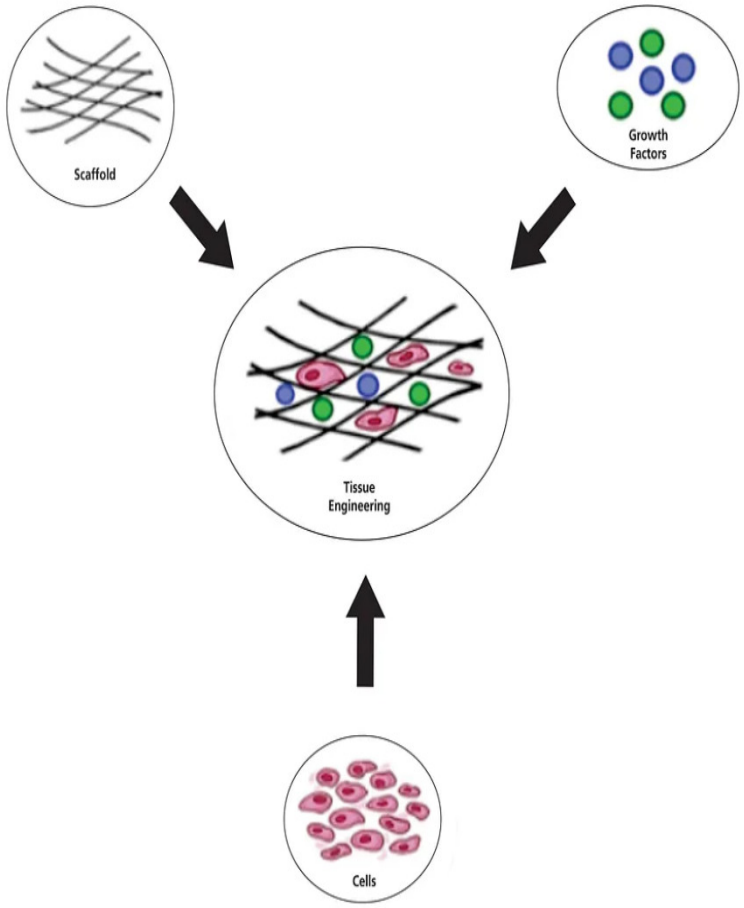
Scaffolds

- A key **concept in tissue engineering (TE)** is using material-based **porous three-dimensional (3D) scaffold**s to **provide physical and structural support** and deliver micro-environmental cues to cells **to enable or facilitate tissue formation** by mimicking the natural 3D structure of the tissues and organs.

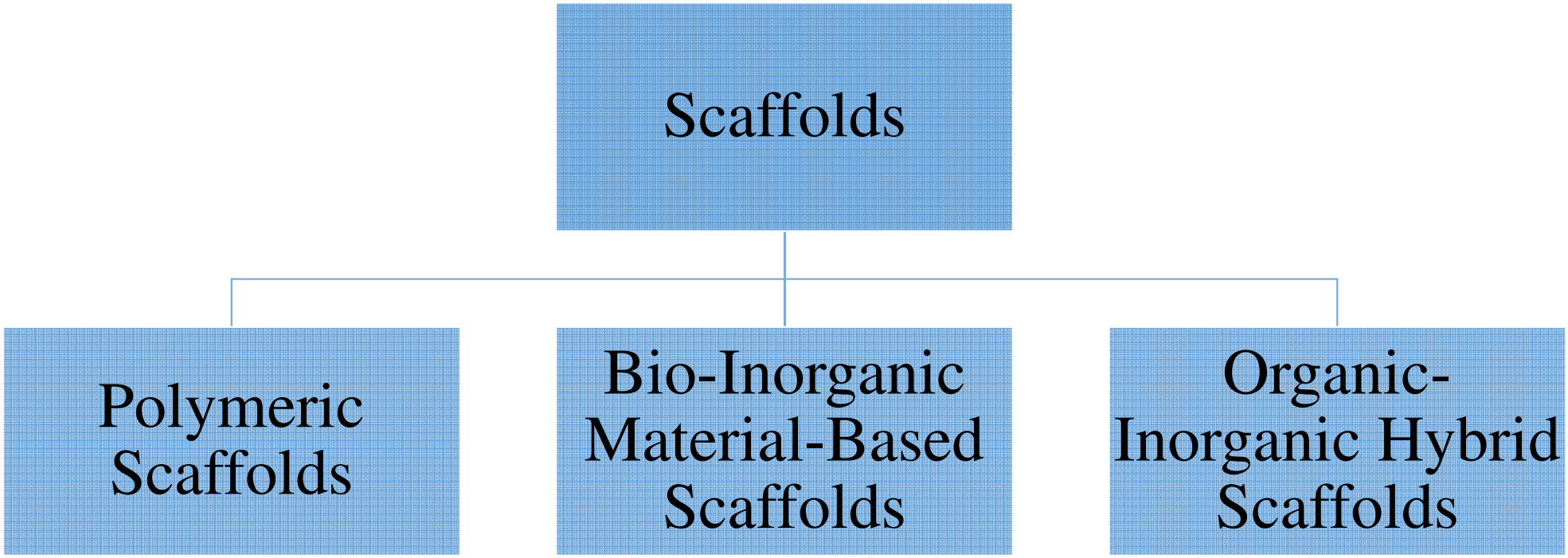
- **Scaffolds** are materials that have been **engineered to cause desirable cellular interactions** to contribute to the **formation of new functional tissues for medical purposes.** They **hold the cells at the defect site** until the wound heals and is covered by the newly formed tissues.

- **Scaffolds can be seeded** with different types of cells, including **stem cells, progenitors, mature differentiated cells, or co-cultures of cells for de novo tissue construction in vitro**; scaffolds can also be **directly implanted in vivo (within the living) to deliver soluble/insoluble and temporal/spatial cues** to guide the regeneration of defected tissue in situ (in the original position or place)



