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Development And Formulation Of Drug Loaded Hydrogel For Bone Regenerative Potential

S. Aravind[1], Dr. T. S. Shanmugarajan[2]

[1,2]*Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science, Technology, And Advanced Studies (VISTAS). Pallavaram-600117, Chennai, Tamil Nadu, India,*
[1]*Email: aravindsenguttuvan15@gmail.com*

***Corresponding Author**: Dr. T. S. Shanmugarajan*
**Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science, Technology, And Advanced Studies (VISTAS). Pallavaram-600117, Chennai, Tamil Nadu, India,*
[1]*Email: hodpceutics@velsuniv.ac.in*

Abstract

Bone defects resulting from trauma, disease, or congenital abnormalities represent a significant clinical challenge, necessitating advanced regenerative therapies. This study presents the development and formulation of a drug-loaded hydrogel as a novel approach for bone regeneration. The hydrogel matrix is engineered to provide structural support and controlled release of therapeutic agents to enhance bone healing. Various biocompatible polymers and crosslinking strategies are investigated to optimize the hydrogel's mechanical properties, degradation kinetics, and drug release profiles. Furthermore, the study explores the efficacy of different drugs, growth factors, and osteoinductive molecules in promoting osteogenesis and bone tissue regeneration within the hydrogel scaffold. The developed drug-loaded hydrogel holds promise as a versatile platform for addressing diverse bone defects and advancing the field of regenerative orthopedics.

Keywords: Drug-Loaded Hydrogel, Tissue Regeneration, Biocompatibility, Gelation Kinetics, Hydrogel Scaffold.

INTRODUCTION

Bone is a highly vascularized, active tissue. The mechanical characteristics of bone are determined by its structure, minerals, and extracellular matrix (ECM) environment [1]. Collagen type I makes up the majority of a bone's extracellular matrix (ECM), making up roughly 90% of the total protein content of a bone. For bone structure, collagen I serves as a supporting scaffold. A little percentage of the bone extracellular matrix (ECM) is made up of non-collagenous proteins such as sialoprotein, polysaccharides, osteonectin, osteocalcin, osteopontin, and fibronectin. About 65 percent of the total mass of bone is made up of inorganic minerals like carbonated apatite and hydroxyapatite (HA). The primary cell types in the bone are osteocytes, osteoblasts, osteoclasts, and osteoprogenitors. In the bone, each type of cell has a specific purpose [2, 3]. Bone integrity is preserved by ongoing remodeling in bone tissue. The absence of \regeneration guarantees the restoration of bone structure and function over time. Small bone defects, self-healing after bone remodeling. However, bone remodeling cannot correct severe traumatic injuries and deficiencies caused by tumors, congenital disorders, and infectious diseases [4,5].

TISSUE ENGINEERING

The field of bone tissue engineering aims to induce new functional bone regeneration through the synergistic combination of biomaterials, cells, and factor therapy. Recent advances in this area focus on developing biomaterial scaffolds, cell-based research, and innovative approaches to enhance bone regeneration [6]. Challenges in bone tissue engineering include issues like insufficient vascularization at defect sites, which drive future research efforts in the field. Studies explore the use of osteogenic cells, scaffolds, and growth factors to regenerate bone defects, particularly in the oral cavity, highlighting the importance of viable cells and signaling pathways in bone repair and regeneration. The review articles emphasize the significance of understanding cellular signaling in skeletal remodeling and the potential impact of growth factors on osteogenic signaling cascade for bone tissue replacement and regeneration [6]. Overall, these studies underscore the promising advancements and ongoing challenges in tissue engineering for bone regeneration, paving the way for innovative solutions in the field.

