

# Hydrogels, Bio-ceramics, and Polymeric Biomaterials in Regenerative Medicine: Chemical Design, Functionalization, and Biomedical Applications

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## ABSTRACT

Regenerative medicine is an inter-disciplinary scientific field that combines biomaterials science, tissue engineering, biotechnology, stem cell biology, and nanotechnology to promote repair and regeneration of injured tissues. Within the broad class of biomaterials being explored for their regenerative properties, hydrogels, bioceramics, and polymeric materials can be highlighted due to their excellent biocompatibility, physicochemical properties, structural diversity, and capacity to mimic the extracellular matrix. Hydrogels represent hydrated three-dimensional networks useful for encapsulation of cells and delivery of therapeutic agents, while bioceramics, which include hydroxyapatite, tricalcium phosphate, calcium silicate, and bioactive glasses represent bioactive materials capable of interfacing with bone and dental tissue. Polymers, natural or synthetic, complement their mechanical, degradation, and functionalization properties by facilitating the fabrication of composite materials. Recent developments in nanotechnology, stem cell engineering, gene delivery, smart biomaterials, immunomodulation, and three-dimensional bioprinting of biomaterials have further broadened the possibilities of use of these materials for tissue regeneration purposes. In this review, particular attention will be paid to chemical design and functionalization of biomaterials, cross-linking chemistry, chemical structure-property relationship, degradation processes, ion substitution and surface chemistry of bioceramics, and molecular interactions controlling the loading and release of therapeutic molecules from biomaterials.

**Keywords:** Regenerative medicine; Biomaterials; Hydrogels; Bioceramics; Polymeric biomaterials; Tissue engineering; Stem cells; Drug delivery; 3D bioprinting; Biotechnology.

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## 1-Introduction

The rapid advancement of regenerative medicine has placed it among the most important scientific fields of the twenty-first century, revolutionizing traditional treatment methods by emphasizing restoration of the normal architecture and function of tissues rather than addressing their malfunction. [1] Unlike conventional therapies which primarily relieve the symptoms of diseases or substitute the injured tissues with artificial structures, regenerative medicine focuses on harnessing the innate healing mechanisms of the body by using biomaterials, stem cells, bioactive substances, and cutting-edge biotechnology tools. [2] This approach has opened up an entirely new avenue for treatment of a range of illnesses, injuries, deformities, and conditions which cannot be adequately treated with traditional therapies. [3]

The rising number of chronic illnesses, aging population, physical trauma and organ dysfunction have resulted in an increasing demand for novel regenerative treatments. [4] According to recent world health statistics, each year millions of people need tissue repair or organ transplants on account of musculoskeletal system disease, cardiovascular illness, neurological injuries, diabetic complications, and large skin wounds. [5] However, the existing organ donor deficiency, organ rejection after transplantations, post-surgical complications and insufficient performance of artificial organs still remains obstacles to treatment success. [6] Therefore, the creation of biologically active biomaterials that will stimulate tissue regeneration has become one of the primaries aims of current biomedical science. [7-8]

Biomaterials constitute the structural and functional basis of regenerative medicine, as they supply



temporary or permanent scaffolding to cells to attach, grow, migrate, differentiate, and deposit extra-cellular matrix proteins. [9] Biomaterials of the present era are not considered to be just passive scaffold structures but rather act as active components of the biological environment by influencing the immune response, signaling pathways, drug delivery, and vascularization. [10] The emergence of such properties of biomaterials has enabled them to become intelligent therapeutic platforms for orchestrating biological events necessary for tissue regeneration. [11]

The most important classes from the range of different biomaterials being investigated at present are hydrogels, bioceramics, and polymeric biomaterials which are responsible for major developments in the field of regenerative medicine. [12] They are highly versatile, biocompatible, and have good controllable degradation as well as replication of physical and chemical properties of natural extracellular matrices. [13] All these materials have shown tremendous potential in different medical applications such as bone regeneration, cartilage repair, wound healing, cardiovascular tissue engineering, neuroregeneration, corneal replacement, and tendon repair. [14]

The high degree of hydration of hydrogels' three-dimensional polymeric network that closely mimics the extracellular matrix of living tissues has drawn immense attention towards this class of biomaterials. [15] The porous nature of hydrogels facilitates easy passage of oxygen, nutrients, signaling molecules, and metabolic wastes, thus ensuring a favorable environment for cell viability and tissue formation. Additionally, the development of injectable hydrogels, self-healing hydrogels, conductive hydrogels, and stimuli-responsive hydrogels through recent research has significantly enhanced their scope in varied medical treatments. [16] Another important class of biomaterials is bioceramics, which have brought a revolution in the field of hard tissue engineering due to their high bioactivity, osteoconductivity, and chemical similarity to bone minerals. [17] Ceramics made of calcium phosphate

materials, such as hydroxyapatite (HA) and  $\beta$ -tricalcium phosphate ( $\beta$ -TCP), display excellent biocompatibility with bone tissues that allows for direct bonding of the implants to the osseous tissues. Bioactive glasses promote tissue repair through biologically active ion release and subsequent stimulation of angiogenesis, osteogenesis, and mineralization of the extracellular matrix. [18] Though intrinsically fragile and featuring low fracture resistance, modern advances in ceramic technology have greatly widened their application range in medicine. [19]

Among all the biomaterials in regenerative medicine, polymeric biomaterials form the most versatile group due to their varying chemical structures, degradability, processability, and mechanical flexibility. [20]

Naturally occurring polymers like collagen, gelatin, fibrin, chitosan, alginate, hyaluronic acid, cellulose, and silk fibroin contain inherent biological receptors for facilitating cell attachment and tissue reconstruction. [21] But their low mechanical stability and variable nature make them less useful from the perspective of commercial application. [22] On the other hand, synthetic polymers like polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), polyurethane (PU), and polyvinyl alcohol (PVA) offer reproducibility, mechanical strength, and controlled degradation rate. [23]

However, most synthetic polymers lack inherent biological activity and thus have to be modified at their surface or made to contain biological molecules to improve their biological performance. [24]