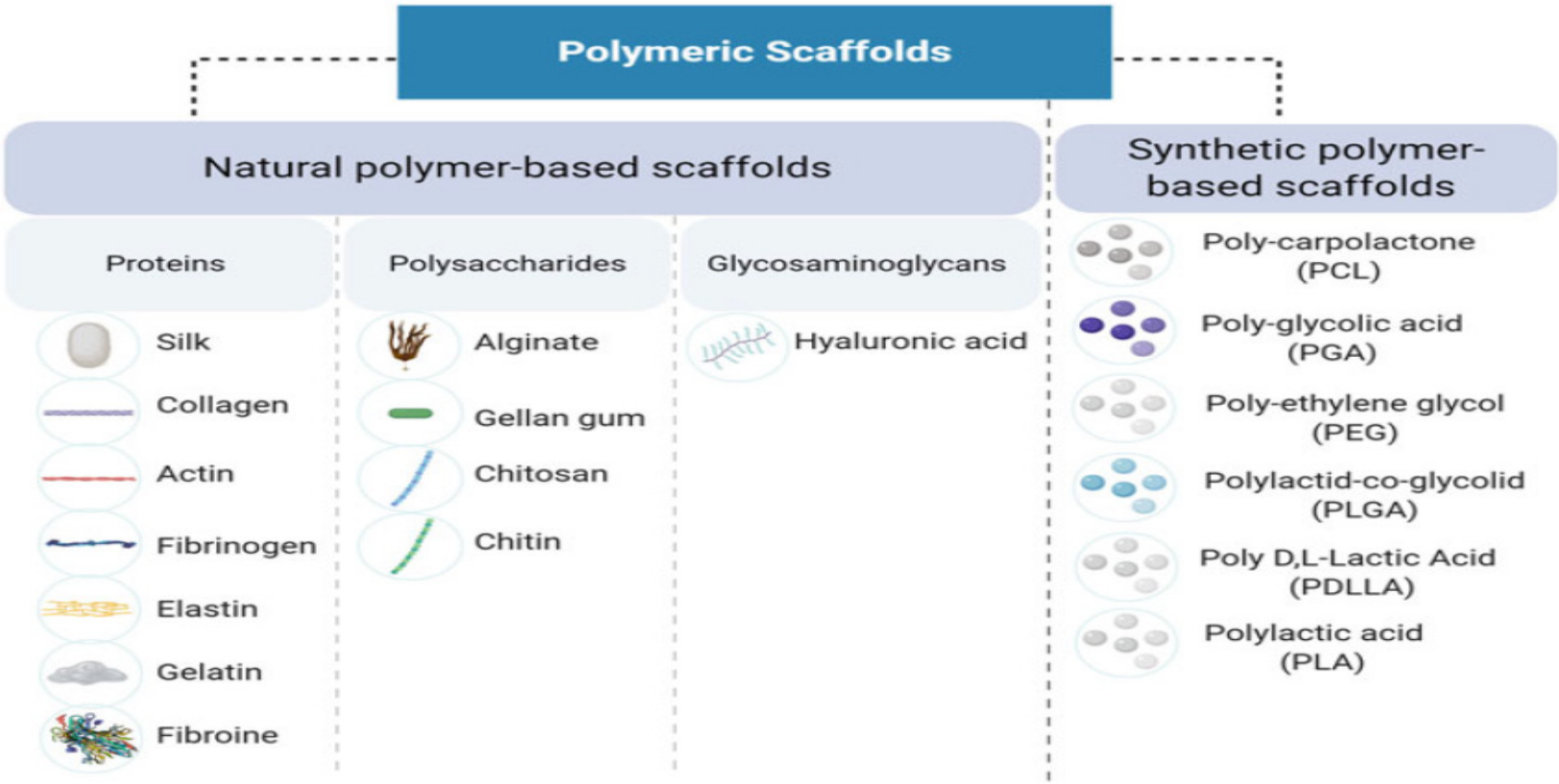
Types of Scaffolds

The scaffold is designed with biology in mind, and according to the type of tissue, the architecture and chemistry differ. Scaffolds can be synthesized from natural or synthetic materials.



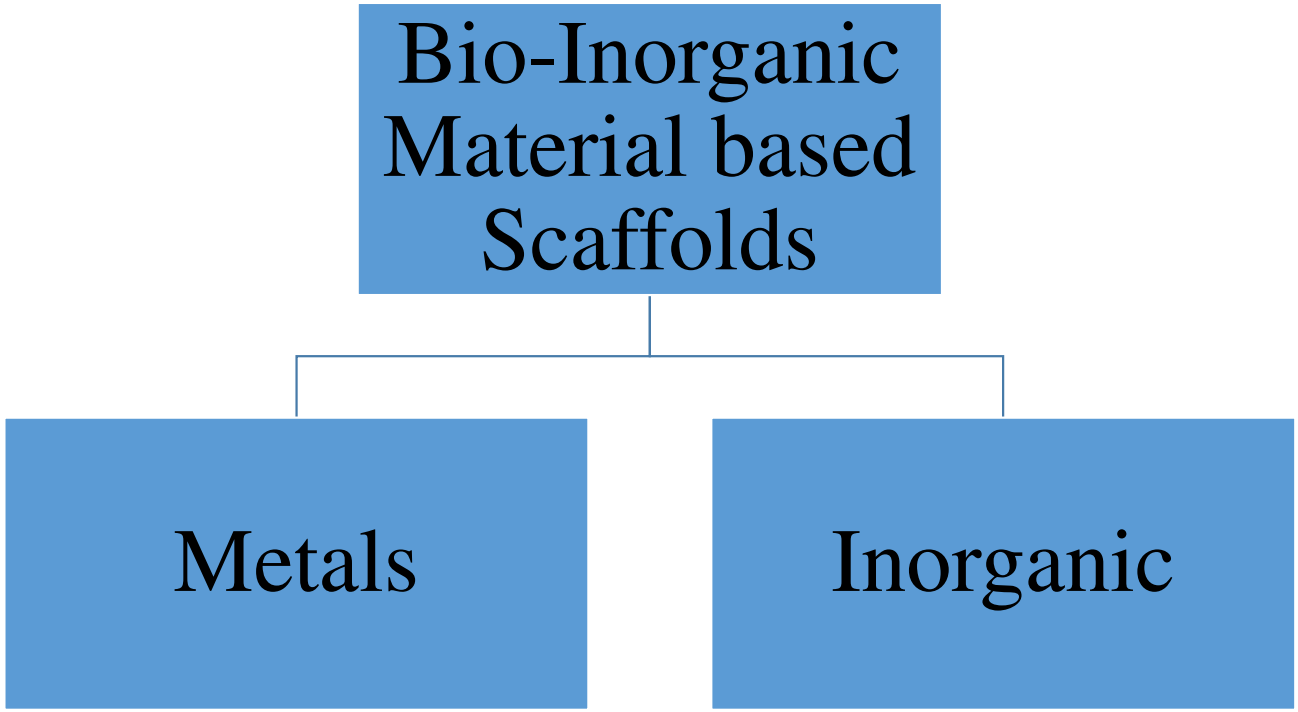
Polymeric Scaffolds

In tissue engineering applications, polymeric scaffolds have been commonly used. Biodegradable polymers provide the benefits of increased inflammatory tolerance, high biocompatibility, and in vivo non-toxic enzyme degradation.



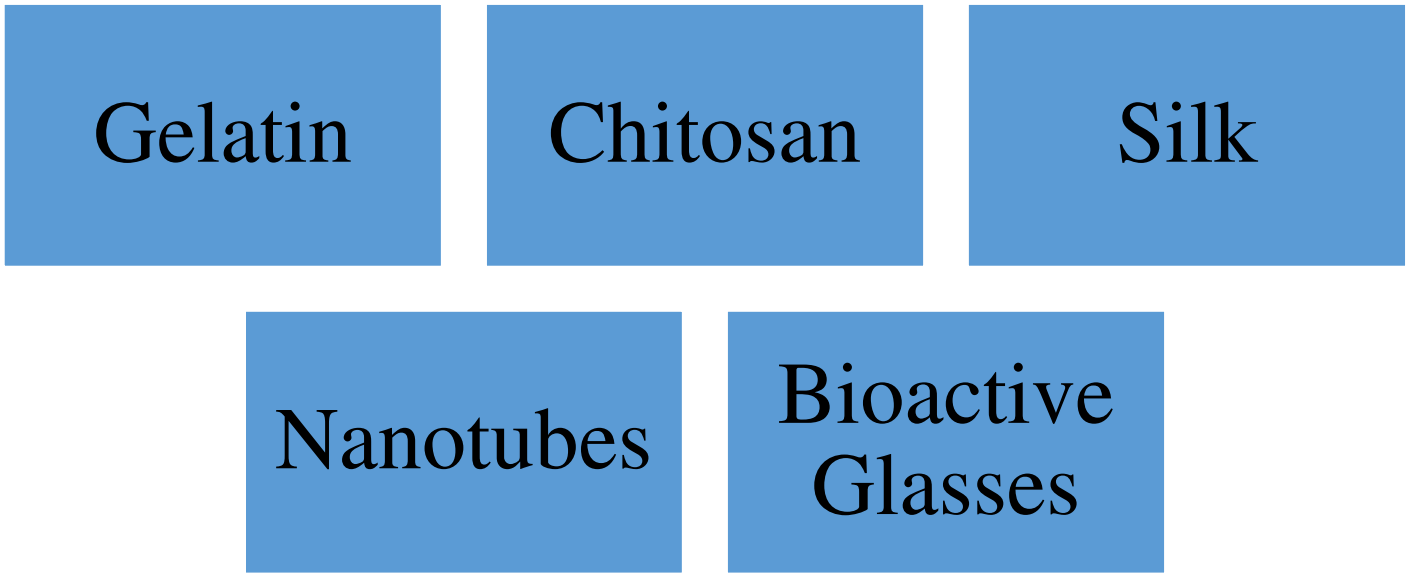
Bio-Inorganic Material Based Scaffolds

Inorganic materials are divided into metals and bio-ceramics according to their structure. They may be further classified as bioinert, bioactive, and bioresorbable material, according to their interaction with host cells and tissues.
Metallic biomaterials have low elastic modulus, low density, and high strength, whereas bio-ceramics possess high osteo-conductivity and biocompatibility. Ceramic material is considered biocompatible because it can gradually degrade into non-toxic products.



