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Hydroxyapatite (HA) and Alginate Combination as Bone Graft Substitute for Bone Defect – A Literature Review

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Abstract
Background: *Bone defects refer to the loss or absence of bone matrix. A critical-sized bone defect is defined as a defect large enough that the bone is unable to heal spontaneously without intervention, making it remains a significant challenge for healthcare professionals. According to the diamond concept of bone healing (osteogenic cells, scaffolds, growth factors and mechanical stability), bone substitutes are an important component for bone defects treatment. Autografts remain the gold standard for treating bone defects; however, the development of biomaterials combining ceramics and polymers has emerged as a promising alternative for bonegraft substitutes. The combination of hydroxyapatite as a ceramic and alginate as a polymer has shown promising results in both in vitro and in vivo studies, demonstrating favorable mechanical and biological properties. To further enhance its osteogenic potential, additional research is needed–either by optimizing the current combination or by incorporating growth factors or mesenchymal stem cells.*
Keywords: *Bonegraft substitute, bone defect, hydroxyapatite, alginate*

Introduction

Bone defects refer to the loss or absence of bone matrix. A critical-sized bone defect is defined as a defect large enough that the bone is unable to heal spontaneously without intervention, appears to be equal to or greater than 20% of the length of a long bone [1]. These defects frequently arise due to infections, trauma, tumor resections, or underlying bone disorders [2]. The prevalence bone defects caused by trauma related events has been reported as 26% in Mulago, Uganda [3]; 11.4% in Edinburgh, Scotland [4]; and 3.6% in Oulu, Finland [5].

A critical-sized bone defects have insufficient bone healing process, and may be complicated furthermore. Under these circumstances, the autografts are the most popular method for bone replacement despite its limitation for filling such large defects as they offer no immunological rejection and are thought to provide the best osteoconductive, osteogenic and osteoinductive properties [6], [7]. Allografts may be used to reconstruct large bone defects as an alternative. The advantages of bone allograft include no morbidity of the donor-site, unlimited use of material, and availability in mechanical support with various shapes and sizes [8]. Its disadvantage is it has no osteogenesis and weak osteoinductivity and may lowering bone union rate [9], [10]. Xenografts are more popular than auto and allografts, but the healing process outcomes are poor due to its limited osteoconductive function only in bone healing [11].

Consequently, management of bone defects remain a significant challenge for healthcare professionals. Due to inability of spontaneous bone healing process, it will lead to prolonged treatment durations, increased risk of complications, and elevated healthcare costs [12]. Inadequate or inappropriate treatment of bone defects can adversely affect patient outcomes in terms of healing and functional restoration, as well as impose significant financial burdens [3].

Bonegraft Substitute

According to the diamond concept of bone healing, bone substitutes are an important component for bone defects treatment. The four parts of the diamond concept are osteogenic cells, scaffolds, growth factors and mechanical stability [7]. The most ideal bone substitute should include the ability of providing a scaffold for osteoconductivity and growth factors for osteoinductivity and should be structurally similar to real bone. The

scaffold for ideal osteoconductivity should exhibit osseointegration and a 3D structure suitable for growing cells and blood vessels. In addition, it should have good biocompatibility, biodegradation, and biomechanics similar to surrounding bone tissues [8]. Since each type of bone graft possesses unique characteristics (Table 1), it is essential to review existing research on these materials to understand how their physical properties influence clinical application [11].

Table 1. Characteristics of bone grafts and bone graft substitutes [11]

Variable	Autograft	Allograft	Xenograft	Ceramics
Osteoconduction	+++	+++	+++	++
Osteoinduction	+++	++	+	-
Osteogenesis	+++	-	-	-
Osteointegration	+++	++	++	+++

Notes: +++, excellent; ++, average; +, poor; -, none

The use of synthetic materials (alloplasts) has been investigated for the repair of bone defects. [13]–[15]. This has created the terms of Bone Tissue Engineering (BTE) using synthetic materials that can act as scaffold. For BTE, the ideal bone graft material or scaffold should be biocompatible, osteoconductive, osteoinductive, osteogenic, resorbable or degradable, and have mechanical properties similar to those of bone at the implant site, to be able to provide temporary mechanical support [16]–[18]. The requirement for a scaffold to be resorbable or degradable derived from the continuous remodeling nature of bone. A scaffold that is non-resorbable or non-degradable may interfere with this natural process, potentially delaying the restoration of normal bone function. [16]. Biomaterials used for BTE should be also angiogenic, with the ability to induce the formation of blood vessels in the construct [19], [20]. In addition to these requirements the scaffold's structure and morphology play a critical role. An ideal bone graft should serve as a template and bridging framework to support and direct the formation of new bone tissue [21]. BTE scaffolds need to be highly porous with a three-dimensional structure to closely replicate the porosity, pore size, and interconnectivity of natural bone. This design supports cell attachment, proliferation, and facilitates new tissue growth and vascularization. However, while high porosity is essential, it significantly compromises the mechanical strength of the scaffold due to increased void space. Therefore, for BTE applications, it is crucial to achieve a balance. Scaffolds should maintain high porosity while preserving adequate mechanical strength and structural integrity [22], [23].