There has been an increased interest in the development of multifunctional composite biomaterials that combine hydrogels, polymers, ceramics, nanoparticles, and biological components in hybrid materials. [25] The combination of these biomaterials allows exploiting advantages of each material while avoiding its drawbacks. [26] For example, the combination of hydrogels and ceramics provides the material with both hydrated environments needed for cells and mechanical strength. The same is true for polymer-ceramic composites. Indeed, such



combinations have proven to be more effective in terms of regeneration than monocomponent biomaterials. [27]

An important feature of today's regenerative medicine is the approach of biomimicry. Natural tissues are characterized by highly organized hierarchical structures, which consist of extracellular matrix proteins, signals, vascular network, and mechanoreceptive environment. [28] Therefore, nowadays, biomaterials not only substitute absent tissues, but also reconstruct those complex micro-environments. [29] Factors such as scaffold structure, pore size, surface topography, stiffness, degradability, and biofunctionality play an important role in cell adhesion, proliferation, migration, differentiation, and matrix production. [30] The optimization of these factors is an important task in biomaterials engineering. New discoveries in stem cell science have also contributed to the progress in creating regenerative therapies. Mesenchymal stem cells, induced pluripotent stem cells, embryonic stem cells, and tissue-specific progenitors all have the ability of self-renewal and multipotent differentiation. [31] Yet, the success of any stem cell therapy requires the right conditions within the biomaterial microenvironment. Scaffold engineering improves cell viability, directs cell lineage determination, induces vasculogenesis, and reduces inflammation after transplantation. [32] Thus, biomaterials are seen as active participants in stem cell fate control rather than simply physical frameworks. Meanwhile, advancements in molecular biotechnology provide the means for adding many different biologically active components to regenerative scaffolds. [33] Controlled release systems for growth factors, cytokines, antimicrobials, nucleic acids, and other therapeutic molecules have greatly increased tissue regenerative capabilities. [34] Bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF- $\beta$ ) growth factors control complicated cell signaling mechanisms in angiogenesis, osteogenesis, chondrogenesis, and wound healing. [35]

The sustained and localized delivery of these factors prevents systemic toxicity and improves the effectiveness of the treatment. Nano-biotechnology has added a new level of revolution in regenerative medicine. [36] Nanostructured biomaterials are more like the nanoscale structure of extracellular matrix in nature, thus improving protein adsorption and cell signaling. [37] Nanoparticles, nanofibers, nanotubes, nanosheets, and nanocomposites exhibit better mechanical strength, antimicrobial behavior, electrical conductivity, and drug delivery systems compared to traditional biomaterials. Moreover, functional nanoparticles allow for real-time imaging, targeted drug delivery, biosensing, and therapeutic applications of multifunctional devices for precision medicine. [38] 3D printing is regarded as one of the promising technologies to develop personalized tissues with high architecture. [39] In contrast to scaffold manufacture methods, additive manufacturing technology permits the layer-wise deposition of living cells, biomaterials, growth factors, and extracellular matrix constituents based on computer-aided design. [40] It allows the precise arrangement of different cell populations in accordance with the geometry of native tissues. Advances in bioink development, printing resolution, vascularization methods, and bioreactor technologies have been driving the rapid advancement of clinical application of printed tissues, especially for bone, cartilage, skin, heart, and liver regeneration. [41] While significant scientific breakthroughs were made, there are still many key challenges that hinder the clinical application of regenerative biomaterials. The mechanical properties suitable for interaction with natural tissues are hard to obtain, especially in cases where it is required to bear a load, such as bone and cartilage restoration. [42] Insufficient vascularization hampers the delivery of nutrients in large engineered tissues, resulting in hypoxia and low cell viability. Besides, it is challenging to engineer the proper degradation kinetics in line with tissue regeneration. [43] Rapid degradation leads to decreased structural stability, while prolonged presence could lead to development of inflammation or fibrosis.



The role of the immune response of the host to treatment cannot be overlooked, too. [44] Despite extensive research in making biomaterials more biocompatible, scaffolds may cause inflammation reaction, foreign body response, fibrosis or degradation of the biomaterial. The novel biomaterials that can actively control macrophage polarization and inflammatory pathways seem promising in this aspect. [45]

With regard to clinical translation, regulatory approval, scalability, sterilization, manufacturing quality assurance, reproducibility and economic aspects present challenges in translating laboratory findings into clinical application. The problem is that many discoveries in the field of biomaterials do not enter the clinical practice due to lack of safety data and high cost of the process. It is important that biomaterials scientists, biomedical engineers, clinicians, regulatory agencies and biotechnology industry work together to solve the problem. [46-48].

## 2. Essential Properties of Biomaterials

### 2.1 Surface Properties

Performance of a biomaterial from a biological point of view is mostly influenced by surface properties because the surface forms the first contact with the biological environment in which the biomaterial is implanted. [49] Attachment of cells, protein adsorption, activation of platelets, bacterial adhesion, immune response and extracellular matrix formation – all this is controlled by surface chemistry, topography, roughness, wettability, charge and energy of the surface. [50]

Surface roughness exerts a great impact on cellular behavior. Micro- and nano-topographic surfaces are able to greatly increase adhesion, proliferation and differentiation of osteoblasts and at the same time affect macrophages polarization towards regenerative type. Hydrophilic surfaces usually favor protein adsorption in biological conformations and hence improve further cellular interactions. On the contrary, highly hydrophobic materials tend to have bad cell attachment and delayed tissue integration. [51]

Several approaches have been devised for modifying surfaces to enhance biological function without changing the bulk properties of the biomaterials used. The approaches include plasma treatment, chemical grafting, UV irradiation, laser texturing, peptide immobilization, growth factor immobilization, extracellular matrix deposition, and nanoparticle functionalization. Surface modifications have helped achieve angiogenesis, osteogenesis, antimicrobial behavior, and improved implant fixation. [52-54]

### 2.2. Porosity and Scaffold Architecture

The architecture of a scaffold is crucial to its effectiveness in the regeneration of tissues. The optimal scaffold must have an interconnected porous structure which promotes oxygen diffusion, delivery of nutrients, waste disposal, vascular invasion, and distribution of cells within the structure. [55] The porosity affects almost all aspects of the functionality of a scaffold such as its mechanical strength, degradability, permeability, and growth of new tissue within it. In general, higher porosity promotes cellular invasion and vascularization but at the expense of reduced mechanical strength of the scaffold. [56] Pore size is also an important criterion. For bone regeneration, pore sizes between 100-500  $\mu\text{m}$  are required for the movement of osteoblasts and blood vessels, while smaller pores might be better suited for the regeneration of cartilage or skin tissue. Due to recent advancements in additive manufacturing, it became possible to achieve high control over pore geometries, pore connectivity, and scaffold architecture, which has led to improved results. Besides porosity, the anisotropy and hierarchical structure of the scaffold have been shown to be an important design parameter. [57-60] Natural biological tissues possess well-organized nano- and microstructures that dictate mechanical forces and cell alignment.