Organic-Inorganic Hybrid Scaffolds

Hybrid scaffolds include natural polymer composites, such as gelatin, chitosan, silk, and collagen, and synthetic polymers, such as PCL, PGA, PLA, poly (lactic-co-glycolic acid) (PLGA), and polyethylene glycol.
Hybrid scaffolds also include bio-ceramics, such as carbon nanotubes, bioactive glasses, and silicates.



2D and 3D Scaffolds

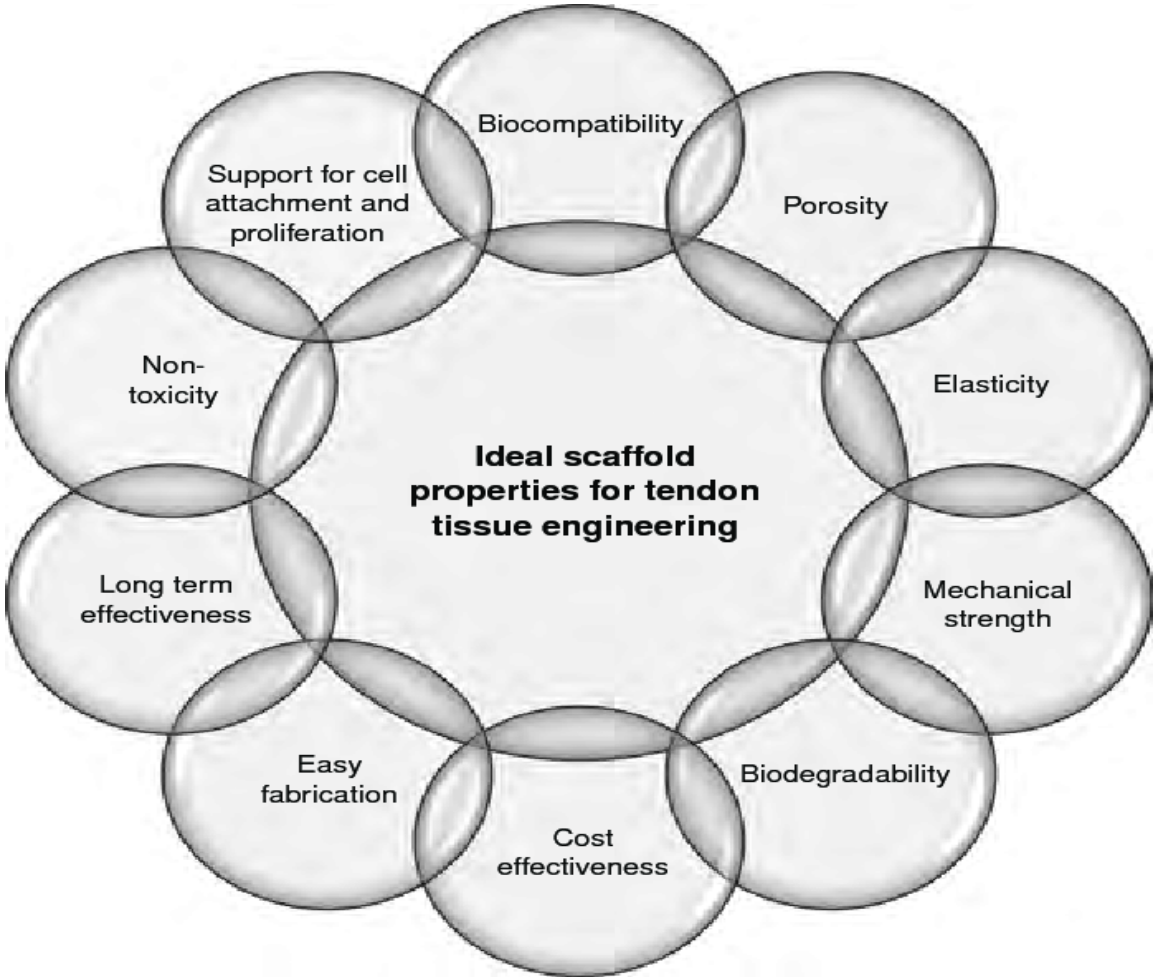
In general, the types of cell culture are explained as **2D or 3D cell cultures in terms of the dimension of cell growth**.**2D suggests cell growth in culture flasks as a monolayer,** usually involving a **single type of cells** growing on a planar surface. Due to factors such as simpler cell observation, direct measurement viability, inexpensive design, primary scope for drug testing, and cytocompatibility, 2D scaffoldss are commonly used in cell research.

A **cellular microenvironment is produced in 3D cell culture** in the interactions of cell–cell or cell–ExtraCellularMatrix, which **imitates the usual real-life scenario**. A 3D tissue culture **offers a broader forum for therapeutic and drug discovery** investigations, It provides a **physiologically relevant morphology, a normal cellular microenvironment, and the possibility of co-culturing a variety of cells**. The choice of **3D cell culture scaffolds depends** on the **type of feature and cell**. The properties of Scaffold vary based on concentration of polymer, ligand density, pore size, shape, strength, rigidity, etc.

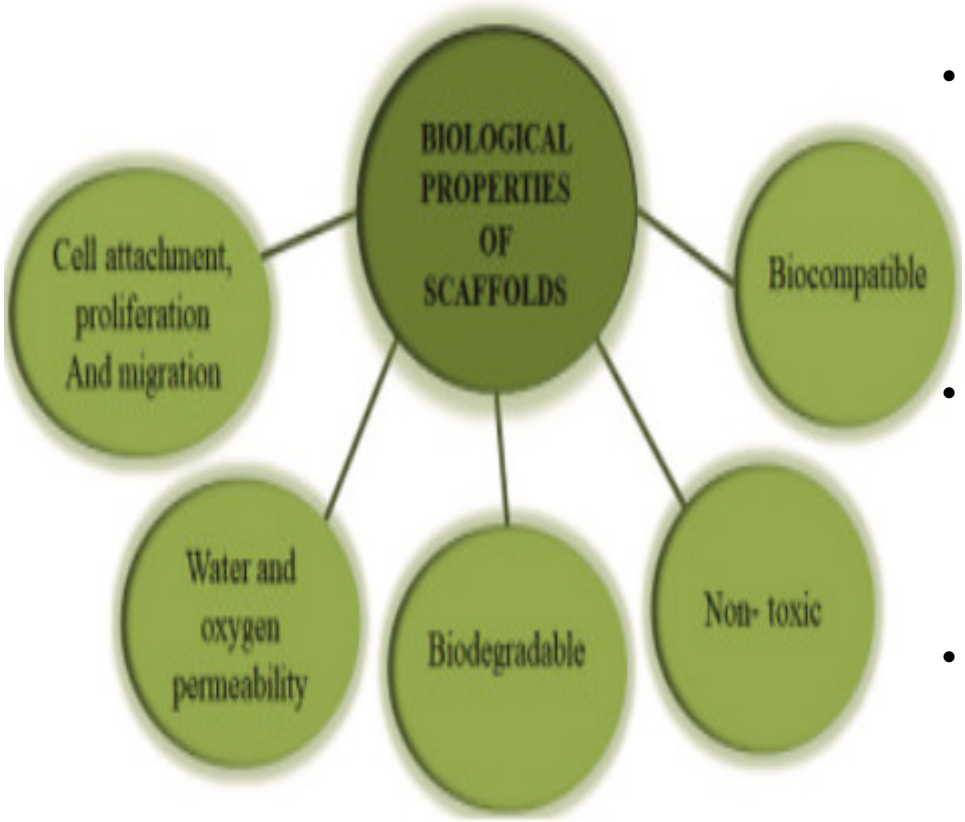
Natural polymers such as **collagen, gelatin, elastin, silk fibroin, chitosan (CS), chitin, fibrin, and fibrinogen are commonly used in the preparation of 3D scaffolds because of their biocompatibility.** Also used in the preparation of 3D scaffolds were synthetic polymers such as polylactic acid (PLA), poly(glycolic acid), polyhydroxyalkanoate, and poly(lactic-co-glycolic acid) (PLGA) due to their porosity, degradation time, and mechanical characteristics easily adapted

Ideal Scaffold Properties

Scaffolds for biomedical applications must meet certain requirements.

