



Review

A Critical Review of Natural and Synthetic Polymer-Based Biological Apatite Composites for Bone Tissue Engineering

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Abstract: During the past decade, there has been a continued increase in the demand for bone defect repair and replacement resulting from long-term illnesses or traumatic incidents. To address these challenges, tissue engineering research has focused on biomedical applications. This field concentrated on the development of suitable materials to enhance biological functionality and bone integration. Toward this aim, it is necessary to develop a proper material that provides good osseointegration and mechanical behavior by combining biopolymers with ceramics, which increase their mechanical stability and mineralization process. Hydroxyapatite (HAP) is synthesized from natural resources owing to its unique properties; for example, it can mimic the composition of bones and teeth of humans and animals. Biopolymers, including chitosan and alginate, combined with HAP, offer good chemical stability and strength required for tissue engineering. Composite biomaterials containing hydroxyapatite could be a potential substitute for artificial synthetic bone grafts. Utilizing various polymers and fabrication methodologies would efficiently customize physicochemical properties and suitable mechanical properties in synergy with biodegradation, thus enhancing their potential in bone regeneration. This review summarizes the commonly used polymers in tissue engineering, emphasizing their advantages and limitations. This paper also highlights recent advances in the production and investigation of HAP-based polymer composites used in biomedical applications.

Keywords: hydroxyapatite; smart polymers; regenerative medicine; bone tissue engineering; polymeric materials



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1. Introduction

Bone concerns, such as osteoporosis, osteoarthritis, and fractures, are major health issues that cause weak bones or even broken bones, leading to decreased mobility, increased falls, and significantly impacting quality of life [1–3]. For these reasons, medical procedures such as bone grafting or even full bone replacement may become necessary to restore bone structure and performance. A big challenge in treating bone diseases is that bones do not grow back easily when badly damaged or with large defects, making replacement a necessity in medical treatments [4,5]. In the past, autografts and allografts were used to replace bones. However, these could lead to serious consequences, such as immune system reactions, risk of spreading diseases, and pain [6]. Tissue engineering (TE) has emerged as a potential new method of fixing and regenerating bones. Tissue engineering aims to produce biomaterials that mimic bone structure and function, as well as help bones heal naturally [7,8]. This method focuses on creating scaffolds that facilitate cells to grow, change, and form mature tissues. These scaffolds are commonly made from biocompatible materials that eliminate long-term health risks, reduce inflammation, and promote cell growth [9,10].

Bone tissue engineering scaffolds are biocompatible, biodegradable, bioactive, osteoconductive, and non-toxic [11]. A scaffold should have a network of pores sufficient to enable cell seeding and cell penetration. In addition to supporting and facilitating bone growth, they should also provide a mechanical structure [12,13]. One of the critical aspects of fabricating TE scaffolds is determining the optimal time to break them down to ensure a proper balance between newly formed bone regeneration and scaffold resorption. [14,15]. A variety of materials have been studied for developing scaffolds, such as hydroxyapatite and fluorapatite [16]. These materials are biocompatible and osteoconductive, enabling their use in tissue engineering applications [17]. Furthermore, apatite forms on their surfaces, facilitating bone attachment to them and contributing to bone growth, which is essential for bone regeneration [18,19].

Bioactive materials like bioceramics and bioactive glasses also stimulate stem cells to proliferate, differentiate into osteoblasts, and promote bone formation [20]. This process is imperative for bone healing. Added substances that act as growth factors or osteogenic agents to the scaffold enhance the rate of regeneration. This is accomplished by activating stem cells to develop into bone-building cells known as osteoblasts, which support bone formation [21,22].

To design a structure, composite materials are created by combining two or more different materials from biocompatible sources that act as bone extracellular matrices. The structure of these biomaterials, natural and synthetic, has similar strength, stiffness, and fracture resistance to natural bone. They are also biocompatible and can be easily implanted into the human body [23]. Additionally, researchers are investigating nanocomposite scaffolds that aid tissue regeneration and repair [24]. This review paper, therefore, concentrates on the investigation and production of composite materials based on biopolymers and hydroxyapatite. Materials such as these can be used to produce advanced scaffolds that promote bone healing. By combining biopolymers and bioactive ceramics like hydroxyapatite, novel scaffolds have been developed that work like natural bone. This review paper, therefore, concentrates on the investigation and production of composite materials based on biopolymers and hydroxyapatite.

2. Biopolymers Used in Tissue Engineering

Biomaterial characteristics must be extensively investigated to ensure their successful application in tissue engineering. Chemical composition, structural and biological properties, degradation rate, and method of manufacture all play a role in material selection [25,26]. Researchers focus on biopolymers to promote regeneration, healing, and reconstruction of injured tissues through scaffolds. Along with the advancement in tissue engineering, biomaterials have emerged as a promising option for bone repair and regeneration, addressing the limitation of the conventional metallic implants and bone grafts [27,28]. Bone transplants, therefore, have significantly increased, exceeding over two million transplants performed worldwide [28]. Biomaterials, including biopolymers, have gained significant interest for their therapeutic potential in bone repair and health owing to their beneficial antioxidant and anti-inflammatory properties. Biopolymers have also been known for their advantageous properties such as biocompatibility, biodegradability, and structural similarity to natural bones [29]. Among biopolymers, chitosan, collagen, and gelatin are extensively researched and applied in bone tissue engineering [29]. Three-dimensional printing combined with nano-biomaterials in scaffolds for bone tissue engineering is expected to further enhance the effectiveness, applicability, and durability of these novel materials. This novel combination will ultimately benefit patients with bone defects, injuries, and diseases [27,30]. Depending on their origins, biopolymers used in TE are grouped into two primary categories [31], naturally derived polymers and synthetic polymers, as illustrated in Figure 1.