### 2.3. Bioactivity

Bioactivity is defined as the capability of a biomaterial to actively trigger biological responses that lead to repair and regeneration of tissues. Different from bioinert biomaterials, bioactive biomaterials develop an



active interaction with tissues via ion exchange, protein absorption, molecular signaling, and cell activation. [61] Bioactive glasses are among the first examples of biomaterials with high bioactivity. After their placement into a body, they quickly release biologically active ions, including calcium, silicon, phosphorus, and sodium. Such reactions result in the development of a hydroxycarbonate apatite layer, which is similar in composition to bone mineral matrix. It leads to a strong adhesion between the implant and host tissue and promotes osteogenic differentiation. The same way, calcium phosphate ceramics dissolve in physiological conditions with the release of calcium and phosphate ions involved in the bone mineralization process. [62] Modern biomaterials have been increasingly created for the delivery of several therapeutic factors, including antimicrobial peptides, anti-inflammatory, angiogenic, and osteogenic substances. [63]

#### 2.4. Sterilizability

It is necessary to mention that sterilization is the key point to consider when dealing with all types of implantable biomaterials. As regenerative scaffolds interact with living tissues, they should not contain bacteria, fungi, viruses, endotoxins, or other microorganisms. At the same time, the sterilization process can affect the structure, molecular weight, crystallinity, mechanical properties, degradation process, and biological activity of the biomaterials. [64] Thus, the choice of the method of sterilization should be made based on the composition of the biomaterials. The most widespread sterilization techniques include steam autoclaving, ethylene oxide gas, gamma irradiation, electron beam irradiation, ultraviolet irradiation, supercritical CO<sub>2</sub> sterilization, and low-temperature plasma sterilization. [65] Hydrogels and biodegradable polymers demonstrate sensitivity to high temperatures; thus, low-temperature sterilization is preferred. Ceramic biomaterials have good thermal stability and can be sterilized using traditional steam sterilization method. [66]

#### 2.4 Biological Response to Implanted Biomaterials

biomaterial and comprise a chain of biological reactions that occur in a highly coordinated fashion. It is necessary to study these biological reactions in order to design future regenerative scaffolds that will enable tissue regeneration without adverse effects. The first step in this chain of biological reactions includes rapid adsorption of plasma proteins that form a provisional protein layer at the biomaterial surface right after its implantation and dictate further actions of the cells. [67] The nature of the adsorbed proteins is important for immune recognition and cell attachment.

One of the first immune cells attracted to the place where the implantation occurs is neutrophil, followed by monocytes that differentiate into macrophages. [68] Macrophages are the key players that determine the fate of implants since they are capable of exhibiting different states – from the pro-inflammatory state (M1) to the regenerative one (M2). M1 reaction causes chronic inflammation and fibrotic encapsulation, while M2 macrophages induce angiogenesis, extracellular matrix secretion and remodeling of tissues. [69] Therefore, much attention is paid to the design of immunomodulatory materials able to control the state of macrophages. [70] After the inflammation stage, fibroblasts, endothelial cells, and tissue-specific progenitor cells infiltrate into the scaffold. Vascularization is becoming more and more necessary with the increase in tissue size since oxygen diffusion does not ensure cell viability after 200 µm due to lack of oxygen supply. In that case, the biomaterials that can induce angiogenesis have become critical elements of the latest methods in tissue engineering. Finally, the new extracellular matrix will substitute the original scaffold through the degradation process. Ideally, both processes take place concurrently to form new tissue without leaving any foreign object. [71]

#### 2.5. Design Criteria for Regenerative Biomaterials

The development of regenerative biomaterials involves the consideration of several biological and engineering factors at once. The perfect scaffold should possess such features as:

Biocompatibility but not toxicity. Biodegradation that is coordinated with the regeneration of tissues. Mechanical characteristics corresponding to those of the targeted tissue. High porosity for vascularization, Controlled release of therapeutics, Surface chemistry for cell attachment, non-immunogenicity, easily sterilized and easy to manufacture on a large scale. [72] As there is no one material that could meet all these criteria, multifunctional composite biomaterials, including polymers, ceramics, hydrogels, nanomaterials, and biological components, are extensively used in modern regenerative medicine. [73]

Figure 1: Schematic representation of the development stages of biomaterials from generation one of bioinert materials to generation four of intelligent biomaterials based on biotechnology, nanotechnology, stem cells, gene therapy, and artificial intelligence.

### 3. Hydrogels

#### 3.1 Definition and Historical Development

Hydrogels are three-dimensional networks of hydrophilic polymers that have the ability to absorb and hold significant amounts of water or biological fluids while maintaining their network structure through either physical or chemical cross-linkage. [75]

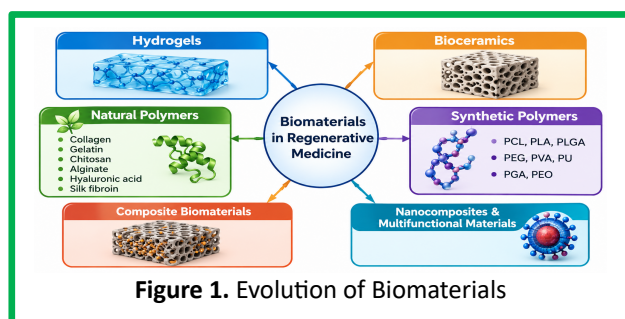


Figure 1. Evolution of Biomaterials

Hydrogel formation enables deposition of the extracellular matrix and hence regeneration of the tissue in physiological conditions. [76]

