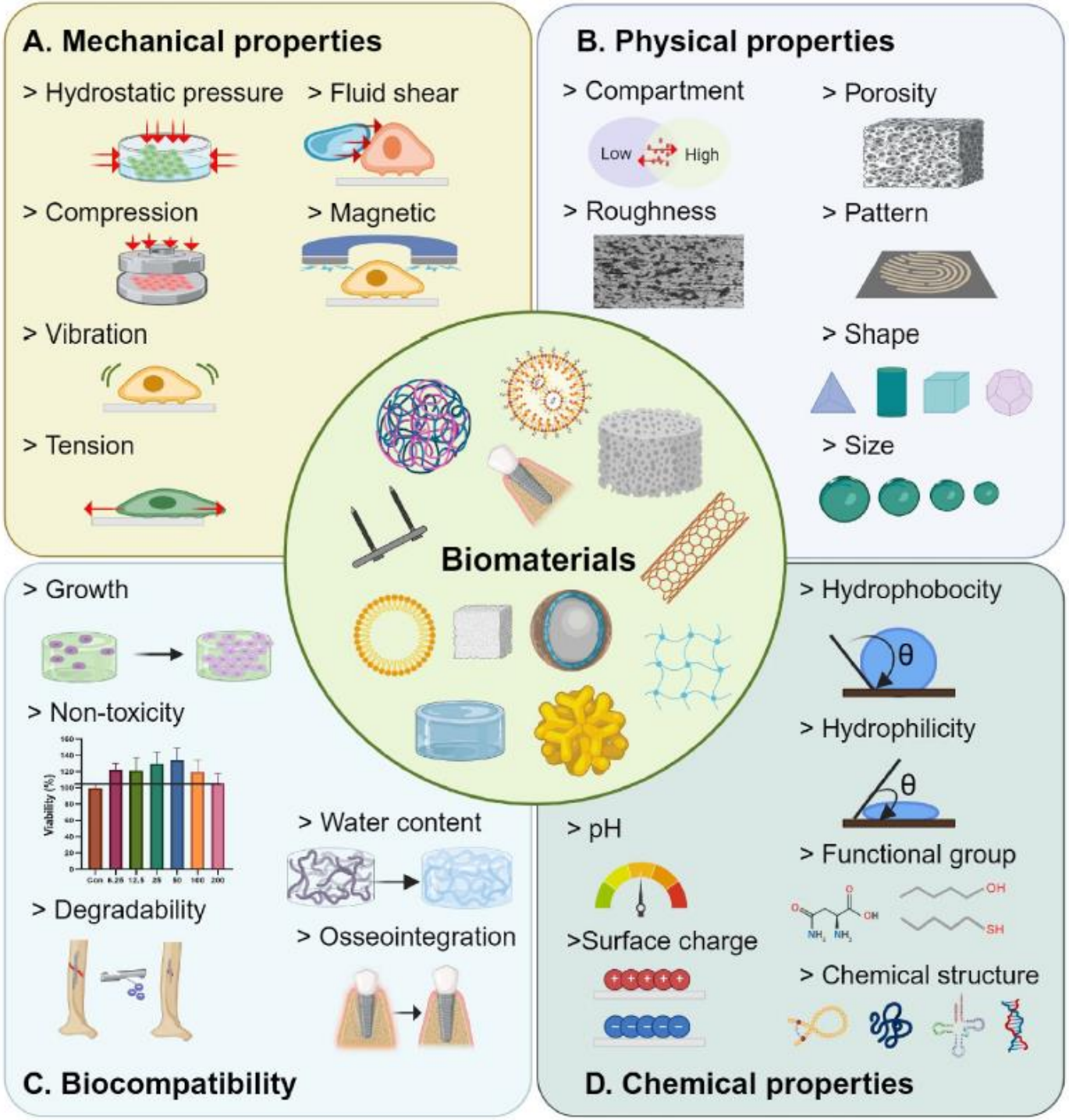

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Review article

Fostering tissue engineering and regenerative medicine to treat musculoskeletal disorders in bone and muscle