BONE TISSUE ENGINEERING SCAFFOLDS

Researchers have shown a great deal of interest in scaffold-based BTE. BTE makes it possible to create implants that include scaffolds, cells, and mechanical/soluble components, providing a longer-term, more sustainable approach to bone reconstitution. In order to support biological delivery and tissue regeneration, scaffolds' fundamental job is to balance transient mechanical functions with mass transfer [11].
Using digital light processing-based 3D printing, scaffolds made of photo-crosslinkable bioglass reinforced akermanite that resemble the Haversian bone were created in a single step, avoiding the cytotoxicity associated with UV or chemical crosslinking. This work demonstrated how changing the scaffold's characteristics could result in a range of mechanical and porosity properties for various ages and illnesses [12]. Biocompatibility in terms of cell attachment and proliferation as well as lack of toxicity and inflammatory reactions, biodegradability for programmed safe substitution of the scaffold material with osteoid, deposition, mechanical properties to bear weight during the amelioration period, proper architecture in terms of porosity and pore sizes for cell penetration, nutrients and waste transfer, and angiogenesis, sterilibility without loss of bioactivity and controlled deliverability of bioactive molecules or drugs [11,12].

BONE WOUND HEALING

A broken bone has the capacity to mend and regenerate. However, delayed fracture healing (also known as delayed union), malunion, or non-union occur in about 10% of fractures. In these situations, the patients endure excruciating pain and eventually need medical attention to help the fracture mend. fracture characteristics, which means that they can be used to create bone tissues with different strengths and architectures to treat patients whose healing can be divided into primary and secondary healing. While secondary healing necessitates minimizing motion (e.g., casting the fracture site), primary healing requires rigid fixation since it can only occur in the total absence of motion at the fracture site. Callus development is aided by reduced mobility at the fracture site during secondary healing. The sequence of events that takes place during fracture healing and bone development has been extensively studied at the cellular and molecular levels. These discoveries have broadened our knowledge of bone repair and may further advance surgical and therapeutic strategies for promoting the repair of damaged bone.[14] The sequence of events that takes place during fracture healing and bone development has been extensively studied at the cellular and molecular levels. These discoveries have raised our knowledge of bone healing and could lead to the development of new surgical and treatment approaches that encourage the restoration of broken bone. [13-14]

SECONDARY HEALING

Secondary healing involves endochondral (EC) ossification, which mediates the stabilization of the injury and restoration of damaged vasculature before regeneration of the tissue during the fracture healing process. This fracture healing process can be divided into 3 overlapping phases: inflammatory, reparative, and remodeling.
where intramembranous (IM) and EC ossification occur during the reparative phase.10 Various cellular components are recruited at different stages in response to growth factors and cytokines. In humans, the inflammatory phase lasts approximately 1 week whereas in mice, the inflammatory phase lasts less than 4 days.11 The damaged vasculature and bone marrow facilitate the influx of primitive mesenchymal stem cells (MSCs) into the fracture site.10 During hemostasis, platelets release transforming growth factor b (TGF-b) and

platelet-derived growth factor (PDGF) for the stimulation and chemotaxis of undifferentiated MSCs and macrophages. Macrophages are initially recruited to remove debris, necrotic tissue, and pathogens at the site of injury [14]. A recent study demonstrated that fracture healing is impaired in macrophage-depleted mice wherein EC ossification is altered.

HYDROGEL

Hydrogels are crosslinked polymer chains with three-dimensional (3D) network structures that can absorb large amounts of fluid, making them suitable for applications in agriculture, biomaterials, the food industry, drug delivery, tissue engineering, and regenerative medicine. Hydrogels can be tailored for biomedical applications by adjusting their biodegradability and biocompatibility through modifications of functional groups or incorporation of natural polymers [46]. The properties of hydrogels, such as surface properties, water content, swelling behavior, nature of the polymer, ionic content, and thermodynamics, influence their biomedical usage and applicability in various fields [47]. Intelligent or environment-sensitive hydrogels continue to be important materials for medical applications due to their unique properties and versatility in drug delivery systems, tissue engineering, wound dressings, immunoisolation, and more [47].