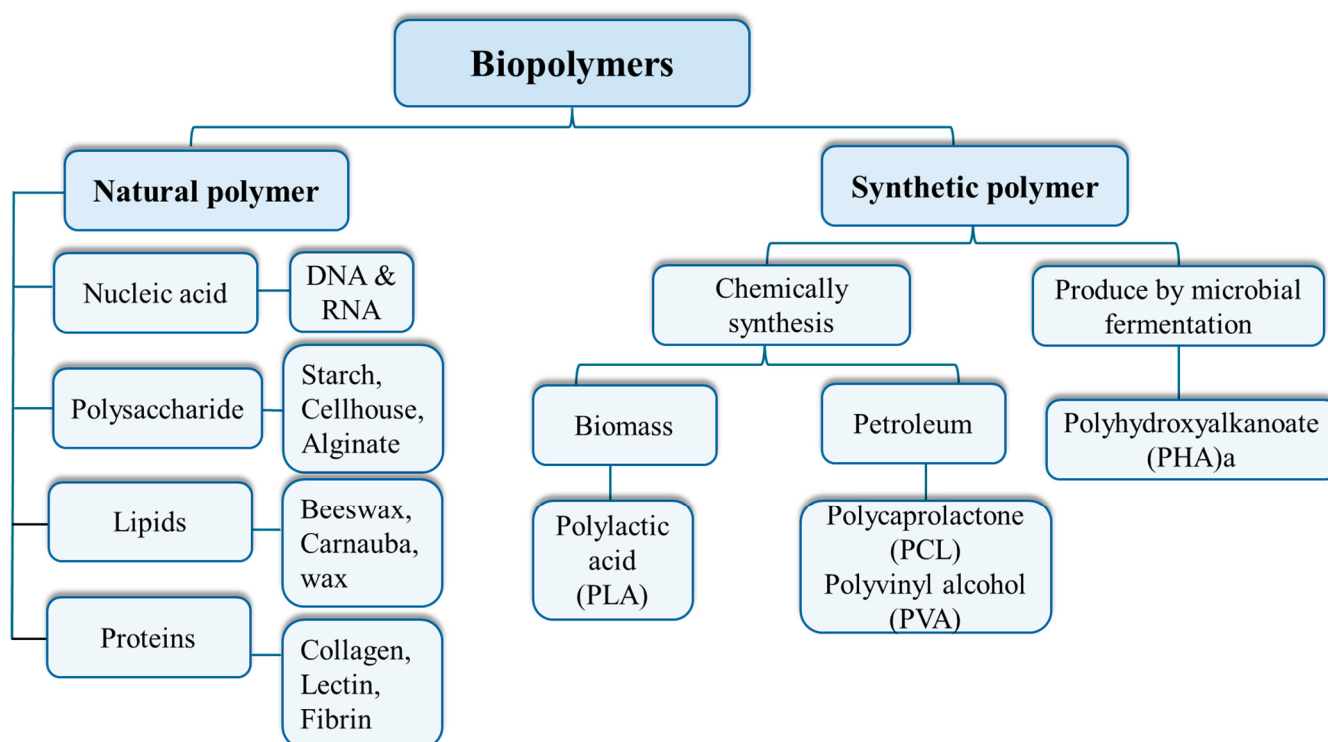


Figure 1. A classification of biodegradable polymers, with examples, based on their source.

2.1. Natural Polymers

Polymers originating from biological sources such as plants, animals, and microorganisms exhibit special properties, including biocompatibility and bioactivity, which improve cell proliferation and adhesion [32]. Examples include proteins, polysaccharides, lipids, and nucleic acids. Each group has special features that make them useful in certain medical treatments or research. Proteins like collagen help form tissues, polysaccharides such as chitosan help heal wounds, and nucleic acids can be involved in genetic therapies [33,34]. The use of natural polymers in TE has considerably increased through the development of multimodal scaffolds that possess distinct shapes and properties, both physically and chemically [35]. However, the strength of these polymers and how quickly they break down make them difficult to control. As a result, they could be less useful for holding weight in the body unless they are altered to support weight [36]. To render these biostructures more efficient in applications required for weight-bearing purposes, some potential alterations may include reinforcing the internal structure, incorporating stronger materials, or redesigning the shape or form to better distribute weight.

These polymers can be combined with other materials to provide a sufficient level of strength and breakdown rate. This is especially beneficial for hard tissues like bones or cartilage. Table 1 shows a summary of the general properties of the natural polymers usually employed in TE. For instance, collagen is an essential protein in the extracellular matrix of the human body. It has a triple-helical structure composed of fibrils serving as nucleation sites for nano-apatite particle growth, facilitating cell adhesion and tissue development [37]. Another effective example is chitosan, derived from crustacean shells. Chitosan possesses several advantageous properties, such as it degrades naturally over time and promotes bone growth. These characteristics make it useful for bone tissue engineering, where it assists with bone restoration and prevents infection [38]. However, these natural polymers have a downside: their limited adhesion strength limits their use in applications requiring durable structural integrity. To address this issue, scientists combine them with synthetic polymers or enhance their properties by incorporating nanoparticles. This enhances natural polymers' performance in medical applications requiring strong, stable materials, such as bone and cartilage repair [39].

Table 1. Natural polymers widely used in tissue engineering together with their main characteristics and applications.

Polymers	Properties		Application	References
	Advantages	Limitation		
Gelatin	Excellent biocompatibility, biodegradable, non-toxic, enhancement of cell adhesion and proliferation	Weak mechanical properties, low stability in physiological condition	Scaffolds for hard tissue engineering	[40]
Alginate	Biodegradability, biocompatibility, bioresorbable, non-toxicity, mimicking the function of extracellular body tissue	Weak mechanical strength, low cell adhesion, difficult to sterilize	Bone tissue applications	[41]
Cellulose	Good water absorption capacity, mechanical performance, structural properties, cell adhesion, biocompatibility	A lower biodegradability in humans	3D scaffolds, bone replacements	[42]
Chitosan	Prevent formation of scar tissue, excellent biocompatibility, biodegradability, and anti-inflammatory	Poor stability, weak mechanical performance	Scaffolds, microspheres	[43]
Collagen	Superior biocompatibility, low toxicity, biodegradable, rough surface, low immunogenicity	Weak mechanical properties, poor stability in an aqueous environment.	Scaffolds, drug delivery systems	[44]
Hyaluronic Acid	High biocompatibility, biodegradability, good cell adhesion, proliferation, differentiation	Weak mechanical performance, high degradation rate	Scaffolds, hydrogel	[45]
Silk Fibroin	Enhanced flexibility, biocompatibility, good mechanical performance	Reduced biodegradation rate	Scaffolds	[46]

2.2. Synthetic Biopolymers

In comparison with natural polymers, synthetic polymers are highly customizable, durable, and easy to manufacture [47]. Unlike natural polymers, artificial ones can be designed to possess specific characteristics such as regulated degradation rates, customized strength properties, and modifiable porosity. These attributes make synthetic polymers particularly well-suited for TE applications, especially in rebuilding bones or cartilage, where the material needs to handle weight [48].

Synthetic polymers can be tailored for specific TE uses. Poly (ethylene glycol) (PEG) is frequently used because of its good biocompatibility and immunogenicity, and its mechanical traits can be fine-tuned to match the needs of the tissue being repaired. Synthetic polymers are limited in that they may not naturally promote cell adhesion or growth, unlike natural polymers [49]. Researchers can optimize synthetic polymer performance in biological environments by incorporating bioactive compounds, nanoscale particles, or growth-stimulating agents. As shown in Table 2, synthetic polymers offer extensive flexibility and control in tissue engineering, but need modifications to improve their bioactivity.

Table 2. Synthetic polymers widely used in tissue engineering together with their main properties and applications.

Polymers	Properties		Application	References
	Advantages	Limitation		
Poly (ethylene glycol) (PEG)	Biocompatible, degradable, non-toxic, non-immunogenic blend with various polymers, enzyme-stable, hydrophilic	Limited rheological, mechanical strength, and bioactivity	Scaffolds, BTE, 3D bioprinting, orthopedic implant	[50]
Poly (vinyl alcohol) (PVA)	Excellent biocompatibility, biodegradability, good mechanical behavior	Low bioactivity and cell adhesion	Scaffolds, drug delivery systems	[51]
Poly(lactic acid) (PLA)	Biodegradability, high mechanical performance, lesser inflammatory response	Low toughness, mechanical support, insufficient biocompatibility	Load bearing applications, orthopedic repair, scaffolds	[52]
Poly (glycolic acid) (PGA)	Proliferation, and differentiation, high crystallinity, excellent mechanical performance, good cell attachment	Hydrophobic	Scaffolds, BTE	[53]
Poly (-caprolactone) (PCL)	Good biodegradability, and biocompatibility, low elastic modulus, tailorable physical properties, low degradation rate	Poor cell attachment, hydrophobic	Scaffolds, BTE, 3D bioprinting	[54]
Poly (methyl methacrylate) (PMMA)	Enhances processability, durability	Undegradable	Scaffolds	[55]
Poly (lactic-co-glycolic acid) (PLGA)	Processability, good mechanical performance, high biocompatibility, adjustable degradation rate, low inflammatory response	Possible inflammatory response, low bioactivity	Scaffolds, orthopedic implants, drug delivery systems	[56]

