

Natural Polymer-Based Nanocomposites: Advances and Applications in Bone Tissue Engineering

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ABSTRACT: Bone tissue engineering (BTE) has emerged as a promising strategy for repairing and regenerating damaged or diseased bone, addressing limitations of conventional grafting techniques. In recent years, natural polymer-based nanocomposites have gained significant attention due to their biocompatibility, biodegradability, and structural similarity to the extracellular matrix, which collectively enhance osteoconductivity and cellular responses. This review critically examines the current advances in the development and application of natural polymer-derived nanocomposites for BTE, highlighting their physicochemical properties, fabrication techniques, and biological performance. Key natural polymers, including chitosan, collagen, alginate, and gelatin, have been combined with various nanomaterials, such as hydroxyapatite, bioactive glass, and carbon-based nanostructures, to improve mechanical strength, bioactivity, and the controlled release of growth factors. The review also explores recent *in vitro* and *in vivo* studies that demonstrate the effectiveness of these nanocomposites in promoting osteogenic differentiation, enhancing bone mineralization, and supporting tissue integration. Challenges such as scalability, long-term stability, and immunogenic responses are discussed alongside future perspectives, emphasizing the need for multifunctional nanocomposites and advanced fabrication methods, including 3D bioprinting and electrospinning. Overall, natural polymer-based nanocomposites represent a versatile and promising class of biomaterials for BTE, with the potential to significantly improve clinical outcomes in bone repair and regeneration. Continuous interdisciplinary research integrating materials science, biology, and engineering is essential to translate these innovations from laboratory studies to clinical applications.

KEYWORDS: Natural polymers, Nanocomposites, Bone tissue engineering, Biocompatibility, Osteogenesis

Date of Submission: 26-03-2025

Date of acceptance: 30-04-2025

I. INTRODUCTION

Bone is a highly specialized connective tissue composed predominantly of a mineralized matrix, primarily hydroxyapatite, embedded in a collagen-rich organic framework. This complex tissue provides several essential physiological and structural functions, including protection of vital organs, facilitation of locomotion through leverage of the musculoskeletal system, housing of bone marrow for hematopoiesis, maintenance of body structural integrity, and storage of essential minerals such as calcium and phosphate (Flores-Silva et al., 2015). The dynamic nature of bone enables it to continuously remodel itself in response to mechanical and metabolic demands, a property critical for maintaining skeletal function and systemic mineral homeostasis.

Globally, there has been a marked increase in the prevalence of bone-related disorders, coupled with a growing need for effective bone grafting and regenerative therapies (Amini et al., 2012). It is estimated that approximately 15 million individuals suffer from bone disorders annually, with direct treatment costs reaching

nearly \$45 billion. Among these patients, 2 million present with osteoporotic bone abnormalities, while an additional 1.6 million experience trauma-induced fractures (O’Keefe & Mao, 2011). In the United States alone, bone grafting procedures for approximately 1.6 million patients account for an expenditure of \$2.5 billion annually (Amini et al., 2012; Bao et al., 2013). Factors such as the aging baby boomer population and increasing life expectancy are projected to nearly double the economic burden associated with bone grafting treatments in the coming decade (Baroli, 2009). These statistics underscore the urgent need for innovative approaches in bone repair and regeneration that are cost-effective, clinically effective, and capable of improving patient outcomes.

Bone tissue engineering (BTE) has emerged as a promising alternative to conventional grafting techniques, offering the potential to regenerate functional bone while circumventing complications such as donor site morbidity, poor vascularization, and immune rejection (Amini et al., 2012; Guo & Ma, 2014). The paradigm of BTE is predicated on the integration of four core components: (a) osteogenic cells, which synthesize the bone extracellular matrix (ECM); (b) biocompatible scaffolds constructed from bioactive materials that emulate the ECM; (c) vascularization, which ensures efficient transport of nutrients and waste products; and (d) morphogenetic signals that regulate cell differentiation and tissue formation (Amini et al., 2012; Guo & Ma, 2014). The overarching goal of BTE is to promote regeneration at the defect site, facilitating the gradual resorption of the biomaterial scaffold while being replaced by newly formed bone tissue. For this process to be successful, the biomaterial must exhibit osteoinduction (stimulation of progenitor cells to differentiate into osteoblasts), osteoconduction (support of new bone growth along the scaffold), and osseointegration (stable integration into surrounding bone tissue) (Stevens, 2008; Hench & Polak, 2002). Additional requirements for biomaterials include manufacturability, mechanical and chemical stability in vivo, non-thrombogenicity, and sterilizability, all of which ensure clinical applicability and long-term functionality.

Scaffolds are the three-dimensional frameworks central to BTE, providing structural support for osteogenic cells while delivering appropriate mechanical and biological cues that stimulate cellular proliferation and differentiation (Williams, 2014). An ideal scaffold must demonstrate biocompatibility, facilitating osteoconduction and supporting cell adhesion, proliferation, and vascularization within its porous architecture. Mechanical properties are equally critical, with cortical bone requiring Young’s moduli in the range of 15–20 GPa and compressive strengths between 100–200 MPa, while cancellous bone requires moduli of 0.1–2 GPa and compressive strengths of 2–20 MPa. Pore size and interconnectivity are vital, with an optimal pore range of 200–350 μm to allow nutrient transport and vascular ingrowth. Bioresorbability is also essential, enabling scaffolds to degrade at a controlled rate that synchronizes with tissue regeneration (Olszta et al., 2007; Murphy et al., 2010). Scaffold characteristics such as crystallinity, surface-to-volume ratio, and porosity play key roles in influencing cell behavior and bone regeneration efficiency (Pina et al., 2015).

Various fabrication techniques have been employed to create scaffolds suitable for BTE, including the foam replica method (Oliveira et al., 2009), electrospinning (Chae et al., 2013), freeze-drying (Liapis et al., 1996), gas foaming (Dehghani & Annabi, 2011), solvent casting/particulate leaching (Hou et al., 2003), phase separation (van de Witte et al., 1996), and molecular self-assembly (Matson et al., 2011). Recently, three-dimensional (3D) bioprinting has introduced a new dimension in scaffold fabrication, enabling the creation of patient-specific constructs that can be directly implanted onto defect sites. Bioprinting allows precise spatial distribution of bioactive molecules, cells, and growth factors, which regulate cellular adhesion, proliferation, metabolism, and differentiation (Pina et al., 2015). Bioactive molecules involved in BTE include mitogens that stimulate cell division, growth factors that promote cell proliferation, and morphogens that direct tissue patterning and regeneration (Pina et al., 2015). Incorporation of these molecules into scaffolds can be achieved through top-down approaches, where the ECM secreted by cells is deposited onto the scaffold to create a hybrid structure, or bottom-up approaches, which involve functionalization with growth factors, cytokines, or peptides, often using nano- or microsphere carriers for controlled release (Kesireddy & Kasper, 2016).