The idea of hydrogels emerged in the early 1960s when researchers developed poly(2-hydroxyethyl methacrylate) (pHEMA). It was a hydrogel used for ophthalmology purposes in order to create soft contact lenses. This breakthrough proved that polymers can

have both a high amount of water and great biocompatibility along with flexibility. The field of hydrogel technology experienced tremendous development owing to the advances in chemistry, biotechnology, nanotechnology, and tissue engineering. Initially, the use of hydrogels was limited to creation of biomaterials, which were mostly used for wound dressings and drug delivery. However, contemporary hydrogels have become much more functional, as they are able to regulate cell behavior, deliver drugs, respond to stimuli, and regenerate tissue. The use of such substances as stem cells, growth factors, extracellular matrix elements, nanoparticles, and bioactive agents has broadened biomedical capabilities of hydrogels. [77-80]

The emergence of new technologies like intelligent hydrogels, injectable hydrogels, self-healing hydrogels, conductive hydrogels, and bioprintable bioinks has made hydrogels an essential part of current regenerative medicine. The emerging materials are engineered to emulate native tissue structure and respond to physiological stimuli, thus making them more efficient for tissue regeneration. [81]

#### 3.2 Structural Characteristics of Hydrogels

Hydrogels are made up of polymer chains that are cross-linked, forming a porous network that allows the trapping of water molecules through hydrogen bonds, osmotic effects, and capillarity. Even though water can make up to more than 90% of the hydrogel, the polymer network makes sure that no dissolution happens. [82]

The structure of hydrogels is important when it comes to their biological and mechanical properties. Factors like pore size, pore interconnectivity, polymer concentration, cross-linking density, molecular weight distribution, and swelling effects determine nutrient transport, oxygen diffusion, degradation rate, and cell penetration. Hydrogels have the viscoelastic property of having both solid and liquid mechanical behavior. [83] This gives hydrogels the advantage of being able to undergo physiological deformation without mechanical



mismatch with the tissue. [84] Moreover, the mechanical properties of hydrogels may be regulated by changing the polymer chemistry, degree of cross-linking, or adding reinforcing nanofibers or particles. One more important characteristic of hydrogels is that they can incorporate live cells and bioactive substances during their fabrication process. Hydrogel formation being performed at mild physiological conditions ensures the survival of incorporated cells while the activity of the proteins, nucleic acids, extracellular vesicles, and growth factors remains intact. That is why hydrogels are very efficient delivery systems for regenerative therapy, which requires biological stimulation. [85]

### 3.3 Classification of Hydrogels

Hydrogels could be grouped in accordance with the various parameters like polymer source, cross-linking type, biodegradation rate, ionic nature, environmental stimuli response capability and structure. Classification will help in rational design of hydrogels for various biomedical applications. [86]

#### 3.3.1 Classification based on polymer source

On the basis of polymer source, hydrogels could be classified as natural, synthetic and hybrid hydrogels. Natural hydrogels are derived from biological polymers like collagen, gelatin, hyaluronic acid, alginate, chitosan, fibrin, cellulose, dextran, silk fibroin, and agarose. [87] They intrinsically possess biological recognition elements that help in cellular attachment, migration, proliferation and tissue engineering. Additionally, structural homology of these biomaterials to extracellular matrix helps in increasing cell-material interaction and avoids immunological problems. [88] However, naturally derived hydrogels suffer from poor mechanical properties, fast enzymatic degradation, inconsistency in production batch, and poor structural integrity. [89] Thus, chemical modification or composites preparation is often used to enhance their mechanical property without affecting their biological performance. Synthetic hydrogels could be prepared from engineered polymers like polyethylene glycol

(PEG), polyvinyl alcohol (PVA), polyacrylamide (PAAm), poly(N-isopropylacrylamide) (PNIPAAm), polyethylene oxide (PEO), and poly(2-hydroxyethyl methacrylate) (pHEMA). [90] The above-listed polymers exhibit outstanding reproducibility, controllable degradation behavior, tuneable mechanical properties, and outstanding scalability of the manufacturing process. Nevertheless, there is no cell-adhesion recognition sequence within most synthetic polymers; hence, additional peptide or protein modification of such substrates can be required in order to improve the cellular adhesion and biological activity. [91] The hybrid hydrogels incorporate both natural and synthetic polymers, resulting in highly versatile structures that offer outstanding biological performance together with superior mechanical stability. Such materials are widely used for complex tissue engineering. [92]

#### 3.3.2 Classification According to Crosslinking Mechanism

Another way of classifying hydrogels is based on the principle governing their network formation into physically crosslinked and chemically crosslinked materials.[93]

Physically crosslinked hydrogels form via reversible interactions which include hydrogen bonding, ionic bonding, crystallization, hydrophobic interactions, host-guest interactions, and electrostatic interaction. The mild reaction conditions used during their formation render these hydrogels highly favorable for encapsulation of living cells and temperature-sensitive therapeutics.[94] Besides, their reversibility gives them the injectable and self-healing properties. However, mechanically, physically crosslinked hydrogels are relatively weak since they are not stable in physiological environments due to reversible cross-linking. [95]Chemically crosslinked hydrogels are made up of permanent covalent linkages which provide high mechanical integrity, dimensional stability, and slower degradation. Different approaches such as free radical polymerization, click chemistry, enzymatic crosslinking, Michael addition, Schiff base reaction, and photo-

**Table 1:** Classification of Hydrogels According to Polymer Origin

| Hydrogel Type       | Representative Materials                               | Advantages                                                | Limitations                                 | Typical Applications                                 |
|---------------------|--------------------------------------------------------|-----------------------------------------------------------|---------------------------------------------|------------------------------------------------------|
| Natural Hydrogels   | Collagen, Gelatin, Alginate, Hyaluronic Acid, Chitosan | Excellent biocompatibility, ECM mimicry                   | Weak mechanical strength, rapid degradation | Skin, cartilage, wound healing                       |
| Synthetic Hydrogels | PEG, PVA, PAAm, PNIPAAm, pHEMA                         | Tunable properties, reproducibility, mechanical stability | Limited biological activity                 | Drug delivery, cartilage engineering                 |
| Hybrid Hydrogels    | GelMA/PEG, Collagen/PCL, Alginate/PEG                  | Combines bioactivity with mechanical reinforcement        | More complex fabrication                    | Bone, osteochondral and composite tissue engineering |

polymerization offer controlled synthesis of chemically crosslinked networks. [96]