One of the commonly used synthetic polymers in TE is poly (glycolic acid) (PGA), which rapidly degrades and has strong mechanical properties, making it ideal for fixing tendons and ligaments [57]. Another synthetic polymer is poly (lactic acid) (PLA), which is well-known for being biodegradable and having an adjustable breakdown rate. PLA is commonly used in cases where a temporary structure is needed, such as fixing bones or cartilage. However, PLA, like other polymers, has weak mechanical performance, restricting its potential applications in the medical field. For this reason, inorganic materials or other polymer matrices are often used to enhance their mechanical properties for bone regeneration purposes [58]. For instance, Bal et al. designed scaffolds based on PLA-PEG to support BMP-2 incorporation. Despite this, HAp was also considered to be introduced to the system, as its mechanical properties were insufficient [59]. In contrast, polycaprolactone (PCL) demonstrates a slower degradation rate and excellent mechanical properties, making it more appropriate for long-term applications such as bone scaffolds. However, synthetic polymers like PCL and PLGA lack innate abilities to promote cell growth. This requires modification through the incorporation of specific bioactive agents or in combination with bioceramics such as hydroxyapatite. This is to enhance cell adhesion and bone tissue formation. Synthetic materials have resulted in enhanced and more targeted tissue healing strategies. These materials are expected to provide increasingly specialized solutions to various tissue regeneration needs [60].

3. Hydroxyapatite as Bioactive Agent in Biocomposites

In human bones and teeth, hydroxyapatite (HAp) is composed mostly of calcium apatite. Bioactivity is approved for its ability to deliver biological signaling that stimulates cell activity and tissue growth. HAp offers a promising opportunity for producing biomaterials at lower costs. Natural sources such as eggshells, shells, and corals provide an effective way of obtaining hydroxyapatite [26,61]. Furthermore, materials with high calcium content from natural origins such as bovine bones, fish bones, and scales can be used as useful resources of HAp (Table 3). In addition, eggshells from chickens, bones from fish, and fish scales serve as favorable calcium sources due to their widespread accessibility and ease of access. Consequently, this results in reduced costs for raw materials, and provides environmental benefits by reusing waste products [62]. Improper control of these substances can foster environmental conditions for bacteria and fungi proliferation, owing to the organic matter. This can result in unpleasant consequences, such as foul odors and the spread of illness.

Table 3. Comparison between different HAp sources.

Source	Types of CaP	Characteristics	Biomedical Applications	References
Animal Bones	Hydroxyapatite	High similarity to human bone, excellent osteoconductive	Bone grafts, dental implants, coatings for prosthetics	[4,63]
Eggshells	Calcium carbonate (CaCO_3), can be transformed into HAp	Low-cost, abundant, easy to process	Bone repair, drug delivery systems	[64]
Corals	Calcium carbonate (CaCO_3), transformed into HAp	Porous structure resembling cancellous bone	Bone scaffolds, tissue regeneration	[22]
Marine Shells	Calcium carbonate (CaCO_3), can be converted to HAp	Biodegradable, porous, naturally occurring structure	Bone regeneration, dental applications	[65]
Fish Scales	Hydroxyapatite	Naturally contains calcium phosphate, biocompatible	Bone implants, regenerative medicine	[66]

Mammalian bones, including horses, camels, pig, and bovine contain a large amount of calcium carbonate (CaCO_3), making them an excellent source for extraction of hydroxyapatite. Bovine bones are commonly employed in HAp extraction. Bones have to be cleaned before preparation by rinsing using hot water or solvents, removing fats, proteins, as well as blood cells by heating the bone to a temperature of 1400 °C during the production stage. Barakat et al. compared the efficiency of three different approaches for HAp production from bovine bones through thermal decomposition, sub-critical water treatment, and alkaline hydrothermal processes. Upon examination of the results, it is obvious that the alkaline hydrothermal process yields pure HAp nanorods, which differ from the nanoparticles produced by the two competing methods [63]. The chemical structure of eggshells, which are organic bioceramic compounds in nature, consists of 95% crystalline calcium carbonate CaCO_3 covered by a 5% protein layer. Thus, it acts as a suitable initial precursor of calcium for HAp synthesis [67]. Azis used a sol-gel process to prepare HAp nanoparticles from eggshells by precipitating calcium carbonate, dissolved using 0.3 M HNO_3 , mixed and stirred with 0.3 M $(\text{NH}_4)_2\text{HPO}_4$, dissolved in aquadest for 3 h. Based on the results, it was shown that the pure hexagonal pattern of HAp was found at one day curing time and pH 9 [64]. Sundaram et al. used a 30-day procedure to synthesize HAp from nanoparticles rich in drug molecules by using ciprofloxacin from eggshells as calcium precursors, eliminating the need for toxic chemicals [68]. Castro et al. produced HAp by hydrothermal eggshell fabrication using ultra-high-frequency treatment techniques [69]. Gergely et al. produced HAp from waste eggshells by thermal processing in a couple of steps [70]. In the initial step, almost all biological substances were calcined, while the

subsequent step included eggshell converted to calcium oxide. A ball mill operating at various rates of speed was used to avoid accumulation and maintain uniform blending of calcium carbonate. XRD, FTIR, and SEM results show that a rotational milling (4000 rpm for five hours) is preferred compared to ball milling (350 rpm for ten hours); thus, at the nanoscale level, uniform HAp is produced, regardless of milling [70]. According to a study by Mohd et al., HAp was synthesized from eggshells by chemical precipitation and calcination processes. The calcination method was carried out at various degrees of heat, and after that, the calcinated powder was dispersed in a solution containing water and a concentration of 0.6 M phosphoric acid. Based on the data, 700 °C was the ideal calcination condition for HAp [71]. Lee et al. analyzed the properties of HAp obtained from eggshells and artificial HAp by FTIR and XRD. In addition, they investigated the bone regeneration efficiency of rabbit fault simulation (in vivo test). Results showed that bone growth in both kinds of HAp were better than the empty group [72]. The similar results were observed by Balázs et al., utilizing nanosized HAp for bone healing in a rabbit and mouse disease experiment, and the findings were examined by TEM [73]. Researchers have found that the prepared HAp extracted from hen's eggshells that are discarded as waste has potential applications in tissue engineering.

Several marine wastes, for example, mollusk shellfish, cuttlebone shellfish, oyster shellfish, and snail shellfish, are of great interest in terms of HAp, as they contain a large amount of CaCO_3 . Natural HAp can be obtained via a thermal calcination process, which involves heat treatment at high temperatures to eliminate volatile substances. For example, seafood residue of green mussel shells was used to produce HAp after calcination at different temperatures for two hours using an infrared treatment method, based on a study by Liand A. et al. [65]. It was shown that the crystalline HAp phase had notable variation among the various specimens [65]. Similarly, Zuliantoni et al. prepared HAp from snail shells using the hydrothermal technique with phosphate hydrogen diammonium. Based on their study, HAp obtained from snail shell has excellent characteristics compared to standard HAp. Nanosized HAp can be also derived from mussel shells. Shells represents about 55% of the weight of mussels, and they are composed of 95–99% aragonite, which is a form of CaCO_3 [66]. Figure 2 summarizes the extraction of HAp from its natural sources. All of these natural sources of HAp offer cheaper, eco-friendly options than man-made materials. Each has special benefits, like strength or working well with bone cells, which makes them suitable for different medical uses.