The chemical and topographical properties of scaffolds play an integral role in cell attachment, proliferation, and differentiation. Surface roughness, porosity, and incorporation of bioactive molecules enhance osteoconduction, osteoinduction, and osseointegration. Studies have demonstrated that natural polymeric composite scaffolds exhibit superior osteoconductive properties compared to their pristine biomaterial counterparts (Tripathi &

Basu, 2012). Nanocomposite biomaterials, which integrate nanoscale fillers into biopolymeric and biodegradable matrices, represent a novel approach to enhance mechanical, chemical, and biological performance. The inclusion of nanofillers increases surface area, mechanical strength, stability, and cellular interactions, providing an optimal environment for bone tissue regeneration (Pina et al., 2015; Bonfield et al., 1981).

Nanocomposite scaffolds can be synthesized using various natural polymers, such as chitosan, collagen, and gelatin. Each polymer imparts specific properties, including biocompatibility, biodegradability, and mechanical strength, which are further enhanced by the type and concentration of nanoparticles incorporated. For example, hydroxyapatite and bioactive glass nanoparticles can improve osteoconduction and mechanical stability, while carbon-based nanomaterials enhance surface area and cellular adhesion (Ullah et al., 2016). The combination of polymers and nanoparticles allows for synergistic effects, optimizing scaffold performance in bone regeneration applications. The incorporation of nanofillers can also influence degradation rates, bioactivity, and immunogenic responses, providing opportunities to tailor scaffolds for specific clinical scenarios (Zhang et al., 2015).

The versatility of nanocomposites extends beyond BTE, with applications in artificial blood vessels, wound dressings, drug delivery systems, cardiac prostheses, stem cell therapy, cancer therapy, and biosensors (Ullah et al., 2016). Advances in nanotechnology, nanobiology, and nanomaterials have facilitated the development of scaffolds that closely mimic the complex hierarchical structure of native bone tissue, thereby enhancing functional tissue regeneration (Gleiter, 2000). Biodegradable polymer-based nanocomposites have been shown to support tissue production in vitro and in vivo, providing a foundation for translational research aimed at clinical implementation (Zhang et al., 2015).

This review focuses on natural polymer-based nanocomposites for BTE, highlighting scaffold fabrication techniques, mechanical and biological properties, and the role of nanofillers in enhancing osteogenesis. The review also examines the synergistic effects of polymer–nanoparticle combinations, emphasizing their potential to improve bone regeneration outcomes. By consolidating current research, this paper provides insights into the design, synthesis, and application of nanocomposite scaffolds, offering a comprehensive understanding of their role in advancing bone tissue engineering strategies.

2.0 NANOCOMPOSITE TECHNOLOGY

Bone itself can be considered a natural nanocomposite due to its hierarchical structure composed of an organic phase of collagen fibers and a mineral phase of nanocrystalline hydroxyapatite (HAp) (Zwingenberger et al., 2012). This sophisticated arrangement imparts remarkable mechanical properties, structural integrity, and biological functionality to bone tissue. The collagen fibers provide flexibility, while the mineralized nanocrystals provide stiffness, creating a composite material that balances strength and toughness across multiple scales, from nanoscale to macroscale as in Figure 2.0 (Mota et al., 2016). Understanding this hierarchical organization has inspired the development of engineered nanocomposite materials for bone tissue engineering (BTE), as these synthetic constructs aim to replicate the microstructural and physicochemical cues that naturally support osteogenesis. Nanostructured materials with surface roughness and pore sizes in the range of 2–100 nm have been shown to elicit natural cellular responses, promoting adhesion, proliferation, and differentiation of osteoblasts, which are critical steps in new bone formation (Zwingenberger et al., 2012; Bernhardt et al., 2008).

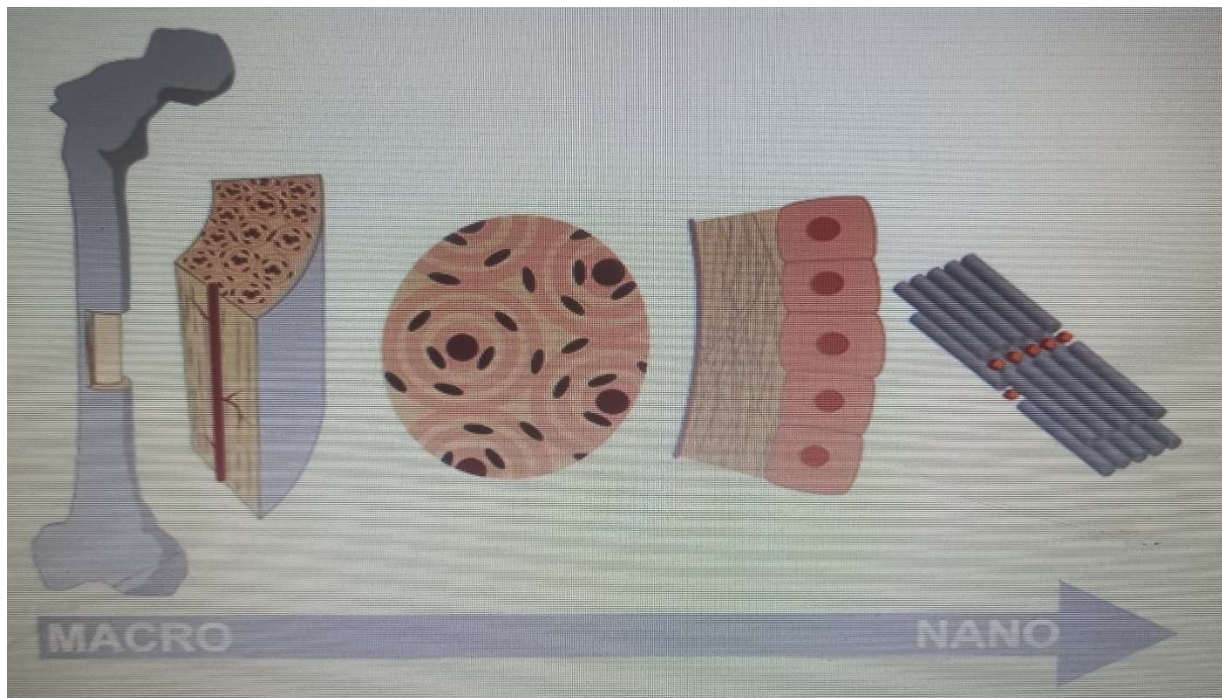


Fig.2.0. The hierarchical structure of the bone (Mota et al., 2016).