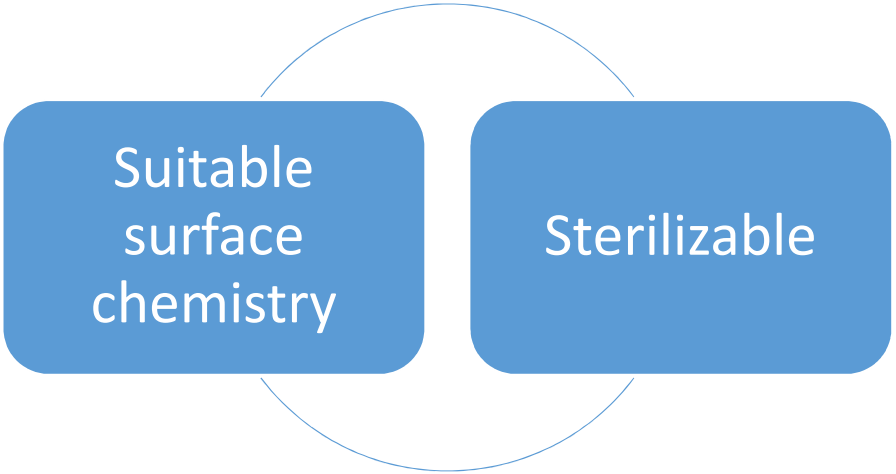


Biological Scaffold Properties



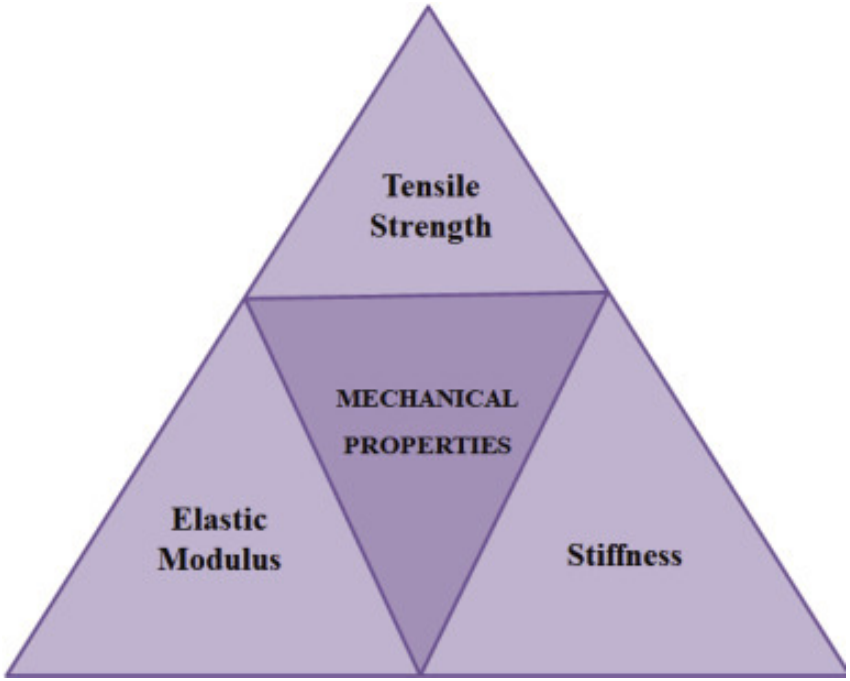
- **Biodegradability** is defined as the scaffold's capability to degrade after being replaced by tissues. Decreasing degradability of some scaffolds, like the amniotic membrane, can be achieved by collagen cross-linking using UV light or treatment with chemicals like carbodiimide.
- **Biocompatibility** is defined as the material's ability to perform its desired function without inducing any undesirable local or systemic effects on the host. The ideal biocompatible scaffold lacks cytotoxicity, genotoxicity, immunogenicity, and carcinogenicity.
- Scaffolds **should not induce deleterious** immune responses or exhibit cytotoxicity and should be **non-toxic** in nature.

Chemical Scaffold Properties



- **Suitable Surface Chemistry** The scaffold's surface topography controls cell attachment, proliferation, and influences cell differentiation potential. Surface roughness can modulate the biological tissue response.
- Scaffolds **should be sterilizable in order to avoid** contamination.

Mechanical Scaffold 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.
- **Ultimate strength-**Maximum value of load bearing after which it may get permanently deformed.
- **Tensile Strength-**load bearing upto which a scaffold could be elongated prior to breaking
  -tested for fibrous materials.
- **Compressive Strength-**degree of compression
  -tested for porous scaffold.
- **Elastic limit-**stress that can be applied to an elastic body without causing permanent deformation. The stress point at which a material will no longer return to its original shape if it is subjected to higher stress.

Forces that can be applied to Scaffolds

- **Tensile**-a force tending to tear it apart
- **Compressive-**A force that squeezes an object's surfaces together and causes its mass to bulge.
- **Shear**-Shearing forces are unaligned forces pushing one part of a body in one direction, and another part the body in the opposite direction.
- **Torsion**-torsion is the twisting of an object due to an applied torque.

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.

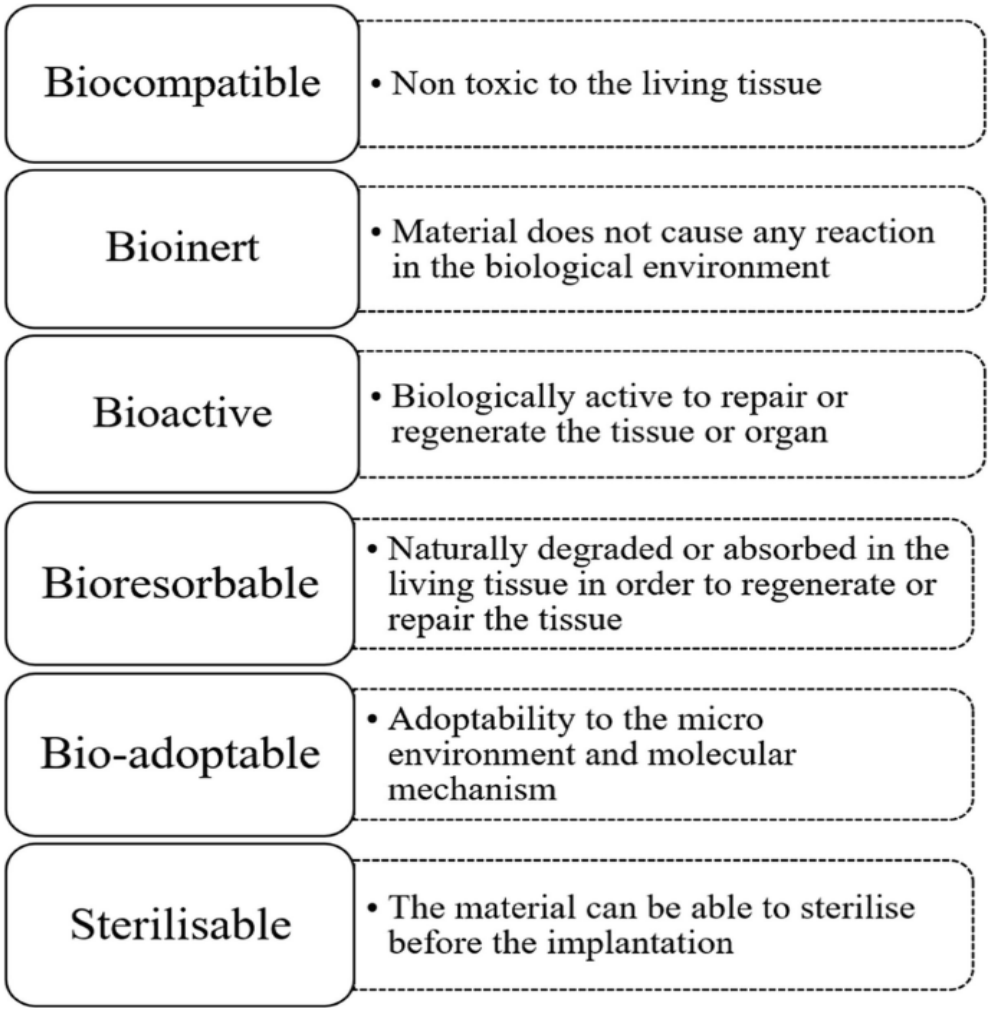
- The pores thus need to be **large enough to allow cells to migrate into the structure**, where they eventually become bound to the ligands within the scaffold, but small enough to establish a sufficiently high specific surface, leading to a **minimal ligand density to allow efficient binding** of a critical number of cells to the scaffold.