Natural Hydroxyapatite

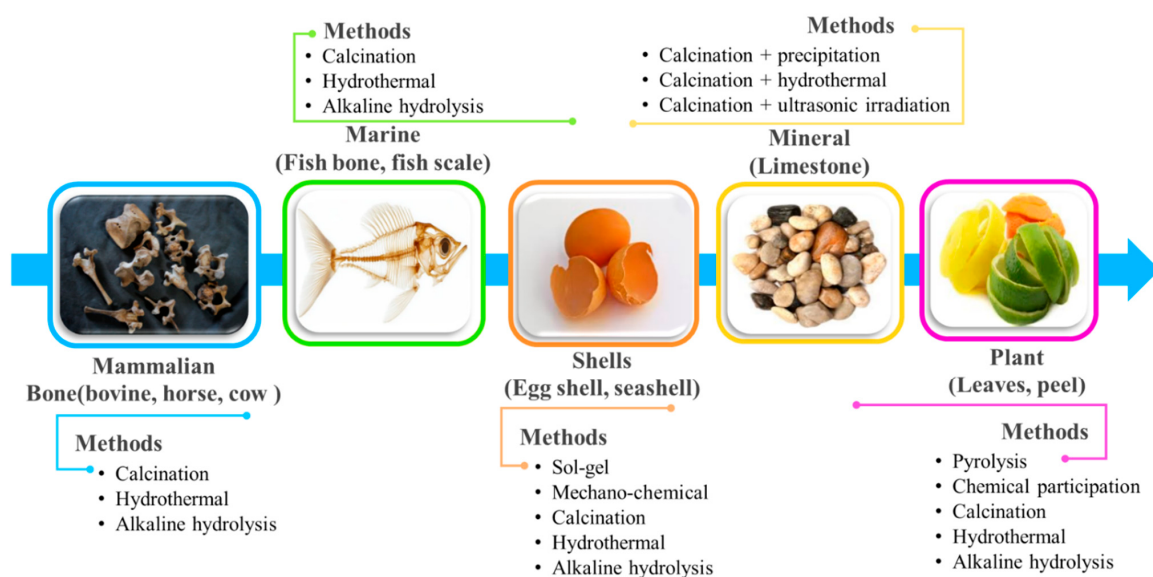


Figure 2. Schematic diagram of natural sources and synthesis methods for HAp.

Hydroxyapatite–Polymer Composites for Bone-Tissue Engineering

In the area of tissue engineering, polymer-based HAp composites are gaining significant traction as they improve the mechanical properties of hydroxyapatite-based implants while still maintaining the desirable bioactive characteristics needed for bone replacement [74]. In addition, investigations have shown that manufactured implants from nanosized HAp exhibit better results than micro-sized ones. HAp particle size, biopolymer content, and synthesis processes can be adjusted to tailor the properties of HAp polymer composites to meet the requirements for biomedical purposes [75]. The produced composites possess superior properties such as biocompatibility, promoting cell adhesion, proliferation, and differentiation, enabling tissue engineering applications. Additionally, HAp nanoparticles remarkably enhance the mechanical performance and bioactivity of biopolymer composite materials. This enhances their suitability for critical applications, for example, load-bearing implants and drug delivery systems. The enhanced performance of polymer composites with HAp as an additive can be because of intermolecular reactions with polymer chains of HAp, as reported in a study carried out on polyurethane. Results showed that increasing concentrations of HAp reduced the mobility of polymer chains [26,76]. In contrast, scaffolds for bone regeneration require specific characteristics, such as biocompatibility, considerable mechanical performance to withstand weights, and biodegradability without emitting toxins into tissues [77].

4. Fabrication of Hydroxyapatite–Polymer Composites

Different fabrication methods are adapted for the producing composite scaffolds from HAp and other calcium phosphates (CaP), which mimic the natural bone properties. This is due to the importance of bioactivity in creating a chemical bond with natural bone. The common techniques to tailor composite scaffolds include freeze-drying, particulate leaching, electrospinning, gas foaming, biomimetic, solvent casting, laser sintering, and 3D printing [78,79].

4.1. Solvent/Solution Casting Method

The process of solvent casting biocomposites consists of a polymeric material dissolved in an organic solvent, and mixed with ceramic granules, which before were molded inside a predefined 3D mold, and then the solvent was allowed to evaporate [80]. A schematic diagram of the solvent casting technique is displayed in Figure 3.

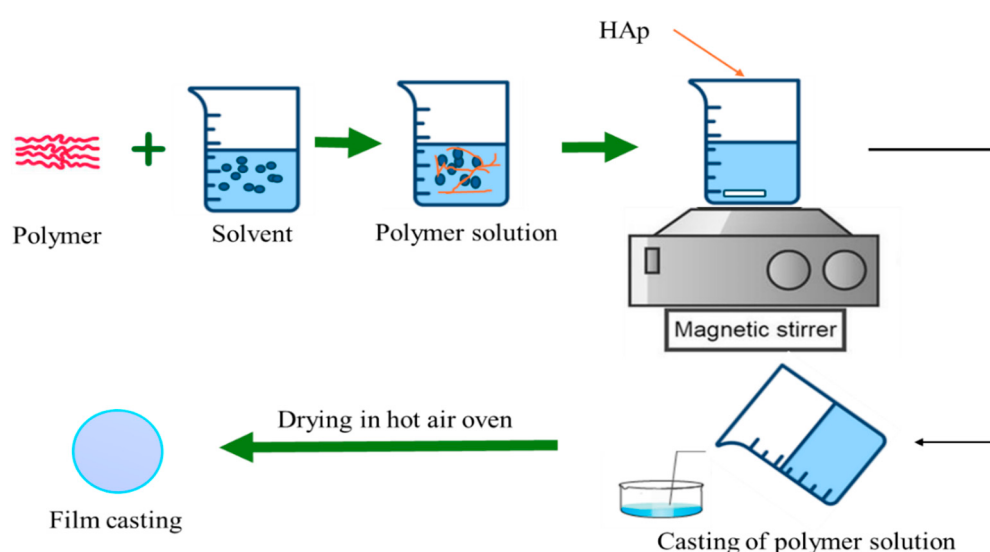


Figure 3. Schematic diagram of the solvent/casting solution process.