Nanomaterials offer several advantages over conventional micrometric materials. Due to their increased surface area, nanomaterials display enhanced mechanical and osteoconductive properties, improved physicochemical characteristics, and greater bioactivity (Bernhardt et al., 2008). The nano-scale topography of these materials can modulate biological processes, influencing protein adsorption, cell signaling, and ultimately tissue regeneration. Nanoparticles can be functionalized with bioactive molecules, including adhesion proteins, growth factors, and morphogens, further enhancing their capacity to regulate cell behavior and promote osteogenesis (Stevens, 2008). However, nanoparticles, especially those smaller than 100 nm, tend to aggregate due to van der Waals forces. Therefore, effective scaffold fabrication requires precise dispersion techniques to maintain nanoscale functionality and prevent formation of larger, less bioactive clusters. Improved nanoparticle dispersion within the polymer matrix directly correlates with enhanced mechanical and biological performance of the resulting nanocomposite (de Lacerda Schickert, 2014).

2.1 TISSUE ENGINEERING

Tissue engineering, officially defined at a 1988 National Science Foundation workshop, is "the application of principles and methods of engineering and life sciences to understand structure-function relationships in mammalian tissues and to develop biological substitutes to restore, maintain, or improve tissue function" (Hasnain et al., 2010). This multidisciplinary field combines principles of engineering, biology, chemistry, and clinical sciences to address therapeutic challenges such as tissue loss or organ failure (Vacanti & Langer, 1999). Tissue engineering employs biomaterials, bioactive molecules, and cells in isolation or combination to stimulate tissue regeneration at target sites, creating constructs that replicate the native microenvironment (Samadikuchaksaraei, 2007; Chan & Leong, 2008). The development of scaffolds with defined physical, chemical, and biological properties is crucial to successful tissue regeneration, providing platforms for cell attachment, proliferation, and organization into functional tissue structures.

Several strategies exist for tissue repair and regeneration, including spontaneous self-healing, autologous tissue transplantation, implantation of cell-free biomaterials, cell therapy, and tissue engineering approaches (Sah & Pramanik, 2012). The choice of method depends on the tissue type, defect site, and the regenerative capacity of the host, which varies with age and health status. Tissue engineering integrates material science and life sciences to generate constructs comprising a biomaterial matrix (scaffold) populated with living cells, providing an environment conducive to tissue formation (Pàmies, 2016). Unlike conventional cell therapies, tissue

engineering emphasizes the creation of functional three-dimensional tissue constructs in vitro, or the implantation of cell-seeded scaffolds in vivo, to facilitate site-specific tissue regeneration (Pàmies, 2016). Biodegradability is a critical feature in tissue engineering, as the scaffold must gradually degrade in synchrony with tissue formation, leaving behind fully functional, native tissue (Amini et al., 2012).

Scaffold biomaterials must satisfy several fundamental requirements to ensure successful tissue engineering outcomes, including biodegradability, porosity, biocompatibility, biointegration, appropriate mechanical properties, ease of manufacture and handling, and cost-effectiveness (Amini et al., 2012). Polymers are indispensable for scaffold fabrication due to their versatility, tunable properties, and ability to support cellular activity. Biodegradable polymeric materials commonly used in tissue engineering include naturally derived polymers such as polysaccharides (starch, alginate, chitin/chitosan, hyaluronic acid) and proteins (soy protein, gelatin, collagen, fibrin gels, silk), as well as synthetic polymers like poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ϵ -caprolactone) (PCL), and poly(hydroxybutyrate) (PHB) (Shin et al., 2003). Copolymers such as poly(lactic-co-glycolic acid) (PLGA) are widely used due to their controllable degradation rates, mechanical strength, and biocompatibility (Fambri et al., 2002).

2.2 BONE TISSUE ENGINEERING

Natural bone exhibits a complex hierarchical structure, comprising an inorganic-organic nanocomposite of collagen fibrils and hydroxyapatite arranged in ordered patterns. The three-dimensional arrangement provides mechanical resilience, flexibility, and biological functionality (Swetha et al., 2010; Mays, 2021). Bone tissue engineering, a rapidly advancing field over the past three decades, focuses on the development of three-dimensional scaffolds capable of supporting osteoblast proliferation, facilitating vascularization, and promoting organized bone regeneration (Swetha et al., 2010). These scaffolds are designed to mimic the microarchitecture of native bone while addressing limitations associated with conventional bone grafts, including donor site morbidity, immunological rejection, limited availability, and risk of pathogen transmission (O’Keefe & Mao, 2011).

The hierarchical structure of bone, extending from the nano to the macroscale, underscores the importance of scaffold design in tissue engineering. Collagen provides tensile strength and flexibility, while hydroxyapatite contributes compressive strength and rigidity. The mechanical properties of bone are therefore closely linked to the ratio of collagen to mineral content, influencing both function and resilience (Mays, 2021). Nanocomposite scaffolds emulate this structure, providing nanoscale cues that enhance cell adhesion, proliferation, and differentiation, thereby promoting osteogenesis. Functionalized nanomaterials offer additional benefits, including increased surface area, improved protein adsorption, and the potential to deliver growth factors or drugs to the defect site (Zwingenberger et al., 2012).

Globally, the demand for effective bone grafting solutions continues to rise. In the United States alone, over a million patients undergo bone repair procedures annually, incurring costs exceeding \$2.5 billion (Baroli, 2009). These demands are projected to increase due to aging populations and evolving healthcare needs. Bone tissue engineering presents a promising alternative to traditional bone grafting, providing a readily available, disease-free source of biomaterials while circumventing limitations associated with donor tissue (Swetha et al., 2010). Contemporary BTE strategies employ synergistic combinations of biomaterials, bioactive molecules, drugs, cells, and growth factors to create biomimetic environments that promote the formation of functional bone tissue.