Up until now, numerous bone substitutes that satisfy these conditions are commercially available in orthopedics. Ceramic and ceramic composites as bone substitutes are typical calcium-based synthetic bone substitutes that are already approved in terms of stability and effect [8]. Today, various types of ceramic products are composed of calcium phosphate ceramics, including hydroxyapatite (HA) and tricalciumphosphate (TCP), or calcium sulfate, or calcium phosphate cements and bioactive glass [24].

Calcium Phosphate Ceramics

Calcium phosphate ceramics are constituted by calcium hydroxyapatites, which is a chemical composition similar to the mineral phase of calcified tissues [25]. As a kind of bioabsorbable ceramic with excellent osteoconductivity, CaP ceramics have received great attention and have been experimented extensively in clinical studies [26]–[29].

Hydroxyapatite (HA)

HA is bioactive ceramic and a main mineral of bone. It is a natural occurring mineral form of calcium apatite with the formula of $Ca_{10}[PO4]_6[OH]_2$ and comprises about 50% of the weight of the bone, which accounts for its excellent osteoconductive and osteointegrative properties [30], [31]. Given its density, HA with a porous structure is easily bio-absorbable and exhibits good osteoconductivity [32]. The primary role of HA in bone graft development is to provide structural stability and facilitate bone regeneration [32][33]. Despite inadequate mechanical properties of highly porous HA scaffolds, its distinct biocompatibility, especially derived from biological raw materials, and bioactivity makes them very convenient for regeneration of bone

tissue [34]. Among other materials, HA stands out as one of the most effective bone substitutes due to its ability to form a strong and stable bond with native bone tissue [24].

Attempts have been made to enhance the mechanical and biological properties of hydroxyapatite (HA)-based nanocomposites. While both synthetic and natural polymers typically offer good toughness and flexibility, they tend to exhibit lower bioactivity compared to bioactive ceramics [35]. Incorporating HA into a polymer matrix has shown promising clinical applications, including use in prosthetic bone cements, joint replacements, dental implants, and bone defect fillers [36], [37]. Therefore, HA/polymer composite scaffolds have attracted significant interest for biomedical applications, and numerous studies have reported on the fabrication of polymer-based composite scaffolds incorporating HA [38].

Tricalcium Phosphate (TCP)

Tricalcium phosphate is an osteoconductive calcium phosphate with a chemical composition closely resembling that of human bone. Compared to hydroxyapatite (HA), it exhibits superior resorption properties. [39], [40]. Tricalcium phosphate (TCP) is more porous than hydroxyapatite (HA), characterized by low mechanical strength and rapid resorption. Highly porous TCP typically degrades within six weeks after being implanted into a bone defect. Due to its compressive and tensile strength being comparable to that of cancellous bone, it is primarily used in non-load-bearing areas. [25].

Biphasic Calcium Phosphate (BCP)

Biphasic calcium phosphate (BCP) is a commonly used commercial ceramic, created by blending hydroxyapatite and tricalcium phosphate in varying ratios to harness the combined benefits of both calcium-based materials [41].

Calcium Sulfate

Calcium sulfate, also known as plaster of Paris, is a kind of osteoconductive and biodegradable ceramics composed of $CaSO_4$ and has been applied in filling void defects as a bone substitute in the late 19th century by Dreesmann [42]. Calcium sulfate typically degrades within 6 to 8 weeks after being implanted into a bone defect. Due to its non-porous structure, it exhibits limited osteoconductivity. Its mechanical weakness and rapid resorption further limit its utility, making it less commonly used than calcium phosphate [25].