This fabrication technique is simple to prepare, low cost, and operates at ambient temperatures without specialized equipment. In one study, nano-HAp/chitosan composite

membranes were prepared by solvent casting. The crosslinking agent is genipin, used with acetic acid as a solvent. Various concentrations of nano-hydroxyapatite/chitosan composite membrane extracts were added to cell wells to determine viability. Results indicated that the developed nano-hydroxyapatite/chitosan composite membranes were noncytotoxic. According to them, these membranes will facilitate the engineering of bone tissue [81]. A study found that different concentrations of HAp were added to the solution containing alginate–gelatin films to make a composite film using the solution casting method to crosslink alginate and gelatin solutions. Physical and mechanical properties of the obtained composite film were examined. The obtained results demonstrated that increased HAp resulted in a rougher film surface. Additionally, Gram-positive bacteria were more susceptible to these drugs than Gram-negative bacteria. It was concluded that the obtained films could be used for biomedical applications [82]. Additionally, Deng et al. investigated the ability of incorporating nHAp into a poly (D, L-lactide) (PDLLA) composite to replace and regenerate bone tissue [83]. In this study, HAp/PDLLA composites were co-blended in N, N-dimethyl formamide solvent and homogeneously dispersed, followed by drying and blending the hybrid material, and compacting with a hot-pressing machine. Improved mechanical properties were observed, which is essential for implant material used in vivo. SEM, as shown in Figure 4 for a composite with more than 20 wt.% HAp, revealed nucleation sites for apatite formation and, as a result, exhibited better in vitro properties [83].

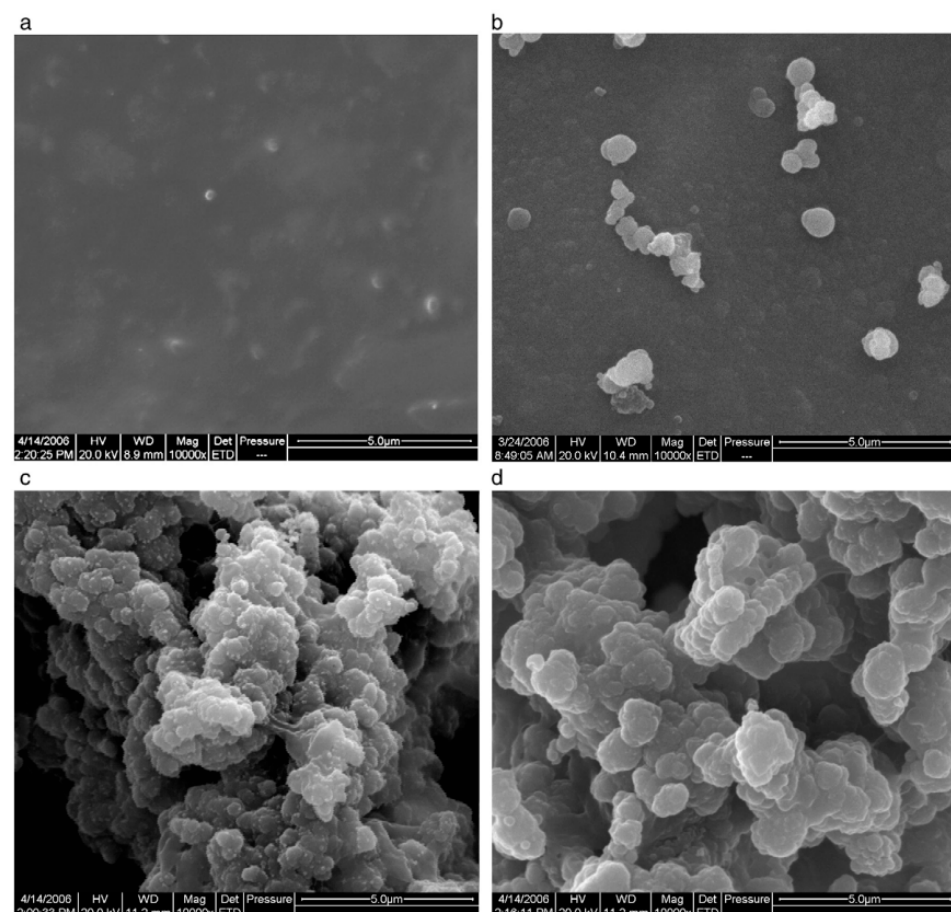


Figure 4. SEM images of composite films immersed in simulated body fluid at a temperature of 36.5 °C for (a) 1, (b) 7, (c) 14, and (d) 21 days. Reproduced with permission [83].

4.2. Freeze-Drying Methods

Freeze-drying techniques involve freezing a material using different methods such as a dry-ice bath to remove residual solvents. The material is frozen after being dissolved in a

solvent, resulting in a solid powder that can be loaded into cells easily. The temperature during this process is kept low to prevent phase separation of polymer solutions. Figure 5 shows a schematic description of the freeze-drying technique used in several studies to develop polymer-based scaffolds for tissue regeneration. Various types of polymers were used in the production of scaffolds, such as chitosan [84,85], gelatin [86], poly (ether ester) [87], silk fibroin/hyaluronan [88], and carboxymethyl cellulose [89]. These are some examples of polymer-based nanocomposite scaffolds for tissue engineering and regenerative medicine prepared by freeze-drying techniques. They include n-HAp/PLLA composite scaffolds with uniformly distributed HAp, and excellent mechanical properties, compression strength, using 20 wt.% HAp content [90], collagen/HAp [91,92], chitosan/HAp [93], or gelatin/HAp [94].

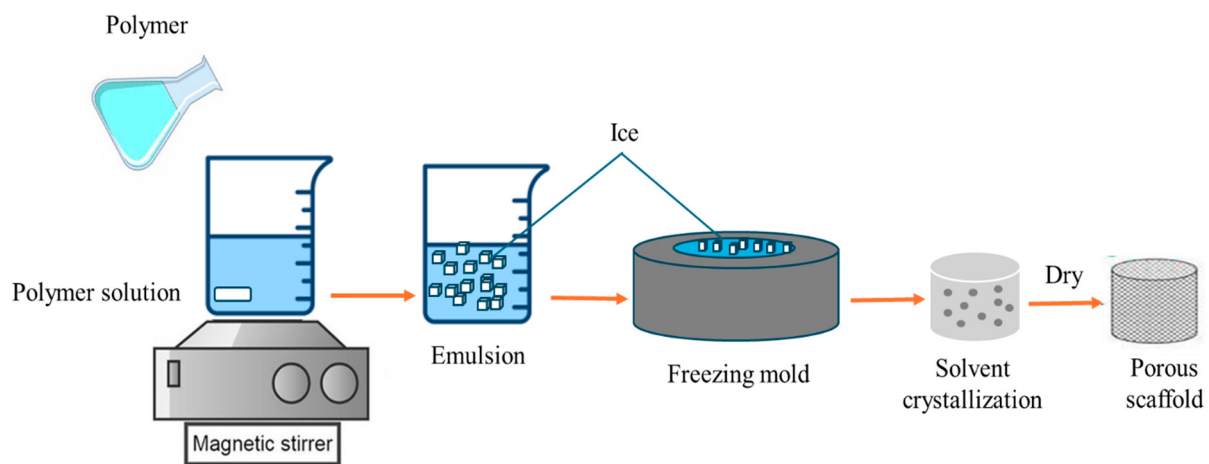


Figure 5. Schematic diagram of the freeze-drying process.

As an alternative, Sun et al. explored the possibility of forming a flexible nHAp/chitosan by combining chitosan with multiple doses of nHAp. A significant difference in surface morphology was detected at freezing temperatures, as shown in Figure 6. Frozen surfaces with a heterogeneous porous structure were achieved at $-20\text{ }^{\circ}\text{C}$ for 4 h, while composites fabricated at $-78\text{ }^{\circ}\text{C}$ showed homogeneity and regularity [95].