HYDROGEL BASED ON BONE REGENERATION

Hydrogels are 3D networks comprising of cross-linked polymer chains with high water absorbability. Owing to the hydrophilic nature and high-water content, hydrogels have been considered as attractive vehicles for encapsulating and delivering cells bypassing cellular post-processing and seeding problems (i.e. non-uniform cellular distribution and deficient cell seeding) [15]. The 3D cultural microenvironment of hydrogels can mimic the native extra cellular matrix (ECM), and thus, are favorable for encapsulating cells such as chondrocytes that can promote the expression of cartilaginous markers [16]. The 3D microenvironment of hydrogels provides desirable chambers for cellular encapsulation, sufficient seeding, and homogenous distribution [17]. Thus, designing a 3D structure with a tuned porous pattern, in which the processing technique for hydrogel fabrication is a determinative factor, is essential for optimizing hydrogel-based bio-applications. It is possible to fabricate a pre-designed pattern by printing hydrogel-type bioinks through which an interconnected porous structure can be achieved layer-by-layer [18]. Since cells represent better depositing and migrating performance in ECM with as aqueous environment, polymers with lower viscosity are better choices as cell-laden bioinks. However, viscose bioinks are mechanically unstable leading to the collapse and deformation of the printed construct [19]. The second limitation faced by 3D bioprinting is the harsh printing conditions (e.g., temperature, pressure, nozzle type) which may affect cellular viability [20]. In order to solve this issue, some complementary manufacturing techniques have been suggested. The main aim of these modifications is providing a 3D hydrogel matrix with shape fidelity and cell-friendly viscosity [21]. The aerosol spraying cross-linking is an imperative method to address the former problem. This method offers an immediate surface gelation of the printed construct to fulfill both the low viscosity and shape fidelity of the scaffold. Upon the cold printing process, the bioink has the opportunity to stabilize its physical shape immediately after printing. Then the printed construct is immersed into the cross-linking solution [22].

1.Bioactive Hydrogels:
Hydrogels play a crucial role in bone regeneration by interacting with cells and regulating stem cell differentiation.
Challenges include optimizing polymer composition, concentration, and crosslinking methods to enhance bone regeneratio[52].

2. Injectable Hydrogels:
- Injectable hydrogels made from biocompatible materials are promising for minimally invasive surgery and bone tissue engineering.
- These hydrogels have adaptable physicochemical qualities and can release drugs in response to external stimuli [52].

3. DNA Hydrogels:
- DNA hydrogels can promote hydroxyapatite growth and new bone formation, making them a potential therapeutic biomaterial for bone regeneration.