Calcium Phosphate Cements

Calcium phosphate cements (CPCs) are composed of one or more calcium phosphate powders that, when mixed with a liquid phase, create a paste capable of self-setting and hardening directly within a bone defect to form a scaffold. A key feature of CPCs is their body-temperature dissolution-precipitation reaction, which enables them to be molded and effectively fill the defect site [43]. It is injectable and enables minimally invasive application and has the ability to serve as a carrier for drug and biological molecule delivery. In recent years, advancements in CPC manufacturing have extended beyond processing technologies, with a growing focus on improving the biological interactions between CPCs and surrounding cells and tissues. This shift highlights the increasing emphasis on their potential in bone tissue engineering applications. [44].

Bioactive Glass

Bioactive glass, or bioglass, is a class of synthetic, silicate-based ceramics. When first developed in the 1970s, its composition included silicon dioxide (SiO_2), sodium oxide (Na_2O), calcium oxide (CaO), and phosphorus pentoxide (P_2O_5) [45]. One study reported that bioglass fiber scaffolds can be fully resorbed within six months in vivo, with minimal inflammatory reaction [46]. Similar to other ceramics, bioglass exhibits brittle and weak mechanical properties, which limits its use primarily to facial defect reconstruction [47].

Polymer

Polymers are composed of repeating structural units known as monomers, and the choice of monomer significantly influences the resulting polymer's properties [48]. In bone tissue engineering scaffold fabrication, biodegradable polymers are generally classified into two main types: natural polymers and synthetic polymers [49]. Natural polymers, such as alginate, chitosan, collagen and hyaluronic acid, have been extensively studied as bone defect repair materials due to their biodegradability, bioactivity and biocompatibility [50]. Some of the most widely used synthetic polymers in bone tissue engineering are aliphatic polyesters, such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL) [49].

Alginate

Alginate is a naturally derived anionic polymer obtained primarily from brown seaweed [51]. It has been widely studied and utilized in biomedical applications owing to its biocompatibility, low toxicity, cost-effectiveness, and mild gelation properties in the presence of divalent cations such as Ca^{2+} [52]. A critical factor in tissue regeneration and bone defect repair is the integration and mechanical strength of the scaffold materials. Due to its inherently low mechanical strength, alginate is often combined with other substances—such as chitosan, hydroxyapatite, gelatin, or natural nanoparticles—to enhance the scaffold's strength and biodegradability [48], [53].

Studies have shown that bioactive compounds encapsulated within alginate hydrogels exhibit greater bioavailability than their pure counterparts, thereby improving their functional effectiveness—a key factor in evaluating material performance [54]. Alginate hydrogels can form crosslinks in the presence of divalent cations such as Ca^{2+}, Ba^{2+}, or Sr^{2+} through a simple and cost-effective process, enhancing their ability to deliver and release therapeutic substances within biological environments. These hydrogels are particularly valuable in bone and cartilage regeneration, as they can be administered via injection and conform to irregular bone defect shapes [55], [56].

Hydroxyapatite (HA) and Alginate Combination

Numerous studies have described the preparation of hydroxyapatite involving biopolymers such as alginate or chitosan [38], [51], [57]–[59]. A study from Jin et al. combining HA with chitosan-alginate composites. The pore structure of the composite scaffolds was similar to chitosan- alginate scaffolds, and the morphology ofuniformmicrostructure was unaffected by the presence of HA. The compressive strength of HA/chitosan-alginate composite scaffolds was to be higher with increasing HA content, which may be associated to the low porosity of scaffolds [38].

Study from Setiadiputri et al. combining HA with alginate and zinc. The optimal composition consists of a hydroxyapatite:alginate:zinc ratio of 88:10:2 (% w/w). The sample exhibited an average pore size ranging from 130.67 µm to 137.43 µm, which is suitable for neovascularization and bone cell growth. It also showed a porosity of 70.142%, meeting the ideal porosity standard for supporting bone cell proliferation in cancellous bone. The highest recorded compressive strength was 9.0107 MPa, which complies with the standard range for cancellous bone compressive strength [59].

Another study from Mahmoud et al. using alginate as a coating for HA scaffolds. Coating the HA porous scaffolds with 3% w/v alginate for 10 minutes was identified as the optimal condition. The resulting composite scaffolds demonstrated 60% porosity, with compressive and flexural strengths of 5.38 ± 0.43 MPa and 1.99 ± 0.08 MPa, respectively. The experimental animals survived without any local or systemic complications, indicating that the implanted scaffolds were biocompatible and did not induce histopathological changes in the surrounding bone tissue. Furthermore, there were no signs of carcinogenicity, inflammation, or adverse effects on liver and kidney function. Six months after surgery, the bone defect was fully regenerated and filled with mature lamellar bone. The calcium-to-phosphorus (Ca/P) atomic ratio of the newly formed bone was 1.64, closely aligning with that of normal bone (1.5–1.65) [51].