### 3.3.3 Classification of Hydrogels According to Stimuli Responsiveness

One of the major breakthroughs in hydrogel science is the development of stimuli-responsive (smart) hydrogels, which exhibit reversible physicochemical changes in response to certain stimuli. Unlike traditional hydrogels, which do not change their structure once implanted in the body, smart hydrogels adapt their swelling capacity, degradability, mechanical properties, permeability, and release profile to certain physiological stimuli or external factors. This makes them more similar to biological tissues and increases the effectiveness of therapies.[97]

Stimuli-responsive hydrogels can be classified based on the type of stimulation into physical, chemical, and biological responsive hydrogels. Physical stimuli include temperature, light, magnetic fields, electric fields, ultrasound, and mechanical stress. The list of chemical stimuli includes pH change, ionic strength, oxidative stress, and glucose concentration. Examples of biological stimuli are enzymes, antigens/antibodies reactions, inflammatory factors, and cell metabolites. [98]

In this category of smart materials, thermo-responsive hydrogels have gained considerable interest due to their liquid nature at room temperature, but a fast change to the gel phase upon heating at physiological

temperatures (37 °C). Thermosensitive polymers, such as PNIPAAm, have a low critical solution temperature (LCST) making them easily injectable and forming a gel in situ. [99] Injectable systems do not require complicated surgical intervention, and, in addition, these systems allow for complete filling of defects of irregular shapes. In pH-sensitive hydrogels, there are ionizable groups that can accept or donate protons depending on environmental pH. Electrostatic repulsion in the polymer network causes either swelling or shrinking depending on pH, thus regulating the rate of drug release and degradation. [100] This kind of hydrogel is useful for delivery of drugs specifically to pathological sites, including tumors, chronic wounds, inflamed tissues, or diseases of the gastro-intestinal tract characterized by abnormal pH.

Another type of smart biomaterial is enzyme-responsive hydrogels. This is due to the fact that pathologically altered tissue overexpresses MMPs, hyaluronidases, collagenases, and proteolytic enzymes, which allows the hydrogels containing enzyme-sensitive peptide cross-linkers to be degraded specifically in the pathological tissues. Such smart material provides specific therapeutic delivery without the need for systemic toxicity. [101].

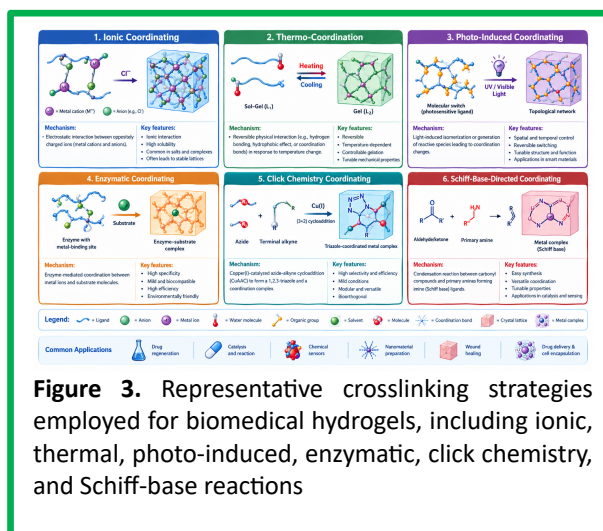
Conductive hydrogels are one of the latest promising materials used to create electroactive tissues such as heart muscles, skeletal muscles, and neurons. Conducting polymers, graphene analogues, carbon nanotubes, or metallic nanoparticles allow to transmit

**Table 2.** Comparison Between Physical and Chemical Crosslinking Mechanisms

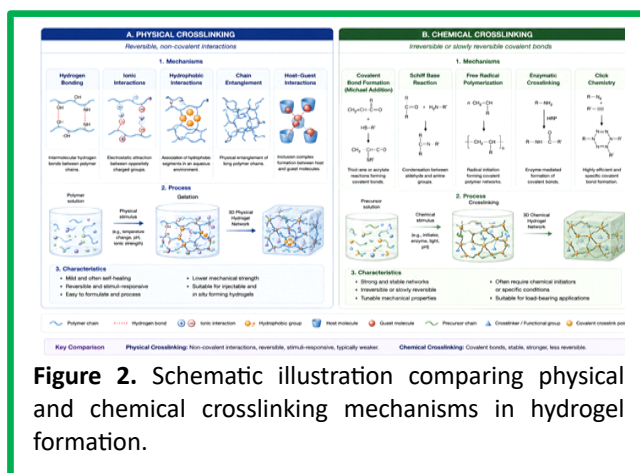
| Feature                | Physical Crosslinking                    | Chemical Crosslinking                  |
|------------------------|------------------------------------------|----------------------------------------|
| Bond type              | Non-covalent                             | Covalent                               |
| Reversibility          | Reversible                               | Permanent                              |
| Mechanical strength    | Moderate                                 | High                                   |
| Cytocompatibility      | Excellent                                | High (depends on chemistry)            |
| Fabrication conditions | Mild                                     | Mild to moderate                       |
| Stability              | Limited                                  | Excellent                              |
| Self-healing potential | High                                     | Moderate–High                          |
| Typical examples       | Alginate, Gelatin, Agarose               | GelMA, PEGDA, Methacrylated polymers   |
| Major applications     | Injectable hydrogels, cell encapsulation | Bioprinting, cartilage, bone scaffolds |

an electric signal without losing the flexibility of the hydrogel. It was found that electrical signals through these conductive networks are able to synchronize cardiomyocytes, promote differentiation of neurons, and skeletal muscles maturation. [102].