Biomaterials

- Biomaterials is a term used to indicate materials that constitute parts of medical implants, extracorporeal devices, and disposables that have been utilized in medicine, surgery, dentistry, and veterinary medicine as well as in every aspect of patient health care.
- The first definition of biomaterial was developed in the 1980s, during the Consensus Development Conference (Chester, UK, 1982) in which the biomaterials were defined as "any substance, other than a drug, or a combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system, which treats, augments or replaces any tissue, organ or function of the body".

	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

Characteristics of an ideal biomaterial



Biocompatibility:

- The very first criterion of any scaffold for tissue engineering is that it must be biocompatible; cells must

    1) adhere,

    2) function normally,

    3) migrate onto the surface and eventually through the scaffold and

    4) 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.
- Biocompatibility testing:
- *In vitro*-

    SEM, Fluorescence microscopy for Cell morphology, attachment and spreading

    Alamar blue assay for Cell proliferation

    MTT assay for cell viability and metabolic activity analysis

- *In vivo*:

    Pre-clinical tests in animal models-creating artificial wound and accessing the response generated by the implant.

- Next step is clinical trial-performed by surgeons in hospitals

Bioactivity

- Ability of a biomaterial to interact with surrounding tissue ensuring cell adhesion, proliferation, and differentiation.
- Generally, biomaterials with chemical composition comparable to the host tissue have a higher bioactivity and can promote cellular recognition evoking specific cellular response to support tissue growth.
- It is possible to modify the surface of the biomaterial by adding extracellular matrix macromolecules, including collagen, fibronectin, and laminin, to produce a biomimetic environment equivalent to the native tissue.
- Also for some applications interaction between the biomaterial and tissue microenvironment should be kept minimal.

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 matrix.
- 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.
- **Degradation rate of scaffold= rate of tissue formation**
- Degradation testing is done by giving enzymes like lysozyme and incubating in a fluid medium (PBS or SBF) for particular time period and measuring change in weight.

Types of biomaterials for tissue engineering

- Typically, three individual groups of biomaterials are used in the fabrication of scaffolds for tissue engineering.

1. Natural biomaterials
2. Ceramics,
3. Synthetic polymers
4. Natural polymers

Natural biomaterials

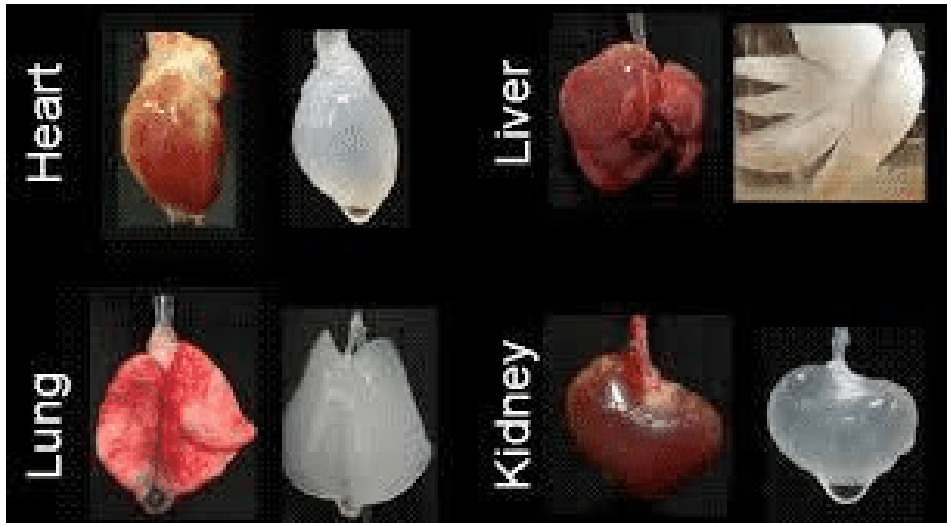
Examples; Proteins (collagen, gelatin, silk), Polysaccharides (chitosan, hyaluronic acid), Extracts of ECM components, de-cellularized extracellular matrix (dECM)

Advantages

1. Chemically similar or identical to molecules in the body
2. Readily degraded in vivo
3. Interact with cells on a molecular level

Limitations

1. Difficult to obtain and purify
2. Vary in properties between batches
3. Difficult to sterilize
4. Alter their properties during storage,
5. Elicit significant immunogenic responses.

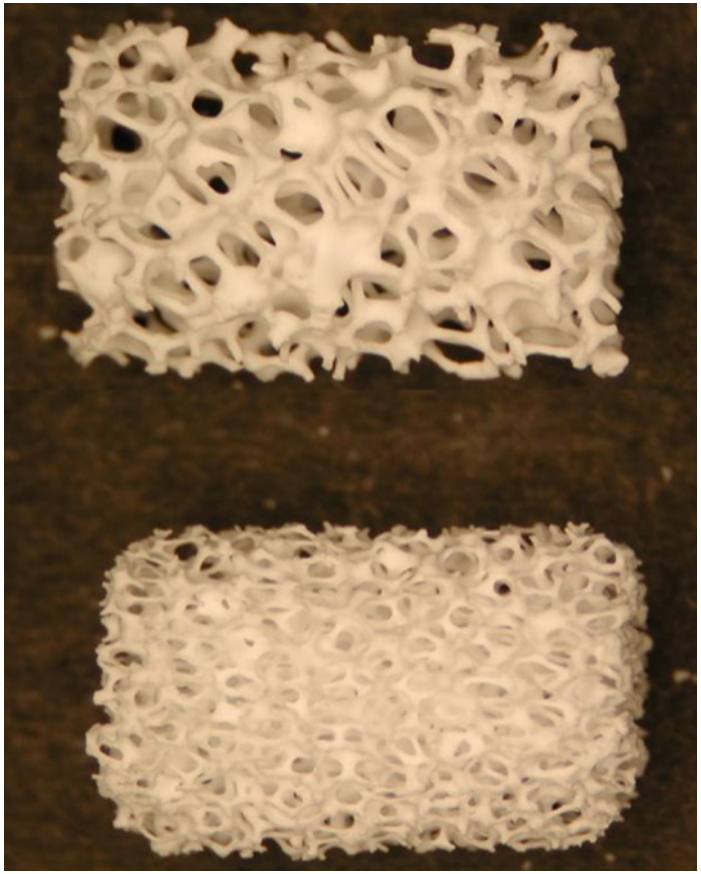


DOI:10.13140/RG.2.2.10342.83528

***In decellularization, cells are removed from allografts or xenografts to reduce immunogenicity but much of the complex composition and architecture of the ECM may be retained

Ceramics

- Although not generally used for soft tissue regeneration, there has been widespread use of ceramic scaffolds, such as hydroxyapatite (HA) and tri-calcium phosphate (TCP), for bone regeneration applications.
- Ceramic scaffolds are typically characterized by high mechanical stiffness (Young's modulus), very low elasticity, and a hard brittle surface.
- From a bone perspective, they exhibit excellent biocompatibility due to their chemical and structural similarity to the mineral phase of native bone.
- The interactions of osteogenic cells with ceramics are important for bone regeneration as ceramics are known to enhance osteoblast differentiation and proliferation.



