
CARBONATE APATITE AS A BONE SUBSTITUTE MATERIAL. A REVIEW

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ABSTRACT

INTRODUCTION: The reconstruction of bone defects, caused by traumas, tumors, infections, and genetic disorders, is a major problem in orthopedic surgery, maxillofacial and oral surgery, plastic surgery, dental implantology, otorhinolaryngology, etc. Although autogenous bone grafts are still regarded as the gold standard in bone reconstructive surgery, alloplastic materials are more commonly applied due to their accessibility. Recently, the utilization of carbonate apatite as a bone substitute has been a matter of interest for researchers in the field, and its physical and biological properties are currently being tested and evaluated.

AIM: The present review aims to evaluate the application of carbonate apatite as a bone substitute concerning its fabrication, physical properties, and biological behavior.

MATERIALS AND METHODS: An electronic search using Google Scholar, PubMed, Scopus, and ScienceDirect was conducted up to November 2022. The article summarizes the current knowledge on the application of carbonate apatite as a bone substitute material, identifies the research gaps in the existing literature, and gives some recommendations for further assessments.

RESULTS: We could assume that the mechanical and biological properties of carbonate apatite depend on the method of its fabrication and recommend further long-term research and evaluation to optimize the synthesis protocols and, thus, the qualities of the material.

CONCLUSION: It has been suggested that carbonate material has the potential to replace autologous bone grafts in bone reconstruction. The material has excellent biocompatibility, resorbability, and osteoconductive capacity, and could be regarded as a promising bone substitute material.

Keywords: carbonate apatite, fabrication, properties, synthesis, bone substitutes

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INTRODUCTION

Bone reconstructive surgery is commonly performed with bone being the second most transplanted tissue worldwide (1). This explains the increasing need for new bone substitutes (2), which should meet the following criteria: osteoconductivity, biocompatibility, resorbability, and degradability (3). Bone grafts are generally classified as autografts, allografts, xenografts, and alloplastic materials. Each

of them has its advantages, drawbacks, and limitations (4). The basic principles of guided bone regeneration require the utilization of bone grafting materials with suitable barrier membranes (5).

Autologous bone grafts are still considered the gold standard but they have the following drawbacks:

1. Deficiency of donor site tissues;
2. Post-operative patient morbidity;
3. The shape of the transplant does not match the shape of the defect in the recipient site. Therefore, alternatives to autografts should be sought for bone reconstruction surgery. The diamond concept in fracture healing suggests that there are 4 essential components—osteogenic cells, osteoinductive growth factors, osteoconductive scaffolds, and mechanical stability (6).

Synthetic bone substitutes have frequently been applied in bone reconstruction surgery. Hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP) have gained popularity in orthopedic surgery (7) since they act as scaffolds that promote bone formation (8). Hydroxyapatite has good osteoconductive properties and biocompatibility. Bone apatite, however, contains carbonate, which means that it is not pure HA (9). The main limitation of HA is that it acts as a foreign material in the bone. It has been reported that two weeks after implantation the material was surrounded by fibrous tissue. On the other hand, β -TCP has been reported to be easily resorbed and to induce collagen deposition (10), which could take up to half a year (11). However, some authors claimed that β -TCP could not be fully resorbed and could cause cyst formation and inflammatory complications (12,13). Therefore, there is a worldwide tendency of developing new bone grafting materials with improved tissue behavior and qualities, of optimizing surgical protocols, and of introducing novel advantageous techniques.

AIM

This review aims to summarize the current knowledge of the use of carbonate apatite (CAp) in bone reconstructive surgery, analyze its physical and biological properties, compare its fabrication techniques, and evaluate its advantages and limitations. We identified the research gaps and uncertainties in the existing literature and gave recommendations for further research.

MATERIALS AND METHODS

An electronic search using Google Scholar, PubMed, Scopus, and ScienceDirect was conducted up to November 2022. The articles that matched the selected keywords were compared, analyzed, and carefully summarized. Some recommendations for further assessment were suggested.