Rajkumar et al. using combination of HA and sodium alginate (SA), synthesised using reagent-grade chemicals such as calcium chloride dihydrate (CaCl2·2H2O, Merck GR, 99.5%), disodium hydrogen phosphate dihydrate (Na2HPO4·2H2O,MerckGR, 99.5%), SA (C5H7O4COONa, M.W=216, Loba) and sodium hydroxide pellets (NaOH, Merck GR, 98%). The process of formation mechanism was shown in Fig. 1. According on the observed results, the incorporation of SA significantly influences the crystallite size, crystallinity, morphology, bioresorbability, and hardness of HA. The improved mechanical strength, morphology, and particle size support the potential of HA/SA nanocomposites for biomedical applications. Nanosized HA was uniformly dispersed within the SA matrix, with the matrix playing a key role in controlling crystal size and morphology—potentially creating a favorable environment for osteoblast adhesion and proliferation [58].

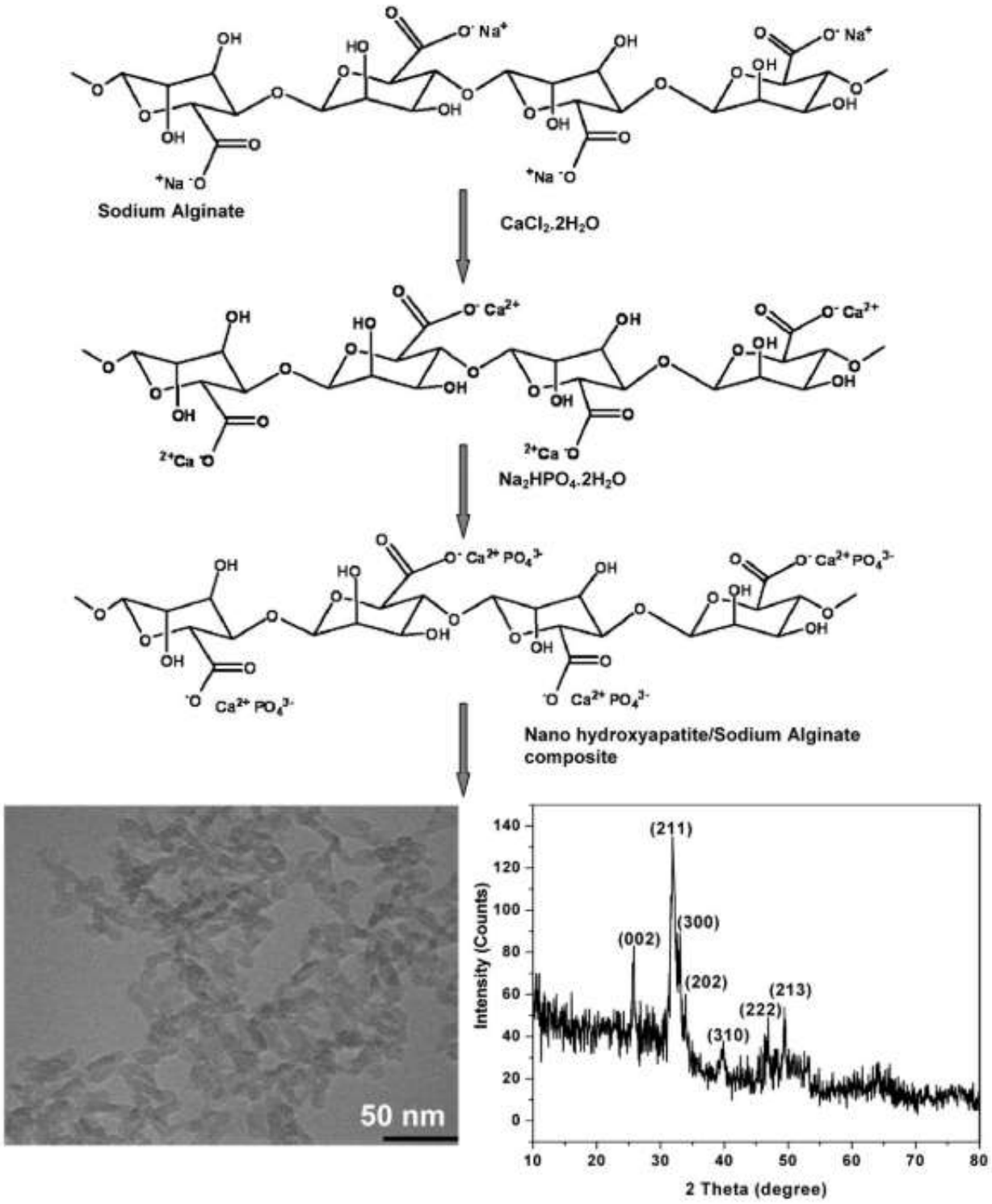

Figure 1. Formation mechanism of nano hydroxyapatite/sodium alginate composite [58]