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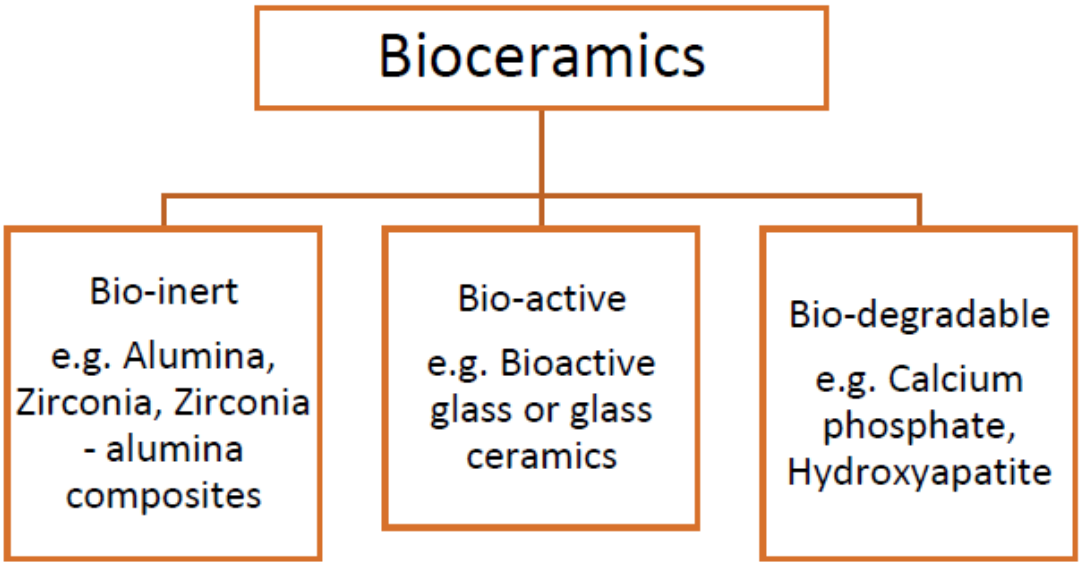
Classification of ceramics



Biomaterials	Density (g/cm^3)	Bond Strength (GPa)	Compressive strength (MPa)	Young Modulus (GPa)
Alumina	3.3-3.9	300-400	2000-5000	260-410
Zirconia	6.0	200-500	2000	150-200
Bioglass	2.5	50	600	75
Hydroxyapatite	3.1	120	500-1000	73-117
Glass Ceramic	2.8	215	1080	118
Bone	3.88	60-160	130-180	3-30

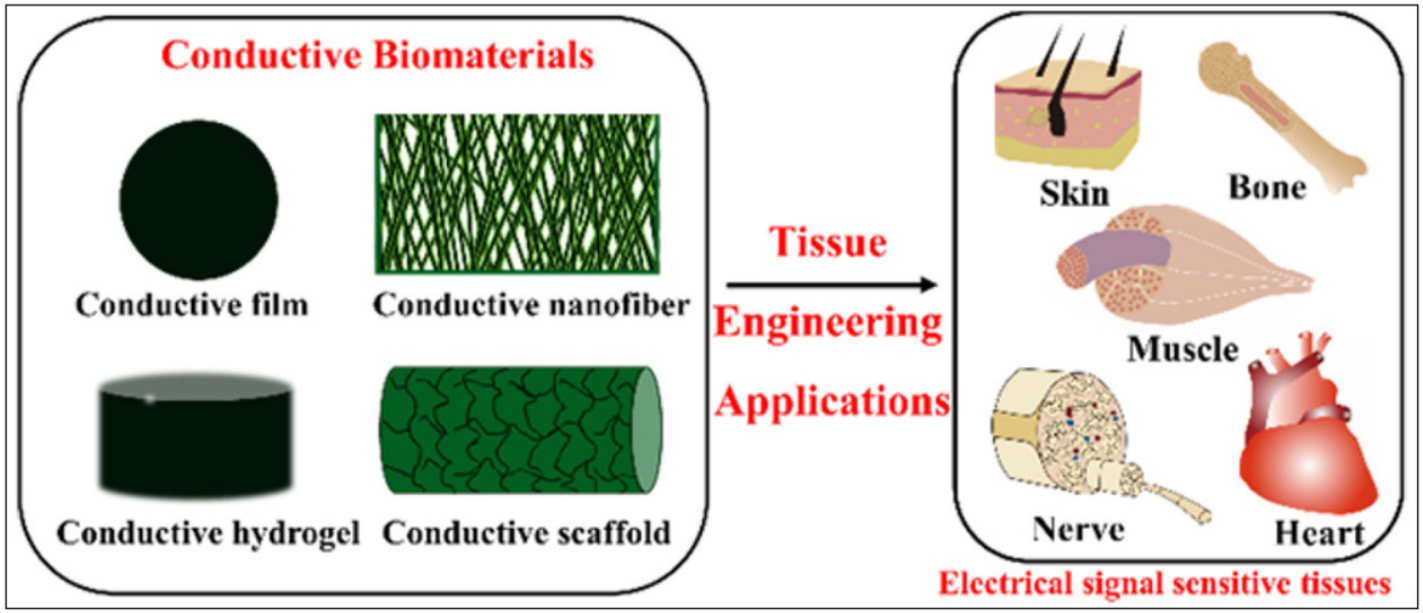
https://api.semanticscholar.org/CorpusID:195782250

Classification of ceramics

- **Bio-enert ceramics** are materials having stable physiochemical properties and makes good compatibility with the hard tissues. When implanted into the body there will be minimal physiological reaction and immunological rejection by body tissue. They keep their physiochemical and biomechanical properties in the host. They repel corrosion and wear. They have a good strength to resist fracture. Bio-inert materials are applied as a structural-support implant for example, bone plates and bone screw.
- **Bio-active ceramics** are materials having a positive effect on living tissues and having ability to induce a response that helps in the regeneration, repair and reconstruction of body tissues. In tissue engineering, bioactive materials are specially used to repair orthopedic, craniofacial (skull and face bones) and dental, chronic osteomyelitis.
- **Bio-degradable ceramics** are materials are proficient to react and broken down rapidly when come in contact with body tissue fluid. These materials do not require secondary surgery for the removal of implants from the body. These are completely absorbed by the body tissues and become the part of hard tissue. The chemicals produced by the ceramic resorption should be able to be treated by the normal metabolic pathways of the body without producing any toxic effect. The controlled degradation of these materials can be more beneficial for the patients.

Polymers

- While use of natural polymers , such as cellulose and starches, is still common in biomedical research, synthetic biodegradable polymers are increasingly used in tissue-engineering products.
- Synthetic polymers can be prepared with chemical structures tailored to optimize physical properties of the biomedical materials and with well-defined purities and compositions superior to those attainable when using natural polymers.