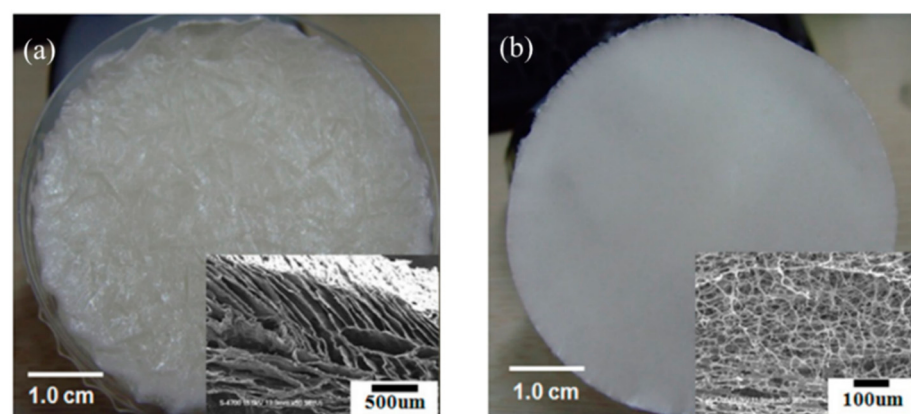


Figure 6. Morphology of (a) a less dispersed film ($-20\text{ }^{\circ}\text{C}$) and (b) a well dispersed film ($-78\text{ }^{\circ}\text{C}$) [95].

4.3. Electrospinning

Electrospinning is an effective process to produce ultra-fine polymeric fibers that range in diameter from micrometers to nanometers. This method is frequently used for tissue engineering applications because of the similarity of these structures in morphology and fiber diameter to the extracellular matrix of human tissues [96]. As an additional advantage, electrospinning is regarded as a simple and low-cost fabrication technique

that generates multiple fiber patterns with high pore size, offers lower fabrication costs, and provides a safe work environment as opposed to other fabrication techniques [97]. The schematic diagram of the electrospinning process is displayed in Figure 7. During this process an electric field is applied to a viscous solution forced through a syringe that controls its flow. This is done to obtain fiber collected on a metal collector plate. Several studies have examined the efficacy of electrospun fibrous biomaterials as tissue regeneration scaffolds. Natural polymers such as collagen, chitosan, cellulose, silk fibroin, and synthetic polymers including polyvinylpyrrolidone (PVP), (PLGA), and (PLA) can be electrospun. Moreover, the combination of natural and synthetic polymers promises better clinical performance [98–104].

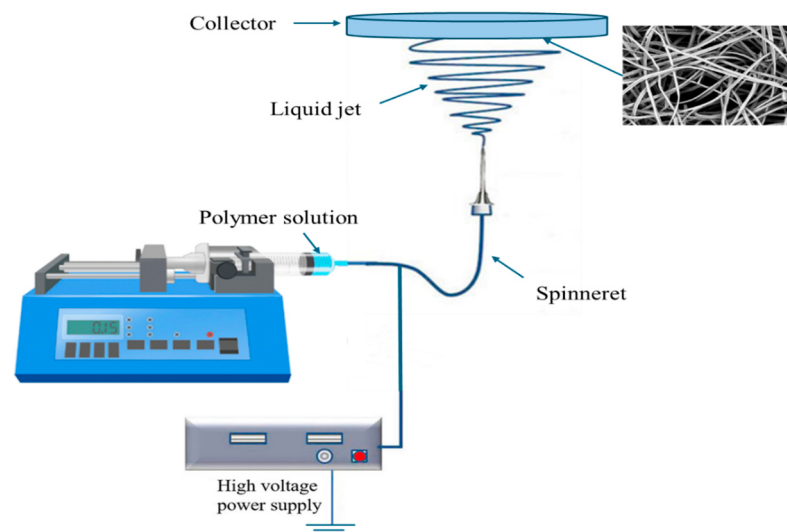


Figure 7. Schematic diagram of the electrospinning process.

For example, HAp substances can be incorporated during the electrospinning process to enhance the characteristics of the final fibers spun for use in bone regeneration [105–107]. P. Gouma et al. fabricated artificial bone tissue scaffolds based on natural hybrids of cellulose acetate and n-HAp particles by employing the electrospinning technique. To mimic extracellular matrix architectures, the performance of in vitro bone generation was investigated for a period of 14 days. The obtained results demonstrated that the produced nanocomposite supports osteoblast binding and growth, as well as stimulates bone cell function, as seen in Figure 8 [108].

Due to chitosan and HAp bioactivity and osteoconductive abilities, PVA/chitosan blended membranes can be utilized for hard tissue engineering. Yang et al. [109] used an electrospinning technique to prepare nanofibrous biocomposite scaffolds of chitosan/PVA with an average diameter ranging from 100 to 700 nm. This is quite like the fiber diameter of natural bone tissues, which ranges from 100 to 450 nm. The results showed that the diameter of the composite fibers increased with an increase in the nHAp content, as seen in Figure 9. Researchers reported that the addition of HAp nanoparticles into nanofibrous chitosan scaffolds yielded significantly enhanced bone formation outcomes, in comparison with pure electrospun chitosan scaffolds [109].

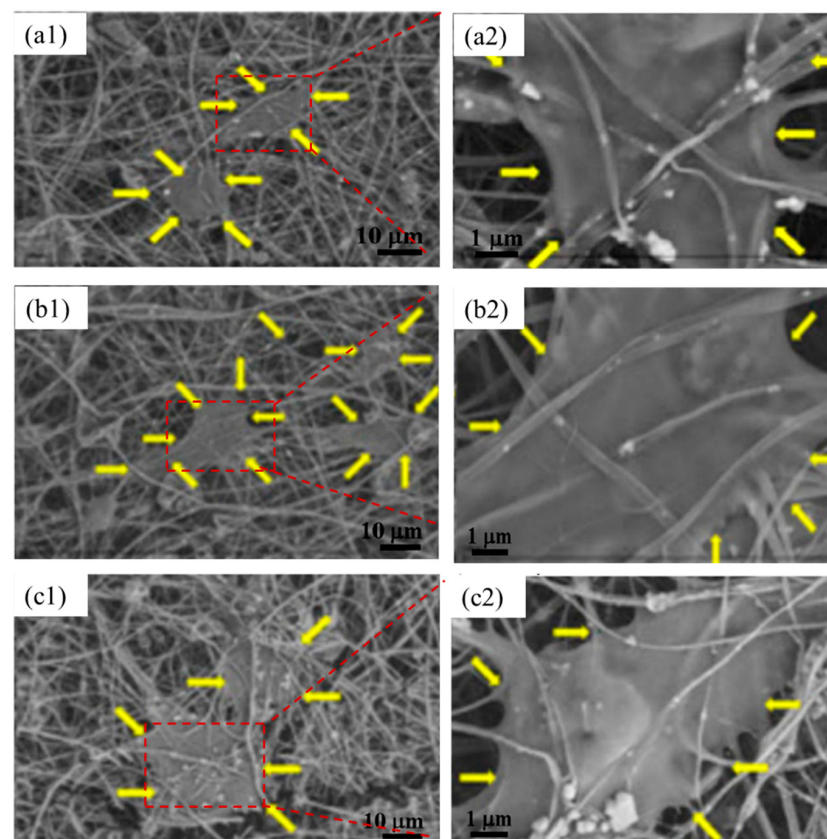


Figure 8. SEM of cell scaffolds cultured, at different magnifications (a1,a2), for 1 day, (b1,b2) 7 days, (c1,c2) and 14 days. Yellow marks show the anchorage sites of the cells. Reproduced with permission [108].