Recent advancements in nanocomposite technology have significantly enhanced scaffold design and performance. Nanoparticles incorporated into polymer matrices confer increased mechanical strength, bioactivity, and surface roughness, closely mimicking native bone microarchitecture (Bernhardt et al., 2008; de Lacerda Schickert, 2014). The inclusion of bioactive nanofillers also enables controlled release of growth factors, facilitating localized osteoinduction and vascularization. Furthermore, nanocomposites allow fine-tuning of degradation rates to match tissue formation, ensuring that scaffolds provide temporary mechanical support while gradually being replaced by native tissue (Pina et al., 2015).

Polymers play a crucial role in BTE scaffolds, providing structural support and a conducive environment for cell proliferation and ECM deposition. Natural polymers such as collagen, chitosan, gelatin, and hyaluronic acid offer excellent biocompatibility, biodegradability, and intrinsic bioactivity, whereas synthetic polymers such as PLA, PGA, PCL, and PHB provide tunable mechanical properties and controlled degradation kinetics (Shin et al., 2003; Fambri et al., 2002). Nanoparticles such as hydroxyapatite, bioactive glass, and carbon-based nanomaterials enhance osteoconductivity, improve mechanical stability, and increase surface area for protein adsorption and cell adhesion (Ullah et al., 2016).

In summary, bone tissue engineering represents a multidisciplinary approach that integrates nanotechnology, biomaterials science, and cell biology to regenerate functional bone tissue. Nanocomposite scaffolds, designed to mimic the hierarchical structure and bioactivity of native bone, offer significant advantages over conventional materials. The synergistic combination of polymers and nanoparticles provides mechanical strength, osteoconductivity, and controlled degradation, making these materials promising candidates for clinical applications in orthopedic regeneration. The continued development of natural polymer-based nanocomposites holds the potential to revolutionize bone tissue engineering, offering innovative, cost-effective, and clinically viable solutions for bone repair and regeneration.

3.0 APPLICATION OF NATURAL POLYMERS USED IN THE DESIGN OF NANOCOMPOSITES FOR BONE TISSUE ENGINEERING.

Natural polymers, also referred to as biopolymers, have become pivotal in the field of bone tissue engineering (BTE) due to their highly ordered structural elements and their capacity to interact with cellular environments (Hasnain et al., 2010). These polymers often contain ligands, which are extracellular materials essential for binding to cell receptors, thereby influencing cellular adhesion, proliferation, and differentiation (Malafaya et al., 2007). Unlike synthetic polymers, natural polymers exhibit higher biocompatibility, enhanced bioactivity, and the ability to support bone mineralization. Their intrinsic ability to integrate into host tissues without necessitating secondary surgical interventions underscores their importance in clinical applications (Pal & Nayak, 2017). Animal- and plant-derived polymers have been successfully employed as scaffold materials for tissue engineering due to their degradability, which aligns with the natural remodeling of tissue, and their minimal induction of adverse immune responses (Hasnain et al., 2010).

Over the past few decades, extensive research has focused on developing natural biodegradable polymer-based nanocomposites that combine biocompatibility, biodegradability, and mechanical robustness for tissue engineering applications (Malafaya et al., 2007; Pal & Nayak, 2017). In addition to bioceramics and metallic materials, naturally occurring biodegradable polymers such as polysaccharides (e.g., chitosan, alginates, starch, hyaluronic acid, dextran, cellulose, fucoidan) and protein-derived polymers (e.g., collagen, gelatin, silk fibroin) have emerged as promising scaffold matrices for bone regeneration (Nayak & Pal, 2015; Pina et al., 2015).

3.1 POLYSACCHARIDES

Polysaccharides are carbohydrate polymers consisting of repeated monosaccharide units, which impart unique chemical and physical properties such as hydrophilicity, biodegradability, and the capacity for chemical modification. Polysaccharides are widely used as scaffolding materials in BTE due to their ability to form porous networks conducive to cell adhesion, proliferation, and differentiation (Lee & Mooney, 2012).

3.1.1 CHITOSAN

Chitosan (CS) is a naturally occurring cationic polysaccharide derived from chitin, which constitutes the exoskeletons of crustaceans and fungal cell walls (Nayak & Pal, 2015). Structurally, it is composed of α -1,4-linked 2-amino-2-deoxy- α -D-glucose (N-acetyl glucosamine) units as in figure 3.1 (Jana et al., 2013). Chitosan possesses intrinsic antibacterial, biodegradable, and biocompatible properties, making it highly suitable for BTE applications (Dash et al., 2011). Its reactive hydroxyl and amino groups allow chemical modifications to tailor scaffold properties, thereby improving osteoconductivity and mechanical strength (Shakir et al., 2018).

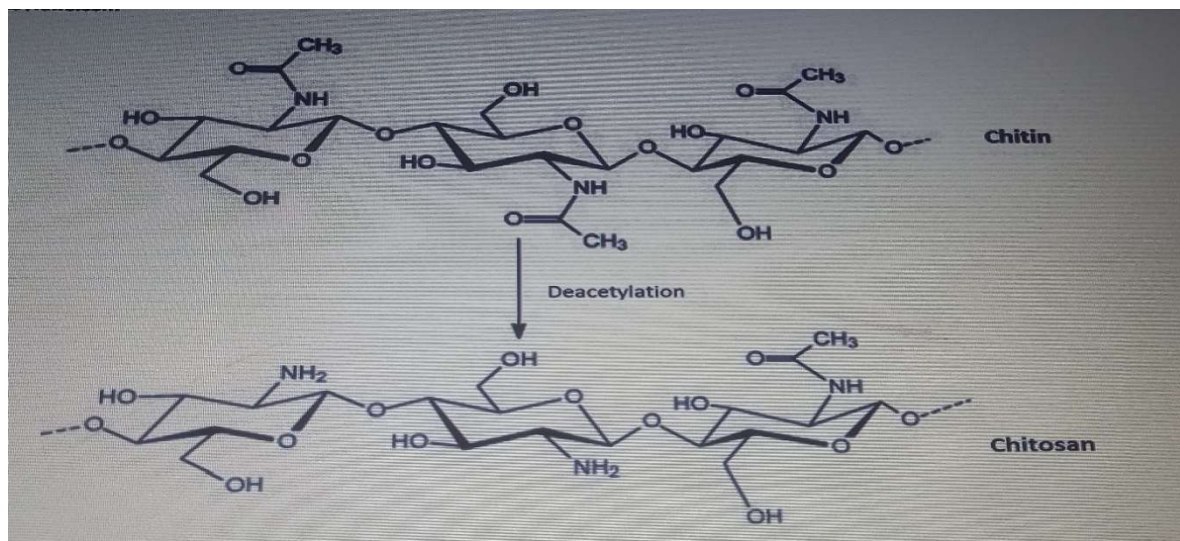


Fig 3.1. Structures of chitosan and chitin (Jana et al., 2013)

Chitosan exhibits good solubility in dilute acidic solutions ($pH < 6.5$), which enhances its processability compared to chitin (Yi et al., 2005). The biological performance of CS is influenced by the degree of deacetylation and the source of chitin. Numerous in vitro and in vivo studies have confirmed that CS scaffolds elicit negligible toxic effects, do not induce inflammatory responses, and are considered safe by the FDA as a “generally recognized as safe” (GRAS) material (Keller et al., 2017; Dash et al., 2011).