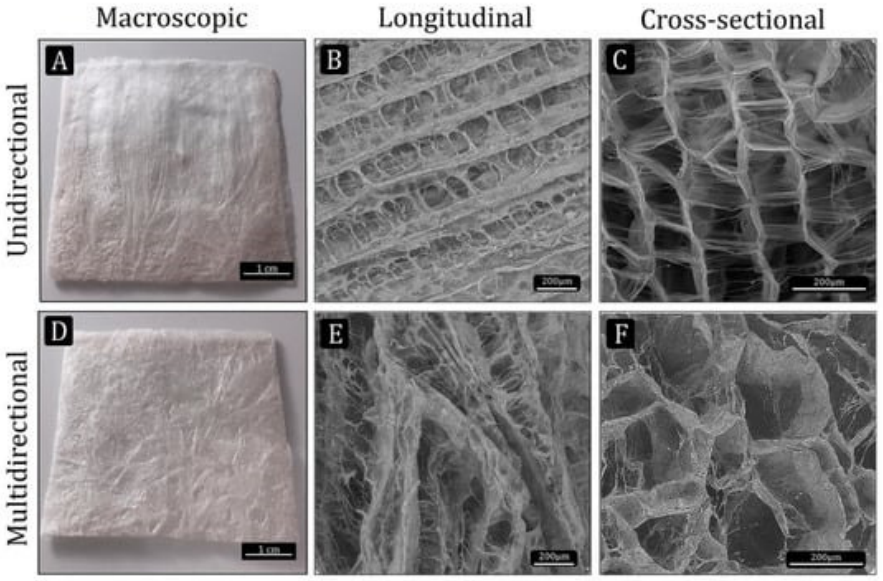


https://doi.org/10.1021/acs.biomac.8b00276

Natural Polymers

- Natural polymers such as collagens, elastin, and fibrinogen make up much of the body's native extracellular matrix (ECM). This ECM provides structure and mechanical integrity to tissues, as well as communicating with the cellular components it supports to help facilitate and regulate daily cellular processes and wound healing
- Blends of collagen and glycosaminoglycans (GAG) have been utilized extensively for dermal regeneration.
- Chondroitin sulfate has been added to collagen type I for dermal regeneration templates and aggrecan (chondroitin sulfate/dermatan sulfate/keratin sulfate) to collagen type II for articular cartilage tissue engineering.

Macro- and microstructure of the collagen scaffolds

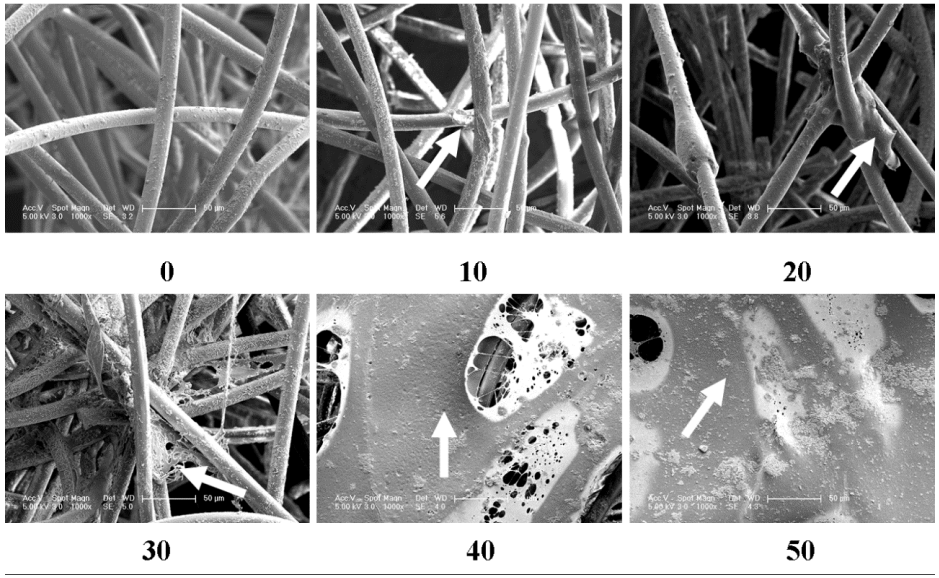


https://doi.org/10.3390/polym13183187

Synthetic polymers

- Synthetic biodegradable polymers with well-defined structure and without immunological concerns associated with naturally derived polymers are widely used in tissue engineering.
- Polylactide (PLA), polyglycolide (PGA), and their copolymer poly(lactide-*co*-glycolide) (PLGA) are commonly synthesized by a ring-opening polymerization of the monomers (lactide and/or glycolide). In addition to their biodegradability and biocompatibility, these polymers are among the few synthetic polymers approved by the U.S. Food and Drug Administration (FDA) for human clinical applications such as surgical sutures and some implantable devices.

Scaffolds with different PLA contents

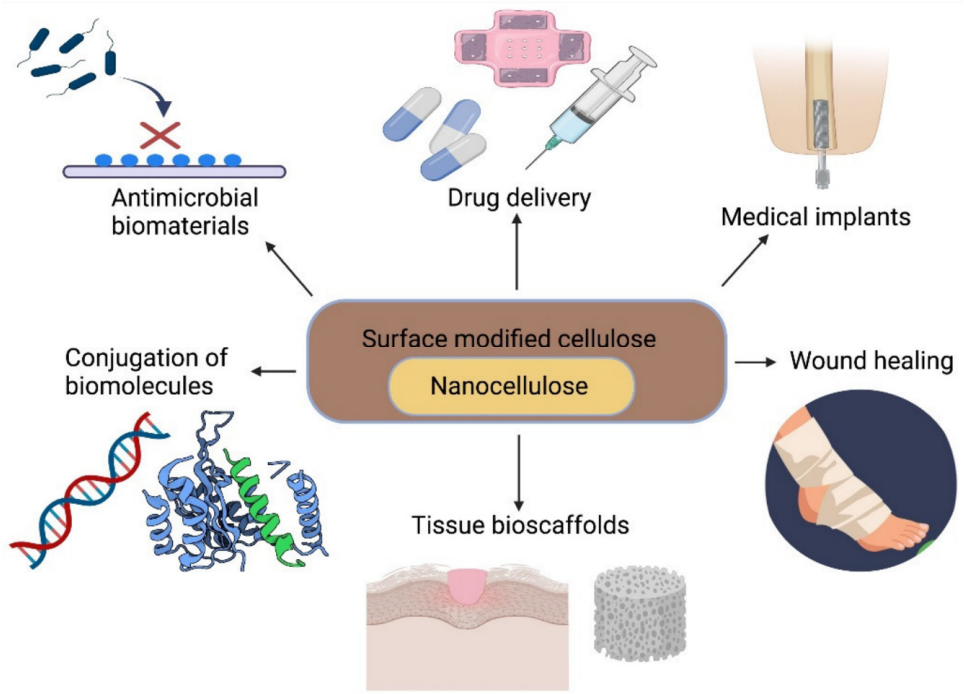


DOI: 10.5772/55540

Advanced biomaterials for tissue engineering

Tailored delivery systems:

- Growth factors, anti-inflammatory peptides, and drugs may be incorporated into biomaterial delivery vehicles for release at the desired time during tissue development.
- Release systems are designed to deliver multiple molecules over different timescales via continuous or pulsatile delivery, which may be programmed or triggered by some change in the local environment.
- Fabricated from biodegradable polymers in the form of
  - micro or nanoparticles
  - capsules,
  - within walls/surfaces of scaffolds or hydrogels



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Advanced biomaterials for tissue engineering

Smart Polymers:

Changes in environmental conditions - changes to the molecular conformation of many materials.