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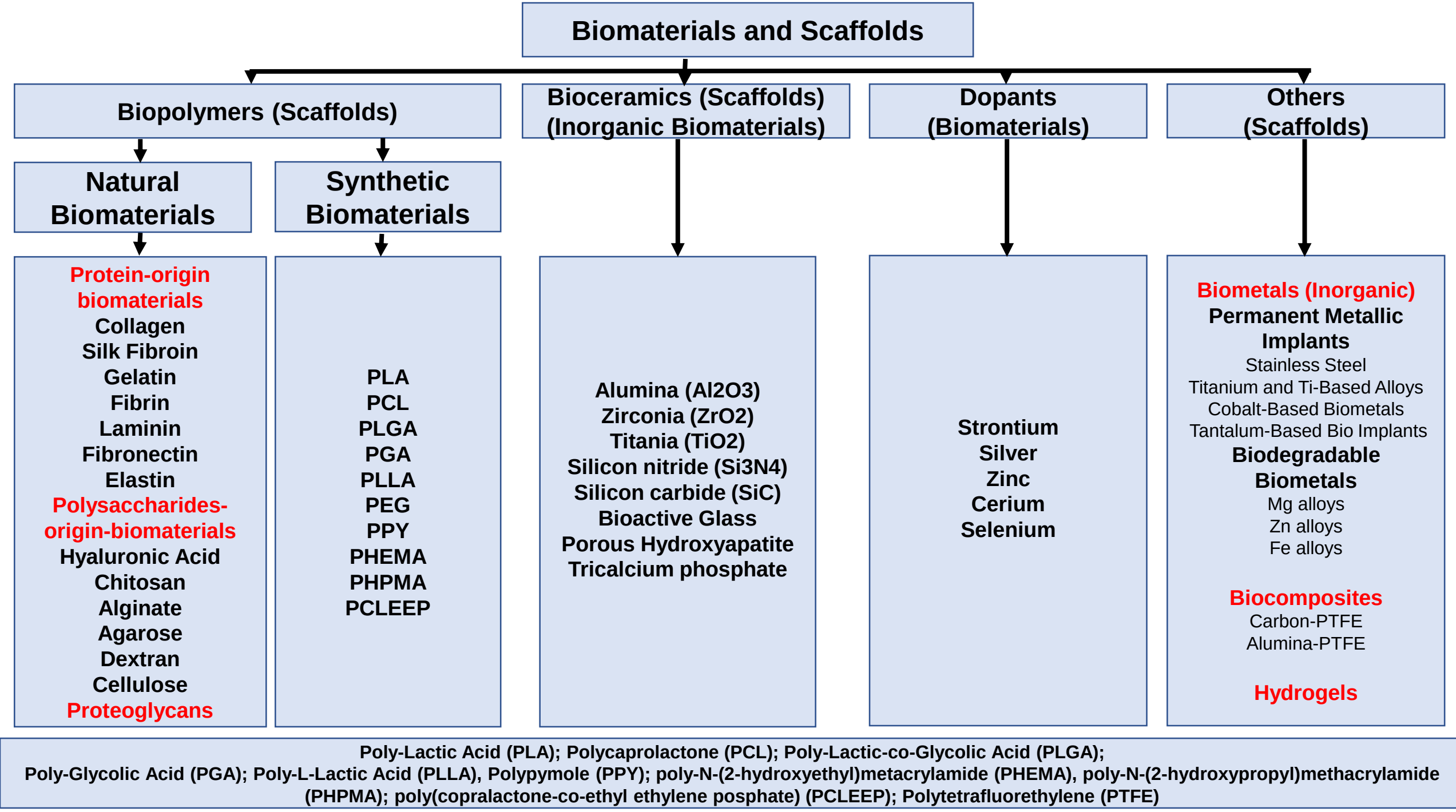
Scaffolds – Important characteristics

- Biocompatibility
- Biodegradability
- Bioabsorbability
- Bioactive
- Non-cytotoxicity
- Surface Morphology (charge and roughness)
- Controlled Swelling
- Pore Size and Porosity
- Mechanical characteristics
- Interconnectivity
- Hydrophilicity (mostly)
- Ease of manufacturing
- Processability
- Cost effective
- Sterilizable
- Antimicrobial
- Cell Adhesion
- Cell Proliferation
- Ethically acceptable
- Biomineralization (for specific tissues)
- Electrical Conductivity (for specific tissues)



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)

	Advantages	Disadvantages	Clinical uses
Ceramics	-Hard surface -High mechanical stiffnes -Chemical-physic refractoriness -High biocompatibility -osteoinductivity	- Brittleness - Slow degradation - Processing difficulties	-Hip prosthesis -Dental prosthesis -Bone and cartilage
Natural polymers	-Biocompatibility -Bioactivity	- Poor mechanical properties - Fast biodegradation	-Bone and cartilage - Tendon and ligament n
Synthetic polymers	- Possibility of modulating porosity and mechanical properties during the synthesis process.	- Low biocompatibility: possible release of ions and other residual particles of polymerization - Low mechanical strength	-Sutures -Catheters -Cardiovascular prostheses -Bone cements
Metals	-Good mechanical properties: high elastic module, yield strength and high ductility	- Reduced cell adhesion to their surface - Possible corrosion mediated by biological fluid	-Dentistry and orthopedic prostheses
Composites	-Biocompatibility -Good mechanical properties	- Processing difficulties	-Hard and soft tissue
Hydrogel	- Biocompatibility - Controlled biodegradation in vivo -Possibility to modulate their parameters [cross-linking density, porosity, pore size and inteconnectivity]		- Hard and soft tissue

Thank you for your attention