RESULTS

Carbonate apatite is a natural mineral part of human bone. The resemblance between carbonate apatite and bone minerals is greater than that of stoichiometric hydroxyapatite and bone (14). The carbonate amount in biological minerals is usually 4–8 wt.% (15–17). It has been demonstrated that carbonate-substituted calcium hydroxyapatite is more effective for bone implants than pure calcium hydroxyapatite (16,17). Incorporation of carbonate into the apatite increases its solubility and reactivity, decreases the crystallinity, and alters its crystal morphology (18–20). Carbonate apatite releases calcium and phosphate ions that induce new bone formation (21–23).

Back in 1938–1940, the geologist D. McConnell (24,25) studied the features and behavior of CAp and found that apatites released carbon dioxide when treated with hydrochloric acid. He observed two types of CAp with different optical responses and called them “type A” and “type B” without clarifying the location of carbonate ions. For some decades there were arguments about the exact structure and the possible carbonate substitutions in the apatite lattice (26).

In 1964, Bonel (27) demonstrated that A-type and B-type CAp are prepared by different methods and have different infrared absorption bands. Fourier transform infrared spectroscopy (FTIR) has become a common method to distinguish both types of CAp (26).

Le Geros suggested that the biological apatite is B-type carbonated HA whereas A-type HA did not exist (28). It was found in 2008 that the apatite in tooth enamel is B-type but the one in dentin is A-B type. It has been recently suggested that both types could be found in biological apatites (29–35).

Recently, Ren et al. conducted combined experimental and computational research and demonstrated that infrared (IR) spectroscopy is not flaw-

less and should be used with caution when identifying the type of CAP. The authors also suggested that biological apatites could be of mixed AB-type (24).

Eriwati et al. observed the mechanical properties and absorption capacity of carbonate apatite blocks produced by the dissolution-precipitation reaction. The precursor they used was calcium sulfate hemihydrate. The authors concluded that the diametral tensile strength and absorption value of the material depends on the molarity and the reaction time (36).

Carbonate ions play a key role in bone metabolism (37) and carbonate-substituted HA has demonstrated better bioactive and osteoconductive properties compared to hydroxyapatite. Yang et al. (38) synthesized stoichiometric HA and nanosized carbonated HA and used IR spectroscopy, chemical analysis, and X-ray diffraction to compare them. The authors found that carbonate substitution led to a material that resembled natural bone better concerning structure and crystallinity. In addition, it was demonstrated that B-type substitution presented reduced crystal energy and increased stability.

Vallet-Regi et al. have stated that the resorption capacity of CAP is due to its low crystallinity and crystalline disorder, caused by the presence of carbonate ions (39). Bioresorption is a process related to osteoclastic activation from the Howship's lacunae and the release of proteolytic enzymes, hydrochloric acid, cathepsin, and matrix metalloproteinase (40).

Le Geros and Tung have demonstrated that the increased carbonate content in the lattice led to increased solubility in a weakly acidic environment (41). Therefore, cell-mediated bone resorption is related to the carbonate content, which explains the limited resorption of HA (42–45). The degradation of CAP provides calcium and phosphate, which are necessary for new bone formation (46–49).

Several protocols for CAP synthesis have been suggested, such as precipitation, hydrothermal activation, and mechanochemical synthesis (50–56).

In 1997, Doi Y (57) investigated CAP sintering and its application as a bone substitute. In vitro and in vivo trials on rat models were conducted. He found that osteoclasts successfully resorbed sintered CAP. In addition, the resorption rate did not exceed the rate of bone formation, and thus, the substitute

served as a scaffold for bone growth. The bony defects were almost filled with new bone four weeks after the surgery without a significant inflammatory response. The author concluded that sintered CAP is more similar to the bone than sintered HA and could be effectively used as a bone substitute. Doi et al. (42) fabricated CAP blocks via sintering techniques while Ishikawa et al. (58) managed to avoid sintering and fabricated low-crystalline CAP through dissolution-precipitation reaction, using precursors.

Carbonate apatite should not be applied in a powder form as it causes inflammatory reactions. It has been sintered into blocks under high temperatures.

Habibovic et al. (59) have introduced two different CAP ceramics sintered at 900°C and 700°C. The first type had a carbonate level of 3 wt.% and large pores whereas the second type had a 5 wt. % carbonate level and micropores. The first sample showed better dissolution and higher new bone formation. However, despite its osteoconductive properties, the first type lost its osteoinductivity due to rapid dissolution. The sample showed good bone ingrowth but failed to promote bridging.