Recent studies have focused on chitosan-based nanocomposites reinforced with hydroxyapatite (HAp), silica nanoparticles (SiO_2), and other bioactive materials to mimic the extracellular matrix of bone tissue. Nazeer et al. (2017) synthesized chitosan-HAp nanocomposites via a sol-gel method and observed homogeneous dispersion of disc-shaped HAp nanoparticles within the polymer matrix. The intercalated morphology of the composites enhanced mechanical stability, while SEM and XRD analyses confirmed successful integration of HAp within chitosan matrices (Figure 3.2). Similarly, Nikpour et al. (2012) produced chitosan-nHAp composites using in situ hybridization, demonstrating favorable compressive mechanical properties suitable for bone replacement.

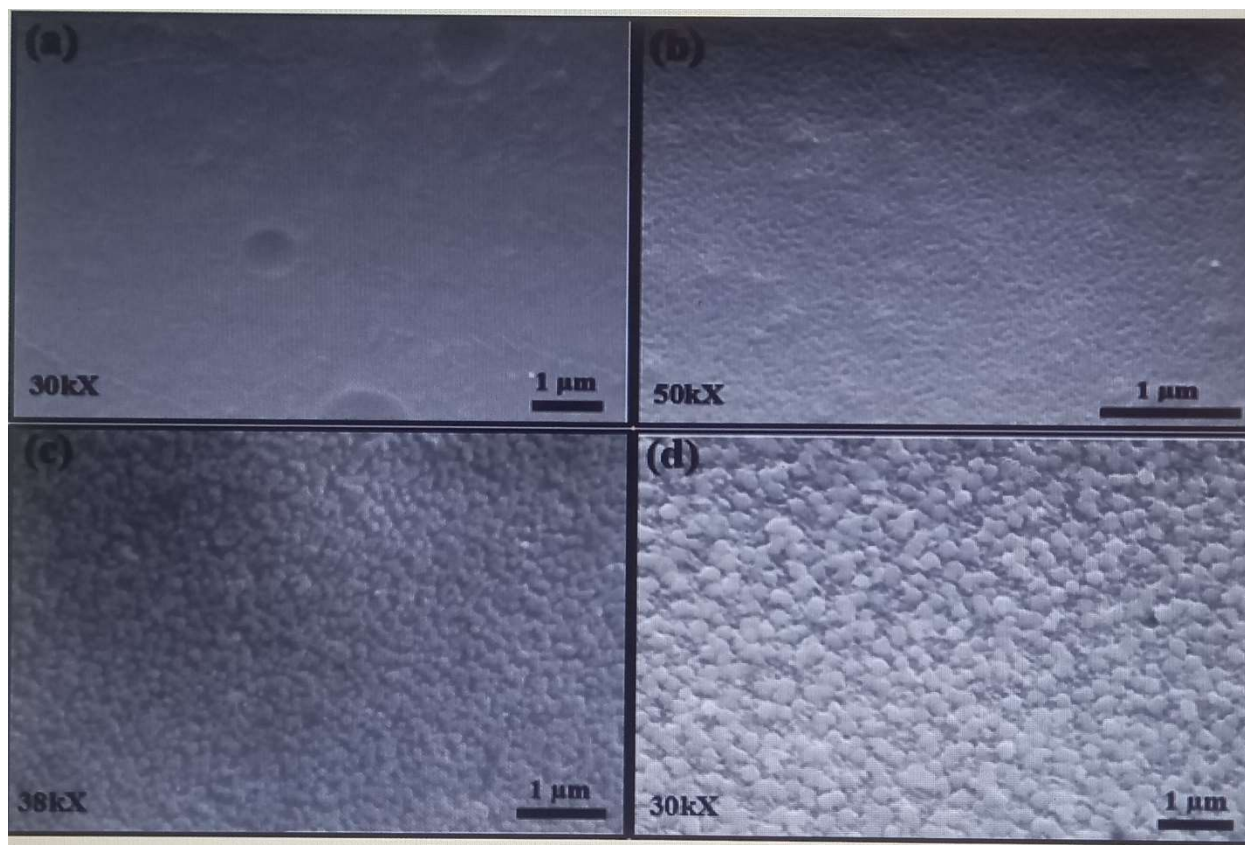


Fig. 3.2. SEM photographs of (A) chitosan film, (B) chitosan-HAp nanocomposite film (containing 5wt% HAp), (C) chitosan-HAp nanocomposite film (containing 10wt% HAp), and (D) chitosan-HAp nanocomposite film (containing 20wt% HAp) (Nazeer et al. 2017)

The incorporation of silica nanoparticles into chitosan scaffolds has also been investigated to enhance mechanical strength and bioactivity. Keller et al. (2017) reported that chitosan-SiO₂ scaffolds exhibited pore diameters of approximately 300 μm and increased compressive resistance by 30%. In vitro studies demonstrated excellent osteoblast adhesion and proliferation, while in vivo implantation in murine calvarial defect models showed enhanced collagen deposition and vascularization.

3.1.2 ALGINATES

Alginates are anionic polysaccharides derived primarily from brown algae and certain bacteria such as *Pseudomonas* and *Azotobacter* (Remminghorst & Rehm, 2006; Pal & Nayak, 2015). Chemically, they are linear copolymers of α-L-guluronic acid (G) and β-D-mannuronic acid (M) (Jejurikar et al., 2012). Alginates form hydrogels in the presence of divalent cations (e.g., Ca²⁺, Zn²⁺), making them suitable for encapsulating cells and growth factors for tissue engineering applications (Sinha et al., 2015).