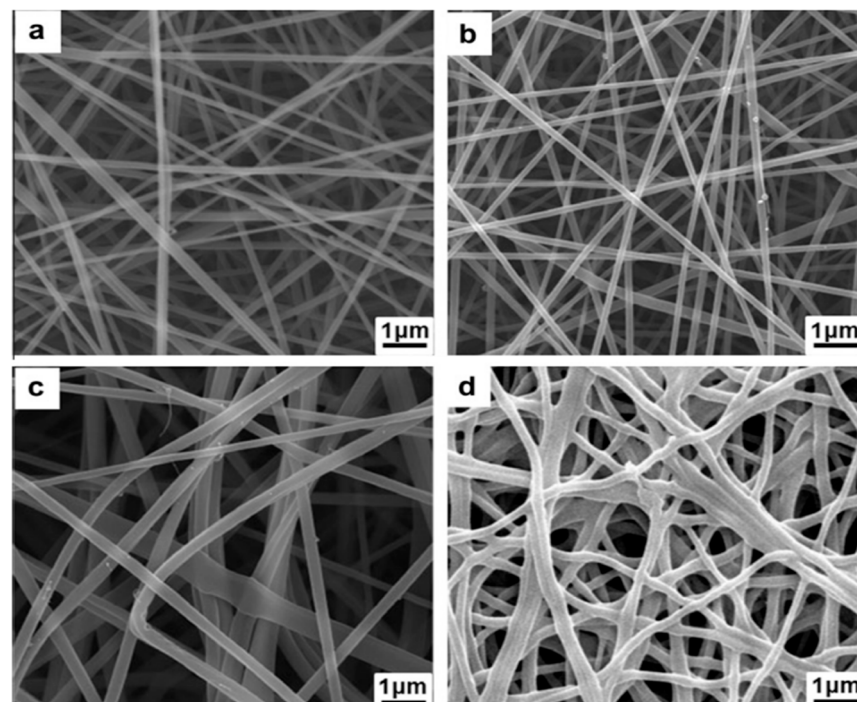


Figure 9. SEM micrographs of scaffolds produced by electrospinning. (a) Chitosan/polyvinylalcohol (Chi/PVA) fibers, (b) Chi/PVA + 2% hydroxyapatite (HAp) (uncrosslinked), (c) Chi/PVA + 5% HAp (uncrosslinked), (d) Chi/PVA + 5% HAp (crosslinked) [109].

4.4. 3D Printing of Microstructure Composites

Using 3D printing technology allows for the rapid production of designs, which can then be visually presented to colleagues and users; 3D-printed scaffolds with controllable internal porosity, pore size, and interconnected structure have attracted increasing attention in recent years in biomedical research. It is essential that scaffolds made from synthetic and natural polymers have excellent biocompatibility and mechanical strength in order to treat critical bone defects [110]. PLA is an eco-friendly clinical polymer having approximately the same compressive strength as natural bone. Despite its excellent mechanical performance, PLA exhibits little bioactivity. PLA and n-HAp combine to enhance biological activity [111]. Heo et al. fabricated PLA/HAp composite scaffolds with nano- and micro-particle sizes of added HAp to produce porous bone tissue scaffolds via 3D printing, as shown Figure 10. The mechanical properties, in vitro performance, and features of PLA/n-HAp and PLA/m-HAp composite scaffolds have been examined and analyzed, and the best type was proposed for critical bone defects. According to the authors, the n-HAp particles were uniformly distributed in the composite and dispersed in the PCL matrix. It was found the coarse and micro-sized HAp particles were dispersed in the PCL matrix, including some rough micropores in the composite. While the nanosized HAp scaffold had a smooth surface, the micro-sized HAp had a rough surface [112].

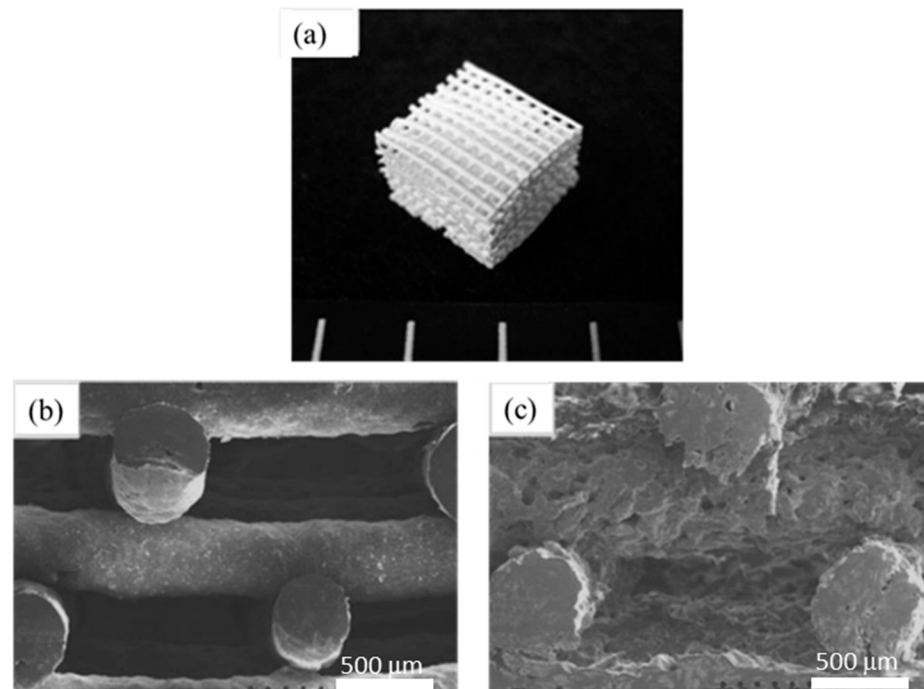


Figure 10. (a) Photograph of n-HAp/PCL, (b) SEM micrographs of n-HAp/PCL, and (c) m-HAp/PCL [112].