Magnetically responsive hydrogels using superparamagnetic iron oxide nanoparticles further provide an avenue for the modulation of drug delivery, mechanostimulation, hyperthermia treatment, and imaging from afar. The use of external magnetic fields allows one to control scaffold deformation, molecular transport, and cellular mechanotransduction non-invasively and precisely. [103]



**Figure 3.** Representative crosslinking strategies employed for biomedical hydrogels, including ionic, thermal, photo-induced, enzymatic, click chemistry, and Schiff-base reactions



**Figure 2.** Schematic illustration comparing physical and chemical crosslinking mechanisms in hydrogel formation.

### 3.5 Preparation Methods of Hydrogels

The synthesis of hydrogels depends on the nature of the chemical structure of the precursor polymers, the desired mechanism of cross-linking, the target tissue application, and the physical and biological properties that are needed. [104]

Choosing an optimal approach for the synthesis of hydrogels is vital especially in the field of regenerative medicine due to the fact that the conditions used in their synthesis can have direct effects on the polymer's structure, porosity, degradation rate, cell viability, and bioactive molecule stability. [105].



### 3.5.1 Solvent-Based Fabrication

Solvent-based hydrogel formation is achieved through dissolution and/or dispersion of the polymer materials in an appropriate solvent and then through cross-linking or solvent removal or phase transformation. The process is quite simple and can be used with a variety of both artificial and natural polymers. Yet, the presence of solvent is a major drawback if the final hydrogel is meant to be implanted. Hence, the choice of solvent and further purification become essential. [106]

### 3.5.2 Bulk Polymerization

Bulk polymerization results in the formation of hydrogels by polymerizing and cross-linking monomers or macromers in the whole volume of the precursor. It yields relatively homogeneous network and facilitates the control of the concentrations of polymers and cross-linking. Nevertheless, heat production during polymerization and the possible cytotoxicity of unreacted monomers/initiators might influence encapsulated cells. [107].

### 3.5.3 Freeze-Drying and Cryogelation

Freeze-drying is widely used for the preparation of highly porous hydrogels. Solvent crystals are formed during the freezing process and serve as temporary porogens. Subsequent sublimation results in the formation of interconnected pores in the polymer matrix. It is possible to control the structure of the pores using polymer concentration, freezing temperature, freezing rate, and drying parameters. [108] The method of cryogelation involves cross-linking at partially frozen state. Macropores are formed by ice crystals whereas polymer chains are concentrated in the unfrozen phase, thus resulting in highly porous scaffolds with good fluid transport and cellular invasion capacity. Interconnected structure of the cryogels is especially appealing for applications where rapid cellular migration and vascularization are required. [109] However, relatively low mechanical properties of highly porous cryogels may necessitate the use of reinforcing nanomaterials, fibers, ceramics, or polymer network.

### 3.5.4 Microfluidic Fabrication

The field of microfluidics technology makes it possible to create hydrogel microspheres, microfibers, and sophisticated microstructured materials. The control over fluid flows makes it possible to control the size, shape, composition, and number of cells encapsulated in these hydrogels. Hydrogel microspheres created by means of microfluidic technology have great potential in drug delivery, cell delivery, and modular tissue engineering. [110]

### 3.5.5 Injectable In Situ Gelation

Intracellular injectable hydrogels are intended to undergo a gelation reaction in situ after their application to the defective site. The method is especially effective for minimally invasive procedures since the precursors are injected in solution through a catheter and then gel in situ. [111] Gelation is initiated through thermal, ionic, enzymatic, photoinduced, and dynamic covalent chemical reactions.

The gelation reaction should take place fast enough not to allow the hydrogel to disperse while allowing the accurate delivery and filling of the defect site. Injectable hydrogels are especially useful in the case of defects with irregular shapes since such sites could hardly be treated by prefabricated scaffolds. [112]

### 3.5.6 Three-Dimensional Bioprinting

Three-dimensional bioprinting is an example of highly advanced methods for the creation of hydrogels. This technique is characterized by sequential layer-by-layer deposition of cell-containing hydrogel compositions, which are called bioinks according to computer models. [113] Various extrusion-, inkjet-, and light-based bioprinters were widely studied. Extrusion bioprinting appears especially convenient for the deposition of viscous hydrogels, while the ability to deposit cell-containing materials with high density is a main advantage of this approach. High-speed printing and good control of droplets size are main advantages of inkjet bioprinters, while digital light processing and stereolithography techniques offer very high spatial resolution due to the process of localized



photopolymerization. [114] The main problem is to balance printability and biological activity of bioinks. Higher concentrations of polymers and crosslinks increase structural fidelity but decrease cell migration, diffusion of nutrients, and remodeling. [115]

### 3.6 Nanocomposite Hydrogels

Nanocomposite hydrogels use nanofillers that are added to polymer networks for the improvement of mechanical, biological, electrical, optical, antimicrobial, or drug delivery properties. This approach helps to solve one of the key problems of the traditional hydrogels – low mechanical strength. Nanoparticles such as hydroxyapatite, silica, gold, silver, iron oxide, and bioactive glass can be added to hydrogels to provide specific biological features. Carbon nanomaterials such as graphene derivatives and carbon nanotubes are especially effective for electrical and mechanical strengthening of hydrogels. Hydroxyapatite-containing hydrogels attract much attention from researchers due to their resemblance to the mineral components of natural bones. [116] The combination of hydroxyapatite with collagen, gelatin, alginate, or synthetic polymers makes it possible to obtain biomimetic scaffolds with high osteogenic activity. The silver-containing hydrogels are also a good example of nanocomposite materials. The nanoparticles of silver have bactericidal properties that can be used in wound healing and prevention of implant infections.

Nevertheless, excessive amounts of silver in hydrogels can cause cytotoxic effects. [117]

The application of magnetic nanoparticles such as iron oxide nanoparticles allows for magnetic targeting, imaging, stimulation, and heating of tissues. Their use in hydrogel can thus help develop multi-functional systems able to combine regeneration with diagnostic and therapeutic functions. [118]

### 3.7 Hydrogels for Drug and Growth-Factor Delivery

One of the major uses of hydrogels in regenerative medicine is controlled drug delivery of bioactive molecules. In conventional systemic drug delivery, there is a fast clearance, poor targeting, and undesired systemic side effects. These problems could be solved by delivering bioactive molecules using hydrogel delivery systems that offer targeted and sustained drug release in the damaged tissue area. Drug release can occur via diffusion, hydrogel swelling, polymer degradation and erosion, and stimulation-induced release. [119].