Alginates demonstrate biocompatibility, biodegradability, and non-antigenicity, but their poor mechanical strength necessitates reinforcement with bioactive nanomaterials. Alginate-based nanocomposites incorporating halloysite nanotubes, hydroxyapatite, or carbon nanotubes have shown improved mechanical properties, porosity, and osteogenic potential (Venkatesan et al., 2015; Liu et al., 2015; Kawaguchi et al., 2006). For instance, Liu et al. (2015) demonstrated that alginate/halloysite nanotube composites achieved over 96% porosity and enhanced compressive modulus, while in vitro cytocompatibility studies with mouse fibroblast 3T3 cells showed improved adhesion and proliferation.

Chae et al. (2013) developed alginate-HAp nanocomposite fibrous scaffolds using electrospinning and in situ deposition of nHAp on collagen fibers, achieving homogeneous distribution of mineral particles. Rat calvarial osteoblasts exhibited spindle-shaped morphology and strong attachment, highlighting the scaffold's potential for

bone regeneration. Furthermore, hybrid alginate/collagen/nHAp composites have been shown to support osteogenic differentiation and mineral deposition in vitro and in vivo (Sangeetha et al., 2013).

3.1.3 STARCHES

Starches are plant-derived polysaccharides comprising amylose and amylopectin, both high molecular weight polymers (Zobel, 1988; Nayak & Pal, 2017). Starch-based nanocomposites offer biodegradability, biocompatibility, and tunable mechanical properties, making them suitable for bone tissue scaffolds (Chung et al., 2010; Meskinfam et al., 2011).

Meskinfam et al. (2011) demonstrated that nHAp-starch biocomposites synthesized via biomimetic approaches formed rod-like HAp structures within the starch matrix, mimicking natural bone mineral. In vitro assays showed enhanced osteoblast proliferation and no adverse effects on cellular morphology. Similarly, Raafat et al. (2013) developed starch/N-vinylpyrrolidone-HAp hydrogels using γ -radiation-induced grafting and cross-linking, exhibiting high gelation, compressive strength, and bioactivity suitable for BTE.

3.1.4 HYALURONIC ACID (HA)

Hyaluronic acid (HA) is a nonsulfated glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine (β -1,3 and β -1,4 linkages) found abundantly in connective tissue extracellular matrices (Pina et al., 2015). HA demonstrates excellent water solubility, viscoelasticity, and biocompatibility, but its enzymatic degradability necessitates chemical modifications to enhance stability (Schanté et al., 2012).

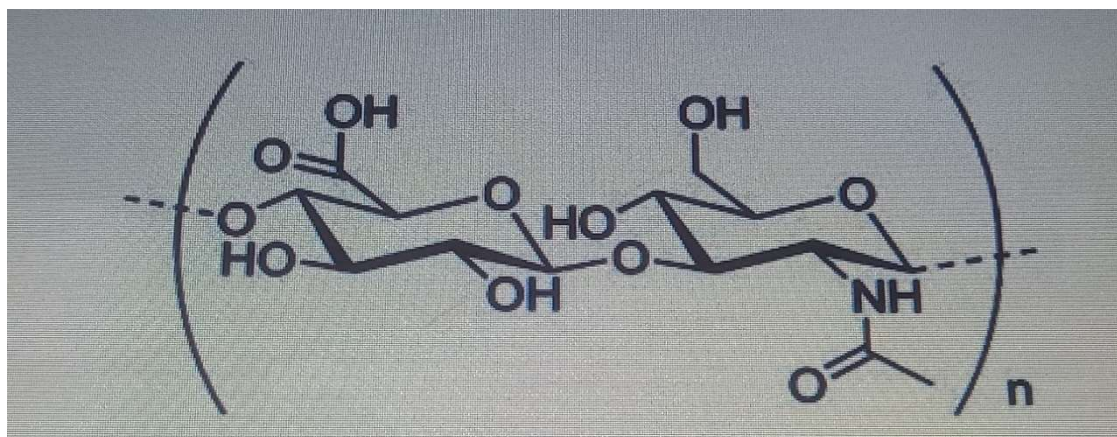


Fig 3.6. Structure of hyaluronic acid (Pina et al., 2015)

Recent studies have explored HA-based hydrogels, fibers, and foams reinforced with nHAp or collagen for bone regeneration. Subramaniam et al. (2016) reported that HA-nHAp-calcium sulfate composites encapsulating collagenase facilitated bone remodeling in alveolar bone defects in rats. Huang et al. (2016) demonstrated injectable nHAp/glycol chitosan/HA hydrogels with porous structures (100–350 μ m), showing strong osteoblast attachment and enzymatic degradability, indicating their potential in minimally invasive bone repair.

3.1.5 DEXTRAN

Dextran, a 1,6-linked glucose polysaccharide, exhibits biocompatibility, biodegradability, and stability for tissue engineering applications (Fricain et al., 2013). It can be combined with pullulan and nHAp to form macroporous nanocomposite scaffolds, which enhance osteogenic differentiation and mineralized bone tissue regeneration in vitro and in vivo (Fricain et al., 2013). Dextran-based scaffolds maintain growth factors such as BMP-2 and VEGF, enabling sustained osteogenic activity across animal models.

3.1.6 CELLULOSE

Cellulose is a linear polysaccharide of β -(1,4)-linked D-glucose units, abundant in plants and bacteria (Young & Rowell, 1986; Klemm et al., 2005). Its biocompatibility and mechanical properties make it suitable for BTE, often in combination with other polymers due to poor solubility and low mechanical strength in pure form (Beladi et al., 2017; Saber-Samandari et al., 2013). Cellulose derivatives such as carboxymethyl cellulose (CMC) and bacterial cellulose have been extensively studied for scaffold fabrication (Novotna et al., 2013; Saber-Samandari et al., 2016). Incorporation of nHAp into cellulose scaffolds enhances apatite formation, mechanical strength, and in vitro osteoblast proliferation (Fragal et al., 2016; Ao et al., 2017).

3.1.7 FUCOIDAN

Fucoidan, a sulfated polysaccharide derived from brown seaweed, exhibits osteogenic potential by stimulating ALP activity, collagen type I, and osteocalcin expression (Fitton, 2011). Composite scaffolds incorporating fucoidan, chitosan, and nHAp promote biomineralization, high porosity (>90%), and enhanced cell proliferation, as demonstrated by in vitro and in vivo studies (Venkatesan et al., 2014; Lowe et al., 2016; Young et al., 2016). Fucoidan/nHAp scaffolds showed superior osteogenic differentiation compared to HAp-only scaffolds in human adipose-derived stem cells, supporting new bone formation in animal models.