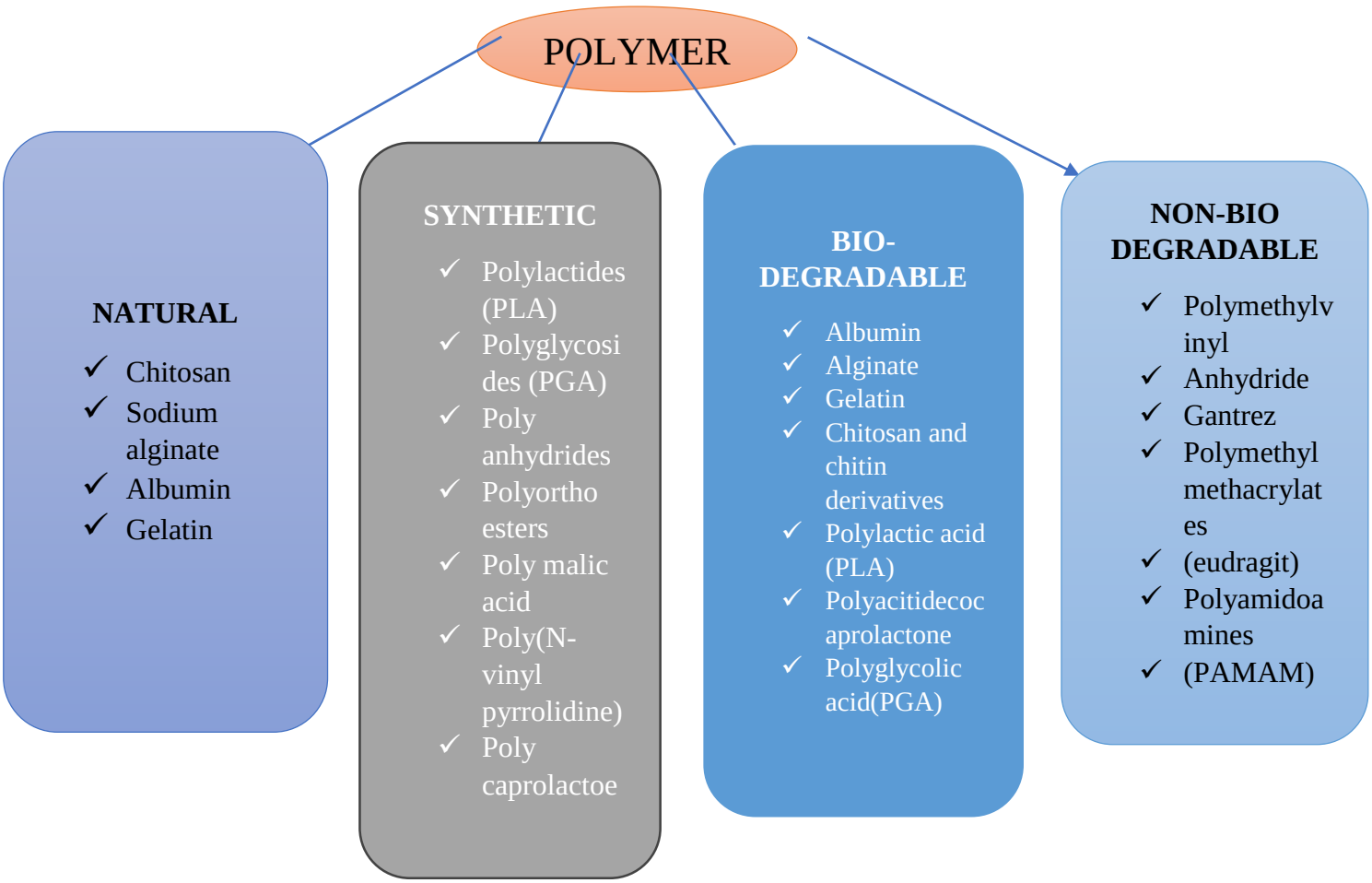
- They are synthesized at room temperature and have shown promising results in promoting bone regeneration in rat calvarial defects [53].

4. Hydrogel Microparticles:
- Hydrogel microparticles are versatile entities for bone tissue regeneration, offering capabilities that make them promising for this application.
- They have been used in bone tissue engineering and have shown potential in repairing bone defects caused by various factors [54].

5.Hyaluronic Acid-Based Hydrogel:
- Acrylated hyaluronic acid (HA) hydrogel has been used as a scaffold for bone morphogenic protein-2 (BMP-2) and human mesenchymal stem cells (hMSCs) for bone regeneration.
- The hydrogel, when combined with BMP-2 and hMSCs, demonstrated enhanced cellular viability and showed promising results in calvarial defect regeneration[5].

POLYMER

Polymers are classified into two main types: natural and synthetic or man-made polymers. Natural polymers include substances like chitosan, collagen, silk, and hemoglycin found in meteorites. On the other hand, synthetic polymers are widely used and include polyethylene, polypropylene, polystyrene, polyvinyl chloride, synthetic rubber, phenol formaldehyde resin (Bakelite), neoprene, nylon, polyacrylonitrile, PVB, silicone, and more [48]. The process of polymerization combines small molecules called monomers into a covalently bonded chain or network. Two main methods of synthesis: step-growth polymerization and chain polymerization. In step-growth polymerization, chains of monomers may combine directly with each other, while in chain polymerization, monomers are added to the chain one at a time only [49].