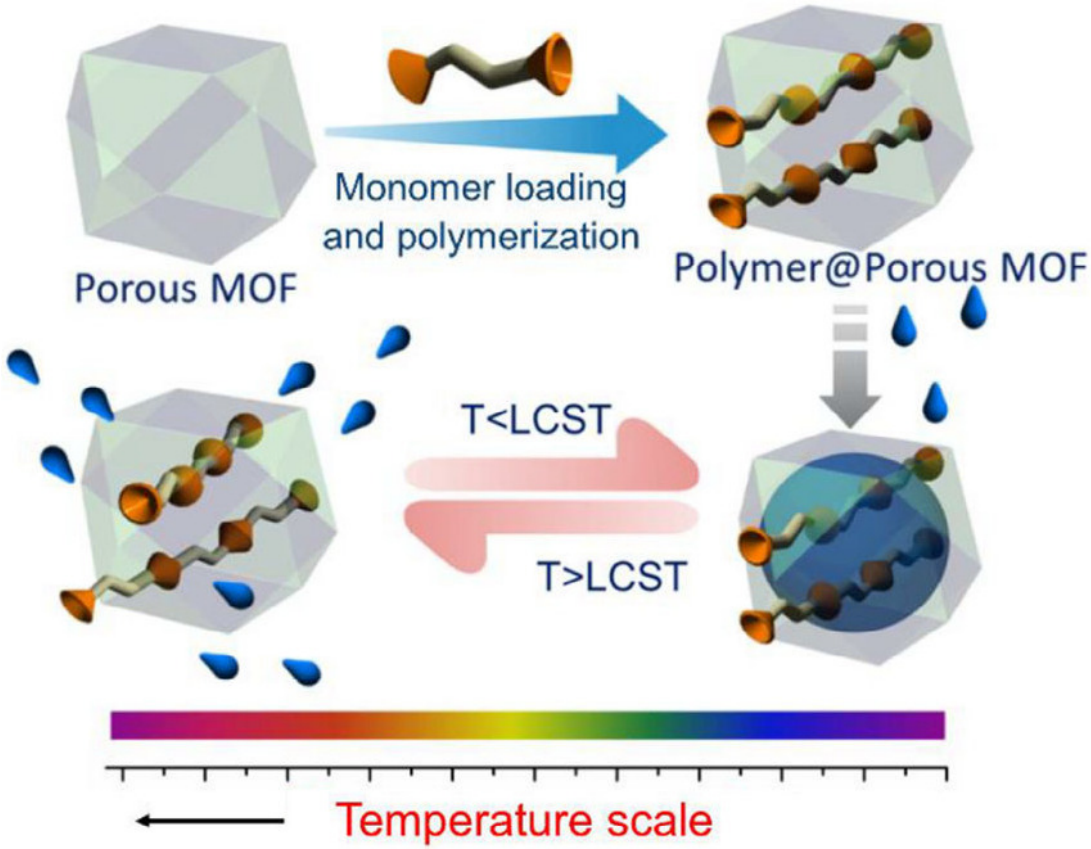
Environmentally-induced changes may be harnessed, thus smart polymers.

Used to:

- encapsulate and release payloads of cells or drugs,
- form gels upon injection in vivo
- for cell sheet engineering.

Example - Thermo-responsive polymer N-isopropylacrylamide (NIPAM)

Used to grow confluent cell sheets and then to detach the intact sheet along with the ECM that the cells have deposited.

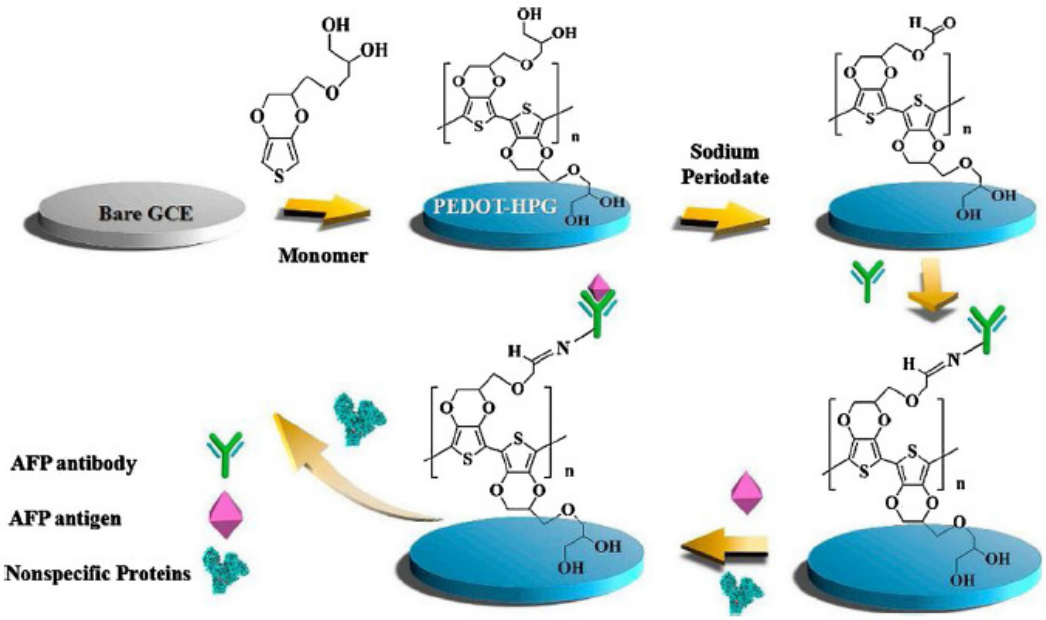


https://doi.org/10.1002/anbr.202100014.

Advanced biomaterials for tissue engineering

Non Fouling Materials:

- Use of chemical surface modifications.
- Initial stage of FBR (foreign body reaction) is the adsorption of a complex layer of biomolecules from body fluids that can be denatured and lead to an immune reaction.
- Non-fouling materials (or stealth materials) resist the adsorption of these proteins.
- One way of producing non fouling materials is by coating with non-specific antifouling materials
- New generations of non-fouling materials - active area of research Zwitterionic polymers, Mixed charged polymers, Polyoxazolines

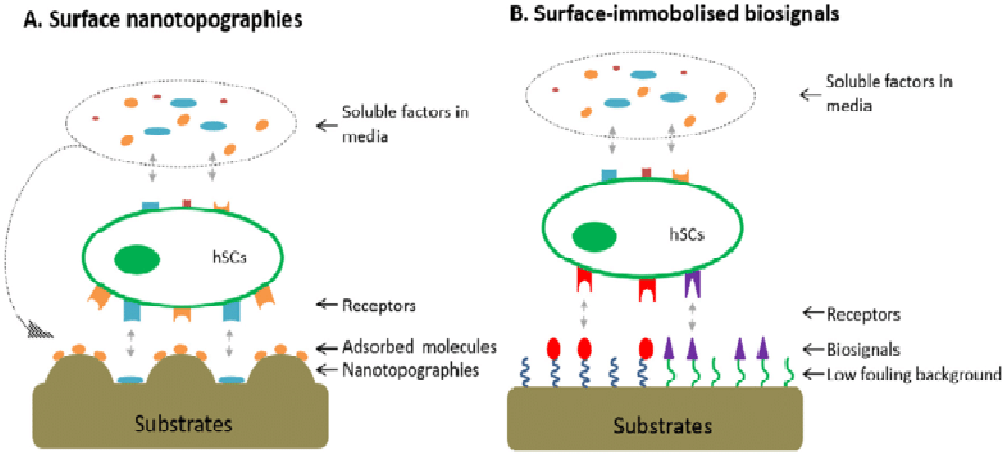


https://doi.org/10.1016/j.porgcoat.2020.105860

Advanced biomaterials for tissue engineering

Bio-functionalized materials:

- It is based on a "blank slate" from the non fouling materials/surfaces.
- Decorated with bioactive molecules, through covalent immobilization.
- These bio-functionalized materials interact with receptors on the cell surface and drive cellular behavior with biological specificity.
- Most common strategy - materials with ligands that engage specific integrin receptors.
- Thus, only cells that express the appropriate integrin are able to adhere to the material.



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