3.2 PROTEIN-DERIVED POLYMERS

Protein-derived polymers have gained significant attention in biomedical applications, particularly in bone tissue engineering (BTE), due to their inherent biocompatibility, biodegradability, and ability to mimic the extracellular matrix (ECM) of native tissues (Mienaltowski & Birk, 2014). Among these polymers, collagen, gelatin, and silk fibroin (SF) have emerged as promising materials owing to their unique biological and structural characteristics. These natural polymers not only provide a supportive framework for cellular attachment and proliferation but also promote tissue regeneration through biochemical signaling (Pina et al., 2015; Balani et al., 2007).

3.2.1 COLLAGEN

Collagen is the most abundant structural protein in the human body and serves as a critical component of the ECM, contributing to the structural integrity of bone, tendon, and cartilage tissues (Fratzl, 2008; Mienaltowski & Birk, 2014). Its widespread presence in connective tissues and natural biodegradability make collagen an ideal biomaterial for scaffold fabrication in BTE. Collagen exhibits several desirable properties, including biocompatibility, low antigenicity, and minimal inflammatory response, which are essential for successful tissue regeneration (Meghezi et al., 2015). These attributes allow collagen to support the adhesion, proliferation, and differentiation of bone cells, thereby facilitating osteogenesis (Mele et al., 2016).

Traditionally, collagen can be extracted from mammalian sources such as bovine and porcine tissues; however, concerns regarding zoonotic disease transmission, particularly spongiform encephalopathy, have led researchers to explore marine sources as safer alternatives. Fish by-products, including scales, skins, and bones, as well as other marine organisms such as cuttlefish and jellyfish, are increasingly utilized due to their abundant collagen content and lower risk of pathogen transfer (Senaratne et al., 2006; Lalezarliliana et al., 2019). Marine-derived collagen exhibits bioactivity comparable to mammalian collagen, supporting cell adhesion, proliferation, and tissue regeneration while demonstrating reduced immunogenicity (Mao et al., 2015).

Despite these advantages, pure collagen scaffolds face inherent limitations, including low mechanical strength, rapid biodegradation, and high swelling rates, which restrict their standalone use in load-bearing applications (Balani et al., 2007; Türk et al., 2018). To overcome these challenges, researchers have developed collagen-based nanocomposites and hybrid scaffolds by integrating collagen with inorganic components such as hydroxyapatite (HAp), tricalcium phosphate (TCP), or bioactive glass. These composites aim to mimic the natural composition and hierarchical structure of bone, improving both mechanical and biological properties (Cunniffe & O'Brien, 2011; Pina et al., 2015).

For instance, Pek et al. (2008) fabricated porous collagen-apatite nanocomposite scaffold foams that exhibited structural, chemical, and mechanical characteristics analogous to trabecular bone. The scaffolds consisted of collagen fibers interspersed with apatite nanocrystals, which enhanced mechanical strength and provided structural support for surrounding hard tissue. Their nanoscale architecture and apatitic composition closely resembled natural bone, promoting osteoconduction and facilitating cellular adhesion and proliferation. In vivo studies confirmed the osteoconductive nature of these scaffolds, demonstrating successful repair of critical-sized bone defects in pig tibia and nonunion fractures in rat femur models.

Similarly, Kikuchi et al. (2001) synthesized HAp/collagen nanocomposite scaffolds through a coprecipitation method using porcine atelocollagen, calcium hydroxide, and orthophosphoric acid. The resultant nanocomposites exhibited self-assembled nanostructures similar to natural bone, allowing osteoclast-mediated resorption and osteoblast-driven new bone formation. These HAp-collagen scaffolds demonstrated structural and compositional properties sufficient to serve as substitutes for autologous bone grafts. Zou et al. (2005) also prepared porous β -TCP/collagen composites, showing integrated structures conducive to bone tissue regeneration. Such studies emphasize that collagen-based nanocomposites can provide both structural support and bioactive cues, making them ideal candidates for scaffold-based bone regeneration.

One effective strategy to enhance collagen's functionality is through the development of semi-interpenetrating polymer networks and nanocomposite scaffolds, which combine collagen with other natural or synthetic polymers to improve mechanical strength and degradation rates (Pina et al., 2015). These advanced scaffolds aim to replicate the ECM's architecture and biochemical microenvironment, facilitating controlled cell proliferation and differentiation, nutrient diffusion, and waste removal (Cunniffe & O'Brien, 2011).

3.2.2 GELATIN

Gelatin, derived from the partial hydrolysis of collagen, retains many of the parent protein's bioactive properties while offering additional processability and plasticity (Djagny et al., 2001). It is a biodegradable, non-antigenic protein that can mimic aspects of the natural ECM, supporting cell adhesion, proliferation, and differentiation (Azami et al., 2012). Gelatin has been extensively investigated as a scaffold material in BTE, both as a stand-alone matrix and in combination with inorganic fillers such as hydroxyapatite, bioactive glass, or tricalcium phosphate, to enhance mechanical and biological performance (Chen & Yao, 2011).

Advancements in scaffold fabrication have led to the development of nanofibrous gelatin-based composites, produced via techniques such as thermally induced phase separation (TIPS). Nanofibrous structures provide a high surface area-to-volume ratio, promoting osteoblast attachment and proliferation while replicating the architecture of natural bone ECM (Liu et al., 2009). Recent studies highlight that gelatin-based nanocomposites display superior mechanical strength and porosity control, essential for supporting new tissue formation.

For example, Johari et al. (2016) explored a three-dimensional bioactive glass/gelatin nanocomposite scaffold seeded with osteoblasts as a potential bone replacement material. Scanning electron microscopy (SEM) confirmed the uniform morphology and enhanced cell adhesion characteristics of the scaffolds. In vitro assays demonstrated excellent cytocompatibility, with increased osteoblast proliferation and viability, while in vivo implantation in critical-sized calvarial defects in rats revealed effective bone regeneration and scaffold biodegradation.

Similarly, Samadikuchaksaraei et al. (2016) developed a gelatin/nHAp composite scaffold conditioned with osteoblast cells to evaluate its regenerative potential. Using layer solvent casting, freeze-drying, and lamination techniques, the scaffold maintained structural integrity while supporting osteoblast adhesion and proliferation. The conditioned scaffolds exhibited enhanced osteoinductivity and biocompatibility, facilitating accelerated collagen deposition and near-complete defect healing within three months post-implantation. These findings suggest that gelatin-based scaffolds, particularly when combined with osteogenic cells and inorganic fillers, can serve as effective platforms for bone tissue regeneration.