Minh et al. have introduced a novel method for the one-step synthesis of carbonate-substituted calcium hydroxyapatite. As initial materials, they used calcite, calcium hydroxide, and orthophosphoric acid and noted that the ratio of the first two influenced the carbonate content of the product (60).

A study suggested that carbonate specimens could be processed at low temperatures (600–750°C) (61). Their carbonate content (12 wt.%) was higher than those retained by bone apatite (4–8 wt.%). It was demonstrated in follow-up research that the dissolution rate of sintered CAP in an acidic environment was ten times lower compared to that of bone apatite (42). The latter has a big surface area and low crystallinity which accelerates the dissolution of the apatite. However, in an acid media, the carbonate content of CAP makes its reactivity higher than that of HA.

Matsuura et al. (62) have researched to evaluate the long-term effect that CAP-collagen scaffolds with different carbonate content had on the bone formation rate. The authors demonstrated that bone grafts with 6 wt.% carbonate presented the highest bone formation and recommended their application.

Mano et al. (63) have also suggested that carbonate content may be a key factor for the osteoconductive capacity of apatitic bone substitutes. The authors investigated the effect of carbonate contents of different bone substitutes—synthetic HA, a bovine-derived xenograft, and synthetic CAP with 0.1 mass%, 5.5 mass%, and 12.0 mass% for the reconstruction of alveolar bone defects with simultaneous implant placement in dogs. The findings demonstrated that the bone-implant contact ratio was the largest in the CAP group. Furthermore, the continuity of the alveolar crest was fully restored at 12 weeks only in the CAP group.

Germaini et al. (64) observed that 4.4 wt% carbonates substituted HA induced greater preosteoblast proliferation than pure HA.

Lee et al. (65) stated that extracellular carbonate ions induced the differentiation of osteoblasts, secretion of osteopontin, and matrix mineralization in rats.

As Elsheikh et al. (66) reported that a key prerequisite for bone ingrowth is the porous structure of bone grafting materials, especially the interconnectivity of pores. The authors fabricated two types of CAP blocks with 15% and 30% interconnected pore volumes via a dissolution-precipitation process. The blocks were implanted in bone defects in rats and the new bone was measured at 4 weeks and 12 weeks after the surgery. The results showed faster new bone formation when using CAP blocks with 30% interconnectivity and proved their role in bone regeneration.

Porous architecture provides an increased surface area and better vascularization (66). The final results depend on their shape, size, orientation, and interconnectivity

Akita et al. (67) fabricated CAP granules with different pore sizes and used the materials for the reconstruction of calvarial defects in rabbits. The authors evaluated the new bone formation at 2, 4, and 8 weeks after implantation and concluded that CAP granules with 85 μm pore size demonstrated excellent osteoconductive properties.

Kanazawa et al. (68) have conducted a study to evaluate the effect of CAP blocks on the reconstruction of rabbit bone defects. The graft was prepared from dicalcium phosphate hemihydrate. The authors reported that CAP was replaced by new bone tissue

in 24 weeks, unlike HA. The resorption rate of the material depended on the recipient site given that it has a linear manner; a complete resorption was expected 1–1.5 years after the surgery.

Recently, CAP blocks prepared through a dissolution-precipitation reaction have been introduced (69–80). They have demonstrated osteoclast resorption similar to that of natural bone and osteoconductivity better than HA.

Ana et al. (81) have suggested the fabrication of macroporous CAP in blocks by dissolution-precipitation reaction of gypsum and calcium hydroxide.

Tram et al. investigated the potential of chicken bone by-products as a cost-effective source for the fabrication of CAP bone substitutes.

Fujisawa et al. (82) compared the behavior of Bio-Oss and CAP fabricated through a dissolution-precipitation reaction with calcite as a precursor. According to the x-ray diffraction and FTIR, both materials were B-type CAP. Carbonate apatite had a larger amount of carbonate ions and higher crystallinity. When implanted in bone defects in rabbits, at 8 weeks Bio-Oss remained while the CAP granules were partially replaced with bone. The study highlighted the role of carbonate content in osteoconductivity and new bone formation. The authors suggested the application of CAP as a bone substitute material and a biodegradable scaffold.