4.5. Electrodeposition

Electrodeposition processes are carried out in aqueous solutions to produce a thin and uniform film on a substrate material. During this method, the solvent solution suspends particles, which move in an electric field and deposit on an electrode. The electrodeposition technique has high accuracy, controls film thickness and uniformity, uses low energy and deposition rate, is easy to use and low-cost. Moreover, electrodeposition is efficient for covering surfaces [113]. Additionally, it is a valuable approach to fabricate composite films that play increasingly significant roles in different applications, like TE scaffolds [114]. S. Mahmoodi et al. utilized electrodeposition methods for the deposition of HAp/chitosan nanocomposites on 316L stainless steel. Composite coatings were prepared from suspensions of 2.5, 5, and 10 g/L HAp and 0.5 chitosan at 60 V/cm in different

solvents including methanolic, ethanolic, and isopropanolic suspensions. According to the results observed in Figure 11, it was found that chitosan adsorbed most strongly to HAp nanoparticles in isopropanol and least in methanol. In addition, coatings deposited from ethanolic suspensions with 2 and 5 g/L of HAp had adhesion strengths of 1.53 MPa and 1.97 MPa, respectively. In coatings deposited from an ethanolic suspension containing 10 g/L HAp nanoparticles and 0.5 g/L chitosan, the powder/polymer ratio is high. This results in aggregation due to not having enough chitosan to adhere HAp nanoparticles to each other, and there are many agglomerates [115]. Xin Pang and Igor Zhitomirsky developed a fabrication method to form HAp/chitosan composite films by chemical precipitation and electrodeposition techniques [116]. A variety of cathodic deposits (with a thickness of up to 50 nm) were formed on platinum foils, stainless steel wires, and graphite substrates. They demonstrated that the deposit composition varied by the amount of HAp content in chitosan solutions [116]. Farrokhi R. examined the bioactivity evaluation of pure HAp, HAp/chitosan, and HAp/chitosan/graphene oxide composites by dissolving them in SBF [117]. It was observed that apatite formation occurs on all composite coatings, which reveals bonding between implants and natural bones. Researchers have shown that HAp/chitosan composite coatings deposited at 60 V for 60 s gradually enhance adhesion strength up to 2.30 MPa when more carbon nanotubes are incorporated. Furthermore, carbon nanotubes improve the composite coating's hardness and elastic modulus [117].

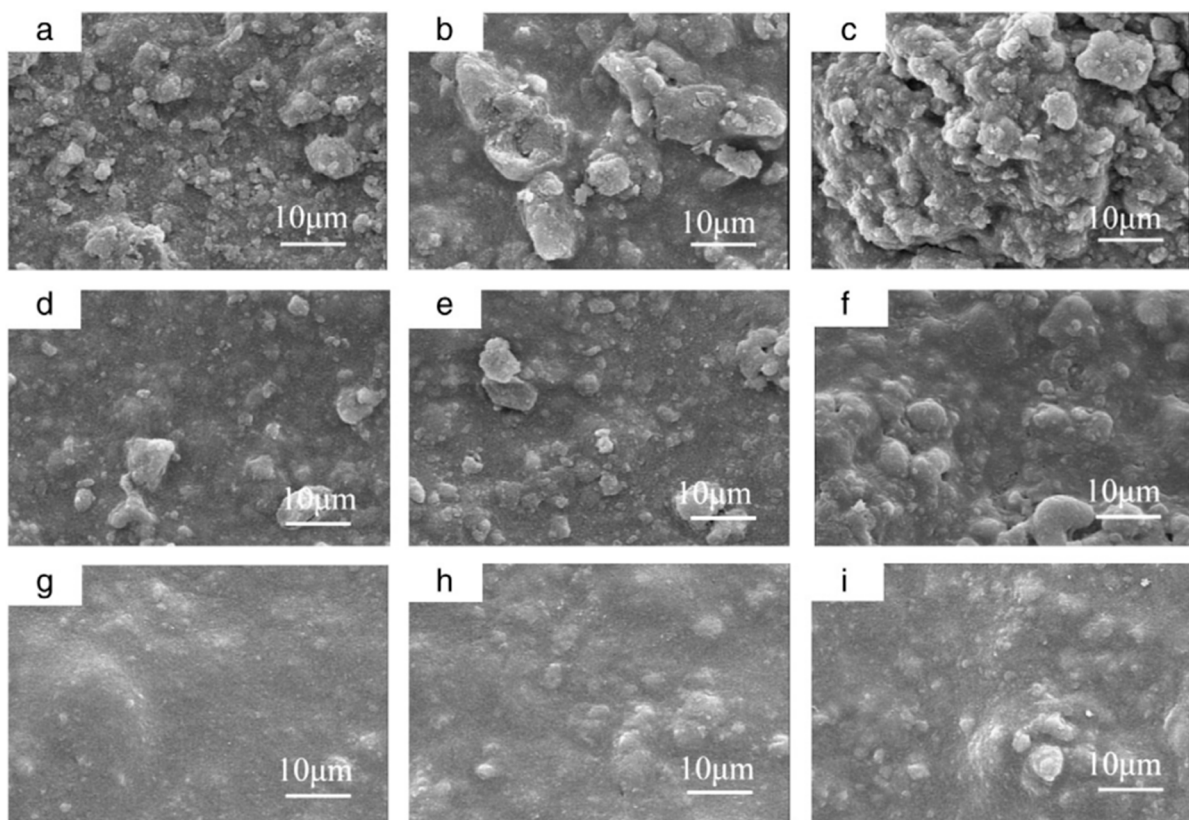


Figure 11. SEM microstructures of coatings deposited from methanolic (a–c), ethanolic (d–f), and isopropanolic (g–i) suspensions containing 0.5 g/L chitosan and 2 g/L (a,d,g), 5 g/L (b,e,h), and 10 g/L (c,f,i) HAp nanoparticles at 60 V/cm. Reproduced with permission [115].

5. Conclusions and Future Direction

Natural and synthetic-based polymers have attracted considerable interest in the past decade in the field of tissue engineering and regenerative therapies by offering a variety of structures to repair an injured organ. Considering polymers are limited in mechanical strength, biocompatibility, and ability to mimic natural ECM structures, there has been

a demand for blending polymers together or incorporation of reinforcing agents into biomaterials. Researchers need to keep improving these materials to take full advantage of their benefits and deal with current challenges. Biological resources such as collagen, gelatin, chitosan, fibrin, cellulose, and alginate are typically sources of natural biopolymers. Their compounds exhibit good compatibility with tissues in the body. Nevertheless, they often lack sufficient strength for applications such as bone scaffolds or other support implants.

Current research has examined the incorporation of bioactive ceramics, for example, HAp, into polymer matrixes to enhance their strength. This will simultaneously help tissue regeneration. HAp is synthesized from different natural resources such as eggshells, fish scales, bovine bones, and marine sources. Hydroxyapatite nanoparticles offer significant benefits as a replacement for base composites found in the human body. HAp-based scaffolds are produced using various fabrication approaches, including solvent/solution casting, electrospinning, freeze-drying, 3D printing, and electrodeposition. This review concluded that HAp-based composite polymers produced by different fabricating techniques could be promising alternative composites in biomedical engineering.

The primary challenge in bone tissue engineering lies in the lack of vascularization. Thus, future research efforts will concentrate on devising an efficient strategy for producing scaffolds that foster the growth of blood vessels and promote vascularization. Primary attention will be directed towards the development of custom-tailored scaffolds utilizing novel biomaterials that are capable of adapting to alterations in the body, providing the desired biological functionalities and mechanical properties for bone regeneration purposes.

Additionally, modeling simulations are useful for developing scaffolds appropriate for bone tissue regeneration requirements. The development of such models can be essential for tackling bone-related issues.

In conclusion, natural and synthetic polymer-based hydroxyapatite composites are promising materials for regenerating damaged tissues and facilitating recovery. Even though positive results have been achieved from polymer-based hydroxyapatite composites in vitro and a few in vivo studies, challenges in translating biomedical research into innovation are still a major concern. Some drawbacks are related to preclinical model inadequacy, infection, expense, and lengthy processes. It is imperative to apply animal models for a correct analysis and ensure animal data are comparable to human data before performing clinical trials. In addition, one of the most important factors is to assess the effectiveness and nature of the interaction of skeletal bone tissue with the damaged part of the body.

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