3.2.3 SILK FIBROIN

Silk fibroin (SF), obtained from the cocoon of *Bombyx mori* and other silkworm species, is a natural fibrous protein known for its impressive mechanical strength, biocompatibility, and tunable degradation profile (Kapoor & Kundu, 2016). SF can be derived from both mulberry and non-mulberry sources, with non-mulberry SF exhibiting enhanced mechanical properties and superior support for osteoblast adhesion due to the presence of integrin-binding Arg-Gly-Asp (RGD) motifs (Patra et al., 2012). SF's combination of structural integrity and bioactivity makes it a valuable material for BTE scaffolds, drug delivery systems, and soft and hard tissue engineering applications.

Recent research has focused on incorporating nanoparticles, such as titanium dioxide (TiO₂), into SF scaffolds to further improve mechanical and biological properties. Johari et al. (2018) reported the fabrication of SF/TiO₂-F nanocomposite scaffolds using phase separation techniques, observing enhanced mechanical strength and osteoblast adhesion at TiO₂ concentrations up to 15 wt%. Increased TiO₂ content improved bioactivity and scaffold mineralization; however, concentrations beyond 20 wt% caused nanoparticle agglomeration, reducing mechanical performance. Degradation studies indicated accelerated scaffold resorption with higher TiO₂-F content, accompanied by improved cytocompatibility and osteoblast viability.

In a complementary study, freeze-dried SF/TiO₂ scaffolds with varying TiO₂ weight fractions (5–20%) demonstrated interconnected macroporous structures suitable for cell infiltration and nutrient diffusion (Johari et al., 2017). SEM analysis showed enhanced directional and mechanical properties with increasing TiO₂ content, despite a slight reduction in porosity due to pore wall thickening. Apatite nucleation on scaffold surfaces confirmed bioactivity, while in vitro assays using osteoblast-like SaOS-2 cells demonstrated increased cell proliferation and alkaline phosphatase (ALP) activity, highlighting SF/TiO₂ scaffolds' potential for BTE applications.

Moreover, 3D-printed SF/hydroxyapatite (HA) nanocomposite scaffolds, prepared using in situ mineral precipitation with sodium alginate as a binder, exhibited uniform particle distribution, high porosity (~70%), and compressive strength >6 MPa (Huang et al., 2019). In vitro studies with human bone marrow stem cells revealed that SF/HA content significantly influenced cell proliferation and ALP activity, with scaffolds maintaining physiological pH throughout the culture period. Drug release studies using bovine serum albumin showed sustained delivery over five days, demonstrating the scaffold's potential as a multifunctional platform for bone regeneration and therapeutic delivery.

Overall, protein-derived polymers such as collagen, gelatin, and silk fibroin provide highly versatile and bioactive scaffolds for bone tissue engineering. By leveraging their biochemical properties, structural features, and compatibility with inorganic fillers and nanomaterials, researchers can design scaffolds that closely mimic the native bone environment, promoting effective osteogenesis and tissue regeneration. These protein-based biomaterials remain central to advancing scaffold technologies and developing clinically relevant solutions for bone repair and reconstruction.

4.0 CONCLUSION

The primary aim of this study was to explore and develop advanced scaffolding systems for bone tissue engineering (BTE), emphasizing biodegradable polymer-based nanocomposites capable of supporting bone regeneration and repair. Bone tissue engineering has emerged as a critical area in regenerative medicine, particularly for addressing bone defects caused by trauma, congenital anomalies, or diseases such as osteoporosis and osteomyelitis (Pina et al., 2015; Meghezi et al., 2015). Effective scaffold development requires a comprehensive understanding of bone's structural, biochemical, and mechanical properties, as well as the interactions between the extracellular matrix (ECM) and resident bone cells, including osteoblasts, osteocytes, and osteoclasts (Fratzl, 2008; Mao et al., 2015).

Recent advances in biomaterials research have facilitated the fabrication of nanocomposite scaffolds that integrate natural and synthetic polymers with bioactive inorganic components such as hydroxyapatite, tricalcium phosphate, and bioactive glass. These nanocomposites exhibit enhanced mechanical strength, improved surface bioactivity, and controlled biodegradability, which are essential for scaffold integration with host bone tissue (Balani et al., 2007; Huang et al., 2019). Natural polymers such as collagen, gelatin, and silk fibroin have been widely utilized due to their inherent biocompatibility, ability to promote cell adhesion and proliferation, and structural resemblance to the native ECM (Mele et al., 2016; Johari et al., 2016; Kapoor & Kundu, 2016). However, pure natural polymers often present limitations, including low mechanical strength and rapid degradation, prompting the development of composite scaffolds that combine these polymers with reinforcing nanofillers to mimic bone's hierarchical structure (Pek et al., 2008; Zou et al., 2005).

Polymer-based nanocomposites for BTE have demonstrated potential not only as structural templates but also as bioactive platforms capable of enhancing bone regeneration through osteoconductive and osteoinductive properties (Kikuchi et al., 2001; Samadikuchaksaraei et al., 2016). These scaffolds provide an appropriate microenvironment for cell adhesion, proliferation, and differentiation, thereby accelerating tissue regeneration while gradually degrading to allow replacement with native bone tissue. Additionally, the incorporation of bioactive nanoparticles such as titanium dioxide and fluoridated hydroxyapatite into polymer matrices has been reported to improve mechanical strength, control degradation rates, and enhance cellular responses (Johari et al., 2017; Huang et al., 2019).

Despite these advances, further studies are necessary to optimize scaffold design for clinical applications. Critical areas for continued research include refining pore architecture, promoting vascularization, and functionalizing scaffold surfaces to better replicate the natural bone microenvironment. Moreover, understanding the molecular mechanisms underlying bone formation and remodeling, and how scaffold composition influences these processes, remains essential for advancing BTE technologies (Cunniffe & O'Brien, 2011).

In conclusion, bone tissue engineering using biodegradable polymer-based nanocomposite scaffolds represents a transformative approach in regenerative medicine. By combining bioactive materials with supportive polymer matrices, these scaffolds offer substantial potential for restoring bone function in patients with bone defects or degenerative conditions. Continued interdisciplinary research integrating materials science, cell biology, and clinical studies is essential to realize the full therapeutic potential of these scaffolds and provide safe, effective, and clinically viable solutions for bone tissue regeneration.

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