In 2000 Linhart et al. (84) introduced a composite material, made of CAP and polyglycolide and suggested its use for bone grafting and as a scaffold for tissue engineering. The authors carried out numerous in-vitro experiments and reported that the negative side effects of both materials were eliminated. In addition, the composite showed excellent biocompatibility.

In 2001 Suh et al. introduced a bone graft material, composed of CAP and bovine collagen (type I) (84).

In 2011 Dewi and Triawan (85) evaluated the healing and regenerative potential of the composite bone substitute CAP-chitosan. Chitosan derives from chitin (an insoluble, cellulose-like organic matrix of exoskeletons) after deacetylation of the latter. It has numerous advantages, such as biocompatibility and degradability, good healing, regenerative and antibacterial properties, and hemostatic potential.

The authors assessed the newly formed bone in the rat tibia on the 1st, 2nd, and 3rd week after the bone grafting procedure with the proposed material. They concluded that the composite bone substitute led to a significant increase in the osteoblast number, faster matrix deposition, and a good interface with the surrounding bone. These qualities suggest that CAP-chitosan could be a promising bone substitute.

Carbonate apatite was reported to upregulate the osteoblast differentiation and osteoclast resorption (86,87).

Since CAP granules are fabricated via chemical reaction, their structure is constant and they do not pose a risk of infection. Furthermore, the resorption rate of the material could be controlled by its carbonate content (88).

In 2019 Sato et al. compared the efficacy of CAP, HA, β -TCP, and deproteinized bovine xenograft in the healing of vertical bone defects in dogs. The authors reported faster bone regeneration in the 3-wall bone defects when CAP was used. It was demonstrated that CAP and the release of calcium and carbonate ions promoted the migration of osteoclasts, differentiation of osteoblasts, and angiogenesis. Moreover, inflammatory cell recruitment was not observed in the CAP group and the material was resorbed along with the new bone formation (88).

The first-in-human clinical trials with CAP bone substitutes were performed in 2019 in patients undergoing sinus floor elevation procedures with simultaneous implant placement (89,90). The histological findings of the specimens taken during the implant placement (between 6 and 10 months after the augmentation) demonstrated that almost all of the material was replaced by new bone (90). There was also osteoid tissue which suggested active bone remodeling. The bone height, gained by the procedure, was sufficient enough for implant placement.

DISCUSSION

Bone defects represent a major challenge in orthopedic, maxillofacial, and oral surgery, dental implantology, otorhinolaryngology, etc. They require adequate and timely transplantation and/or substitution. Autogenous bone grafts are still regarded as the gold standard in bone reconstructive surgery. However, they represent numerous disadvantages, such as the need for a second donor site, tissue de-

ciency, and postoperative morbidity. There is a variety of bone grafting materials, each presenting its advantages and drawbacks. Researchers in the field are constantly developing and testing new materials with optimized properties and, hopefully, being superior to the currently used bone substitutes. A relatively novel concept is the utilization of CAP for these purposes.

We have summarized, compared, and analyzed the current knowledge of the methods used for the fabrication of CAP and concluded that the synthesis technique has a major impact on the mechanical and biological properties of the material. There are numerous features that affect the quality and tissue behavior of CAP, such as carbonate content, crystallinity, surface morphology, and porosity. Therefore, we recommend further research in search of optimized fabrication protocols. Furthermore, treatment success and behaviour of CAP should be evaluated by histomorphometric and CBCT analyses. The latter is the most accurate radiographic method for examination of bone structures and defects (91,92). Since CAP is undergoing the stage of clinical trials, it is a matter of time before fully evaluating its qualities, advantages, and potential.

CONCLUSION

Carbonate apatite is a biomaterial that resembles natural bone (60). Its physical and biological properties have been a matter of interest, observation, and analysis through the years. Various fabrication protocols have been suggested in the search for safe, reliable, and cost-effective methods. According to the literature, the material has excellent biocompatibility, resorbability, and osteoconductive capacity and could be regarded as a promising bone substitute material.

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