Growth factors are among the cargo molecules of great importance. Bone morphogenetic proteins promote osteogenic differentiation; vascular endothelial growth factor stimulates angiogenesis; fibroblast growth factors promote cell proliferation and vasculogenesis; while transforming growth factor- $\beta$  helps in cartilage formation and extracellular matrix formation. [120]

**Table 3.** Comparison of Major Hydrogel Fabrication Techniques.

| Fabrication method        | Main principle                                    | Major advantages                          | Principal limitations        | Representative applications        |
|---------------------------|---------------------------------------------------|-------------------------------------------|------------------------------|------------------------------------|
| Solvent-based fabrication | Polymer dissolution followed by network formation | Simple, versatile                         | Residual solvent concerns    | Drug delivery, polymeric scaffolds |
| Bulk polymerization       | Polymerization throughout precursor volume        | Homogeneous networks                      | Heat and initiator effects   | Tissue engineering                 |
| Freeze-drying             | Ice templating followed by sublimation            | High porosity                             | Limited mechanical strength  | Wound healing, tissue scaffolds    |
| Cryogelation              | Crosslinking under partially frozen conditions    | Macroporosity, rapid fluid transport      | Complex processing           | Cell delivery, tissue regeneration |
| Microfluidics             | Controlled fluid manipulation                     | Precise particle size and morphology      | Specialized equipment        | Cell encapsulation, drug delivery  |
| Injectable gelation       | Gel formation at target site                      | Minimally invasive, defect conformability | Gelation control required    | Bone, cartilage, cardiac repair    |
| 3D bioprinting            | Layer-by-layer deposition                         | Precise architecture, personalization     | Bioink optimization required | Complex tissue engineering         |



However, growth factors have an action only at low concentrations and lose their biological activity quickly in physiological conditions. So, their direct administration requires higher amounts which increases the probability of adverse effects. [121]

The hydrogel encapsulation provides both the protective environment as well as a localized delivery, and this might decrease the necessary dose of the therapy. More sophisticated approaches include using several growth factors and their release at a specific time point. For instance, first the angiogenesis factor is released, and after that osteogenesis factor is delivered. [122]

### 3.8 Hydrogel-Based Gene Delivery

The introduction of nucleic acids in hydrogels has widened the scope of use beyond traditional proteins and drugs delivery. Gene-activated hydrogels are capable of delivering DNA, mRNA, microRNA, siRNA, or CRISPR-associated agents locally to control the cellular processes. Gene therapy can induce endogenous cells to synthesize therapeutic proteins at the site of application, thus eliminating the requirement of continuous injection of recombinant growth factors. [123] Angiogenic genes will stimulate the development of blood vessels, while osteogenic genes will facilitate bone formation. Inflammation regulation by RNA therapy can further improve the regenerative environment. The hydrogel matrix protects the genetic material from rapid degradation and allows sustained delivery. Cationic polymers, [124] lipid nanoparticles, inorganic nanoparticles, and peptides can also help to increase cell uptake efficiency. Still, safety should be a priority since uncontrollable gene expression, immunogenic reactions, adverse off-target effects, and persistence of genetic material require thorough evaluation prior to the clinical trials. [125].

### 3.9 Stem-Cell Encapsulation in Hydrogels

Hydrogels represent an extremely advantageous microenvironment for 3D stem-cell culture and transplantation. [126] The hydrated structure allows cells to remain embedded in a matrix that mimics the

native extracellular matrix. Mesenchymal stem cells (MSCs) have been widely studied in the context of hydrogel-based regenerative medicine owing to their multilineage differentiation potential and immunomodulatory functions. Stiffness, ligand density, degradation rate, and mechanical relaxation of the hydrogels can affect the MSC differentiation into osteogenic, chondrogenic, adipogenic, or other lineages. [1]

Furthermore, hydrogel encapsulation could be beneficial in protecting transplanted cells from mechanical trauma during transplantation. [127] Nevertheless, proper functioning of the cells requires sufficient nutrient and oxygen supply. For this reason, angiogenic signaling molecules, microvessel channels, oxygen-generating elements, or prevascularized cell populations have been increasingly utilized. One of the key benefits of using hydrogels for cell delivery is the ability to localize cells within the defect without any uncontrolled migration. Nonetheless, long-term survival and functional engraftment of transplanted cells still pose significant challenges. [128]

### 4. Bioceramics in Regenerative Medicine

Bioceramics constitute a significant category of inorganic biomaterials that have been developed for interaction with biological tissues. [129] Chemical similarity to the mineral phases of the hard tissues combined with bioactivity and osteoconductivity makes these ceramics a key material used in bone and dental regeneration applications. Unlike other polymeric materials, bioceramics are able to play an active role in the mineralization process and induce the formation of bone-like apatite on the ceramic surface. The biological activity of these materials depends significantly on the chemical composition, crystallinity, specific surface area, porosity, grain size, and degradation. The most thoroughly studied bioceramics are hydroxyapatite (HA), beta-tricalcium phosphate ( $\beta$ -TCP), biphasic calcium phosphate (BCP), calcium silicates, calcium carbonates, and bioactive glasses.



#### 4.1 Hydroxyapatite

Hydroxyapatite is a calcium phosphate ceramic with a chemical structure similar to that of the inorganic mineral content in human bones. [130] The material's high biocompatibility, osteoconductivity, and affinity to bond with bone tissue make it among the most investigated biomaterials for skeletal tissue engineering. Synthetic hydroxyapatites can be prepared by means of precipitation, sol-gel, hydrothermal, combustion, and other routes. Synthesis conditions can be controlled in order to tailor particle morphology, crystalline structure, surface area, and stoichiometry. [131] Nano-hydroxyapatites are specifically interesting due to the similarity of their small scale structure to mineral nanocrystals naturally existing in collagen fibrils in bone. Such structures can enhance protein binding and cell interactions and provide nucleation sites for additional mineral formation. However, hydroxyapatite shows rather low degradation rate and poor mechanical strength. Therefore, it is commonly used in combination with degradable polymers or hydrogels to create composites.