NATURAL POLYMERS

Collagen, gelatin, albumin, fibrin, silk proteins, Matrigel, glucan, hyaluronic acid, chitosan, agarose, bacterial cellulose, and alginate are examples of proteins or polysaccharides that are naturally occurring hydrogels. It is possible to obtain several natural hydrogels by extracting them from their natural source. For instance, the extracellular matrix (ECM) naturally contains components like collagen, fibrin, and hyaluronic acid, whereas sea algae are the source alginate
and agarose. Natural hydrogels are low-immune response and sufficiently biocompatible. Through enzymatic or metabolic breakdown, they are absorbed and facilitate cell adhesion, proliferation, and the regeneration of new tissue. However, inadequate repeatability and restricted control over the mechanical properties are the result of fluctuations in material quality, which are a drawback of any natural material. [23–24]

To date, a variety of biodegradable matrices, or scaffolds, have been created to support the growth of new tissue until it integrates into the transplanted location, recreate the biological microenvironments of the regenerating tissue, and hold it in place. drug's functional cues are regulated by a range of interactions and variables, including as extracellular matrix (ECM), soluble factors, and other cells. Drug perceive mechanical and biochemical signals from the niche they are placed in in Dischер. Acquiring a deeper comprehension of the intricate and ever-changing control over cell fate specification will facilitate accurate phenotypic manipulation and the efficient utilization of stem cells in regenerative medicine. In this case, synthetic or naturally occurring polymers can serve as the foundation for the composite scaffold matrix. [24]

Natural polymers commonly used in bone tissue engineering include collagen, chitosan, gelatin, silk fibroin, alginate, cellulose, and starch. These polymers are utilized either alone or in combination to create scaffolds, hydrogels, and micro-nanospheres for bone tissue regeneration. Natural polymers like collagen, chitosan, gelatin, silk fibroin, alginate, cellulose, and starch offer high biocompatibility, excellent biodegradability, and low toxicity compared to synthetic polymers. [50]

These natural polymers play a significant role in bone tissue engineering by providing a biocompatible and biodegradable platform for developing bone grafts as substitutes for damaged bone structures. [51]

POLYMER	ADVANTAGES	DISADVANTAGES	FORMULATION
Collagen	1. Biocompatibility, 2. biodegarable, 3. Hydroplicsity,high porosity, 4. Facility for combinig for with 5. Other polymer.	1. Weak stiffness, 2. Low antigencity.	1. scaffolds
Chitosen	1. Biocompatibility, 2. Biodegrability,anti- 3. Microbility and no 4. Immunogenicity.	1. Low stability, 2. Weak mechanic strength.	1. Composite scaffold, 2. hydrogels,sponges.
Gelatin	1. Biocompatibility, 2. Biodegrable, 3. Ability to gelation.	1. Low mechanic strength, fastdegradation rate.	1. Hydrogels,micro nano sphere.
Cellulose	1. Biocompatibility, 2. Biodegrability,high 3. Mechanic strength.	1. Low cell binding properties.	1. Composite scaffolds.
Silk fibroin	1. Biocompatibility, 2. Biodegrability,high 3. Flexibility high mechanic strength.	1. Slow degradation rate.	1. Scaffolds.
Starch	1. Biocompatibility, 2. Biodegerability.	1. Low surface area, 2. Brittle.	1. Composite scaffolds.

TABLE 1. NATURAL POLYMER USED IN BONE TISSUE ENGINEEING

COLLAGEN

Thirty percent of the body's total protein weight is made up of collagen. Collagen comes in a variety of forms (25), with type I accounting for 90% of all forms and being extensively distributed in the extracellular matrix (ECM) of native tissues such as dentine, bone, skin, tendon, pancreas, and cartilage (26–28). The collagen triple-helix structure is made up of two α1 and one α2 polypeptide chain (29). Different forms of collagens have different chemical compositions in the α2 chain (15). The polypeptides consist of a repeating triplet of glycine (Gly), proline (Pro), and 4-hydroxyproline (Hyp) (28). Procollagen is the name given to the triple helix structure made up of the three polypeptide chains. Mature collagen is produced when metalloproteinase enzymes split the helix's N- and C-terminals (30). The formation of collagen fibers from monomers is largely affected by environmental factors including pH, ionic strength, and temperatures [29,31].