DISCUSSION

Bone defects remain a clinical challenge and continue to pose difficulties for healthcare professionals. The prevalence bone defects caused by trauma related events has been reported as 26% in Mulago, Uganda [3]; 11.4% in Edinburgh, Scotland [4]; and 3.6% in Oulu, Finland [5].

The use of synthetic materials (alloplasts) has been investigated for the repair of bone defects. [13]–[15]. Among these materials, hydroxyapatite (HA) stands out as one of the most effective bone substitutes due to its ability to form a strong and stable bond with native bone tissue [24]. Despite inadequate mechanical properties of highly porous HA scaffolds, its distinct biocompatibility, especially derived from biological raw materials, and bioactivity makes them very convenient for regeneration of bone tissue [34].

Despite their benefits, bone grafts face several limitations when used to treat bone defects. One challenge is that solid powder forms may not effectively fill the defect area. To overcome this, injectable paste formulations have been developed. However, hydroxyapatite (HA) alone is not suitable for forming a stable paste, as it requires a cross-linking agent to set within a defined time frame. Alginate are biocompatible polymers that can serve as cross-linkers for HA [38], [60]. Numerous studies have described the preparation of hydroxyapatite involving biopolymers such as alginate or chitosan [38], [51], [57]–[59].

In vitro studies have demonstrated that the combination of hydroxyapatite (HA) and alginate enhances the mechanical strength, bioresorbability, porosity, and pore size/morphology of the scaffold. These improvements make the composite a promising candidate for bone defect treatment, owing to its osteoconductive properties that support neovascularization and bone cell proliferation. The mechanical strength is also significant, providing structural integrity comparable to cancellous bone. Additionally, the composite exhibits enhanced osteoinductivity, as it promotes osteoblast adhesion and proliferation.

An in vivo study using the femur bone of albino rats demonstrated that the animals remained healthy without local or systemic complications, confirming the biocompatibility of the implanted scaffolds and the absence of histopathological alterations in the surrounding bone tissue. No signs of carcinogenicity, inflammation, or impairment of liver and kidney functions were observed. In this study, a biogenic hydroxyapatite scaffold was coated with alginate, a natural polymer known for its excellent ability to support endochondral bone formation. Alginate was cross-linked using calcium ions, which serve as nucleation sites and accelerate cartilage ossification during the early stages of defect healing, thereby promoting endochondral bone formation [61]–[63].

The scaffold's backbone, composed of bioactive hydroxyapatite, is recognized for its osteoconductive and osteoinductive properties, facilitating direct bone regeneration through intramembranous ossification. Additionally, the release of calcium and phosphorus during the resorption of biogenic hydroxyapatite by osteoclasts or multinucleated giant cells contributes to bone mineralization, enhancing the maturation of newly formed bone. Therefore, the combination of a porous biogenic hydroxyapatite scaffold with an alginate coating has resulted in a highly advanced bioactive material capable of supporting both endochondral and intramembranous bone formation, achieving excellent critical-size defect repair in the animal model [64], [65]. Six months post-surgery, evaluation revealed complete regeneration of the bone defect, with the site fully filled by mature lamellar bone [51].

CONCLUSION

In conclusion, the combination of HA and alginate as a bone graft substitute shows potential in accelerating the bone healing process in bone defect models. Recent studies have demonstrated that this composite forms a highly advanced bioactive scaffold capable of supporting both endochondral and intramembranous bone formation. The combination enhances osteoconductive and osteoinductive properties while also improving the mechanical strength of the scaffold. However, further in vitro and in vivo investigations are necessary to validate these findings. Additionally, incorporating growth factors such as Bone Morphogenetic Protein

(BMP) or Platelet-Rich Plasma (PRP), as well as mesenchymal stem cells, may further enhance the osteogenic potential of this composite, given its suitability as a scaffold for such bioactive agents.

Conflicts of Interest

The authors declare no conflicts of interest.

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