#### 4.2 Biphasic Calcium Phosphate

Combination of hydroxyapatite and  $\beta$ -TCP ceramics in a single biphasic system known as biphasic calcium phosphate (BCP) allows the combination of relative stability of hydroxyapatite with faster resorption of  $\beta$ -TCP. Adjustment of the HA/ $\beta$ -TCP ratio allows control of the degradation, mechanical, surface chemical and biological properties of the material. More HA in the BCP composition results in increased stability, whereas more  $\beta$ -TCP accelerates the material resorption. Thus, BCP is a very versatile material for bone regeneration applications. Its composition can be selected based on the required regeneration rate, defect dimensions, and mechanical environment. The performance of BCP is greatly affected by its porosity. The presence of highly inter-connected pores facilitates vascular invasion and cell infiltration, but decreases the mechanical strength of the scaffold. Therefore, BCP scaffolds used for load-bearing purposes are usually reinforced with polymers or other components. [135]

angiogenesis, whereas strontium-containing compositions support bone formation by suppressing excessive bone resorption. Copper-containing compositions may provide additional angiogenic and antimicrobial properties, whereas zinc and magnesium ions affect cell proliferation and mineralization. [138].

The biological activity of bioactive glasses results from surface chemical reactions that take place in a series of events. After the material is immersed in physiologically charged fluids, there is exchange of ions at the surface of the glass. The next step includes hydrolysis, deposition of silica layers, calcium and phosphate precipitation, and finally the growth of an apatite-like phase. [139]

#### 4.4 Calcium Silicate-Based Bioceramics

The properties that have made calcium silicate ceramics (e.g., wollastonite and other related phases) attractive are their good biocompatibility as well as the capacity to liberate biologically active calcium and silicon ions. Silicon is a trace element which plays a significant role in the process of bone mineralization and connective tissue formation. Silicon-containing ion liberation is able to enhance the osteogenic behavior and affect the angiogenesis-related signaling pathways. Moreover, calcium silicate materials showed their promising biomedical application in dentistry for endodontic treatment as well as pulp regeneration due to apatite-forming property. [140] Nevertheless, some formulations of calcium silicate possess relatively high alkalinity and fast degradation rates.

#### Surface Reaction Mechanisms of Bioactive Glasses

Surface chemistry of bioactive glasses plays an important role in their bioactivity. In contact with aqueous physiological environment, network-modifying ions like  $\text{Na}^+$  and  $\text{Ca}^{2+}$  may be replaced with  $\text{H}^+$ / $\text{H}_3\text{O}^+$  ions of the solution, after which hydrolysis of unstable  $\text{Si-O-Si}$  linkages takes place. This process forms the hydrated silica-rich surface and leads to the formation of a calcium- and phosphate-containing layer. Formation of the carbonate-containing apatite layer then results in



formation of the chemically active interface between glass and mineralized tissue. Glass composition, network structure, and ion content determine dissolution rates and composition of the released components. [196, 201, 203, 204]

### Chemistry–Structure–Property Relationships in Bioceramics

Comparative analysis for ion-substituted bioceramics may include: the  $\text{Sr}^{2+}/\text{Mg}^{2+}/\text{Zn}^{2+}$  substitutions at the  $\text{Ca}^{2+}$  position alter the coordination structure; the  $\text{CO}_3^{2-}$  substitutions at the  $\text{OH}^-$  or  $\text{PO}_4^{3-}$  position affect the crystalline and solubility properties; silicate additions alter the phosphate-based structure and dissolution property; and the changes in the surface chemical and ion release properties affect interfacial mineralization. These structural properties and applications will guide the comparative analyses of ion-substituted bioceramics in regenerative medicine.

### Conclusions

Hydrogels, bio ceramics, and polymeric materials are three of the essential pillars of contemporary regenerative medicine. While all of them have unique features, no one of them is capable of replicating in its entirety the structure, composition, physical, and biological characteristics of natural tissues. Hydrogels provide highly hydrated and biocompatible substrates that are especially useful in the context of regeneration of soft tissues, cell encapsulation, drug delivery, and 3D bioprinting. Bioceramics feature mineral bioactivity and osteoconductivity, making them vital for the regeneration of bone and dentine tissues.

Limitations of individual materials have encouraged the development of composite materials, such as polymer–ceramic composites, hydrogel–ceramic systems, natural-synthetic polymer blends, nanocomposite hydrogels, and multifunctional bioinks. At the same time, the field is progressing towards more complex manufacturing techniques. Bioprinting allows for precise spatiotemporal arrangement of biomaterials and cells, whereas nanotechnology gives a nanometer-scale level of control over mechanical, biological,

electrical, and antimicrobial properties. Artificial intelligence may be used to accelerate the development of biomaterials and optimize manufacturing parameters. Still, many problems need to be addressed, including inadequate vascularization, mechanical mismatch, degradation-regeneration disequilibrium, infection, immune response, manufacturing variability, long-term safety, and regulatory complexity. Thus, future regenerative biomaterials should be developed not as inert scaffolds but as active biological microenvironments that can control cellular activity, extracellular matrix synthesis, vascularization, immune system response, and tissue remodeling. Molecular chemical design becomes a key unifying factor in this approach: functional group chemistry, cross-linking reaction, polymer degradation, surface chemistry, and ionic substitution allow for controlling the properties of regenerative biomaterials at the molecular level.

Hybrid polymer–ceramic compositions, hydrogel–ceramic constructs, natural-synthetic hybrid polymer systems, nanocomposite hydrogels, and multifunctional bioinks can integrate various advantageous properties into one platform for regenerative engineering. In addition to that, the area is developing in the direction of the implementation of more sophisticated technologies of production. 3D bioprinting allows for the precise spatial organization of biomaterials and cells, and nanotechnology allows controlling properties at the nanolevel in terms of mechanical, biological, electrical, and antimicrobial properties. AI-aided design may serve as an opportunity to accelerate the biomaterial discovery process and optimize production conditions. However, there are several important obstacles that have not been addressed so far. They are inadequate vascularization, mechanical incompatibility, imbalance between degradation and regeneration, infections, immune responses, manufacturing variability, long-term safety, and complexity of regulation. Future biomaterials for regenerative medicine should therefore not act as passive scaffolds.



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