CHITOSAN

Chitin undergoes deacetylation to produce chitosan. Glucosamine and N-acetyl glucosamine are the constituents of chitosan (32). The degree of deacetylation has an impact on the crystallinity of chitosan, a linear polysaccharide with a semi-crystalline structure (33). Because free amino and N-acetyl groups are present, chitosan has a cationic character. Consequently, chitosan can interact with both proteoglycans and glycosaminoglycans (34, 35). For tissue engineering, chitosan is a preferred polymer because of its antibacterial activity, biodegradability, and biocompatibility (36). One enzyme that aids in the breakdown of chitosan is lysozyme. The degradation rate is determined by the quantity of N-acetyl-glucosamine units (36, 37). Chitosan has been shown to possess antibacterial properties against a variety of bacteria and fungi. The structure of chitosan is similar to that of GAGs in connective tissue (19). Research has demonstrated that, when compared to tissue culture plates, chitosan is more effective in MSCs osteogenic differentiation (35)

STARCH

Certain plants, such as potatoes, corn, and wheat, can produce starch. This naturally occurring polysaccharide is found in leaves, fruits, roots, and seeds as 1–110 μm granules (37, 3). Starch is commonly employed in the food business because it can be digested and broken down (by amylases) into oligosaccharides to obtain energy (14). The starch is composed of D-glucose units, which include α-amylose (20%) and amylopectin (70%). Through glycosidic connections, amylopectin forms the branches of the starch structure (40). Starch is biocompatible, biodegradable, and harmless by nature (38). It is water-sensitive, delicate, and has a small surface area. The use of starch in bone repair is restricted by these characteristics (39). In order to avoid these problems, both natural and artificial polymers. In the area with a deficiency, more new bone was formed when starch and polycaprolactone (PCL) were combined (40, 41). The osteoblast-like cells' ALP activity was elevated by starch–chitosan composite.

SYNTHETIC POLYMER

Synthetic polymers play a significant role in bone tissue engineering due to their tunable properties, biocompatibility, and biodegradability. Some commonly used synthetic polymers in bone tissue engineering include:

Poly(lactic acid) (PLA): PLA is a biodegradable polymer derived from renewable resources such as corn starch or sugarcane. It has been extensively used in bone tissue engineering due to its mechanical strength, biocompatibility, and degradation into non-toxic byproducts.[42]

Poly(glycolic acid) (PGA): PGA is another biodegradable polymer commonly used in bone tissue engineering. It degrades rapidly into glycolic acid, which is metabolized by the body. PGA is often used alone or in combination with other polymers to enhance mechanical properties and degradation rates.[43]

Poly(lactic-co-glycolic acid) (PLGA): PLGA is a copolymer of lactic acid and glycolic acid. It combines the properties of PLA and PGA, offering tunable degradation rates and mechanical properties. PLGA scaffolds have been widely used for bone tissue engineering applications.[44]

Polycaprolactone (PCL): PCL is a biodegradable polyester with a slower degradation rate compared to PLA and PGA. It provides excellent mechanical properties and is often used.[45]

CONCLUSION

The field of bone tissue engineering aims to induce new functional bone regeneration through the synergistic combination of biomaterials, cells, and factor therapy. Recent advances in this area focus on developing biomaterial scaffolds, cell-based research, and innovative approaches to enhance bone regeneration. Challenges in bone tissue engineering include issues like insufficient vascularization at defect sites, which drive future research efforts in the field. Studies explore the use of osteogenic cells, scaffolds, and growth factors to regenerate bone defects, particularly in the oral cavity, highlighting the importance of viable cells and signaling pathways in bone repair and regeneration. The review articles emphasize the significance of understanding cellular signaling in skeletal remodeling and the potential impact of growth factors on osteogenic signaling cascade for bone tissue replacement and regeneration. Overall, these studies underscore the promising advancements and ongoing challenges in tissue engineering for bone regeneration, paving the way for innovative solutions in the field**.**

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