Scripta Scientifica Medicinae Dentalis, 2025;11(1):7-17
DOI: 10.14748/ssmd.v11i1.9879

ISSN 2367-7236 (Print)
ISSN 2367-7244 (Online)

REVIEWS

METHODS FOR OBTAINING SYNTHETIC CARBONATE APATITE FOR BONE REGENERATION: A REVIEW

Ivaylo Parushev[1], Tsvetalina Gerova-Vatsova[2]

[1]Department of Clinical Medical Sciences, Faculty of Dental Medicine, Medical University of Varna, Bulgaria
[2]Department of Periodontology and Dental Implantology, Faculty of Dental Medicine, Medical University of Varna, Bulgaria

ABSTRACT

This review examines the most common methods for the preparation of synthetic carbonate apatite for bone repair in the last ten years. The article is organized into two sections. First, the methods for obtaining powdered carbonate apatite with nanosized particles by wet chemical precipitation reactions are presented, considering the starting reagents and the conditions of the chemical reactions. The second section presents the preparation of carbonate apatite blocks and/or granules by dissolution-precipitation reactions. The transformations occurring in these interactions are systematized according to the type of precursor, soaking solution, and reaction conditions.

Keywords: *carbonate apatite, carbonated hydroxyapatite, carbonate hydroxyapatite, bone, regeneration*

INTRODUCTION

The development of methods for obtaining and evaluating biomaterials for bone regenerative therapy is a widely discussed topic. Calcium phosphate bioceramics are some of the most frequently used in the field (1–9). In recent years, carbonated calcium hydroxyapatite (carbonate apatite, CO_3Ap) has gained popularity (10–18).

Carbonate apatite is calcium hydroxyapatite in which hydroxyl and/or phosphate groups are replaced by carbonate ions. According to the type of substitution, three types of carbonate apatite are distinguished: A-carbonate apatite (with substitution of PO_4 groups), B-carbonate apatite (substitution of OH groups), and AB-carbonate apatite (when both groups are substituted) (18–20).

The inorganic component of hard tissues in the human body (bones and teeth) consists mainly of carbonate-substituted hydroxyapatite of type B and AB (B > A) with 2–8% carbonate content (20,21). This determines the great interest in developing methods for obtaining synthetic carbonate apatite and studying its application in orthopedics, oral surgery, dental implantology, and other biomedical fields (18). Dental implantology is a rapidly developing science with a continuous improvement of the used techniques and the search for new ones. There is a growing need for biomaterials with improved biological and mechanical properties to provide a shorter treatment duration (22). Maximum bone preservation and regeneration are aimed at the tooth extraction stage. For this purpose, autogenous grafts (bone and soft tissue), bone substitutes, autologous platelet concentrates, and barrier membranes are used (23–26).

Evaluation of its biological behavior is based on histomorphometric and imaging studies, such as cone-beam computed tomography (CBCT). It is among the most preferred imaging techniques for three-dimensional diagnosis of bone structures with

Address for correspondence:
Ivaylo Parushev
Faculty of Dental Medicine
Medical University of Varna
84 Tzar Osvoboditel
9002 Varna, Bulgaria
e-mail: Ivaylo.Parushev@mu-varna.bg

Received: December 3, 2024
Accepted: January 27, 2025

© The Author(s) 2025. This is an open access article distributed under the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

high resolution and about 90% lower radiation dose than conventional CT (27,28).

The synthesis of carbonated hydroxyapatite, which is a powder, is carried out by various methods such as: solid-phase syntheses, chemical precipitation, sol-gel methods, hydrothermal methods, etc. (5,21,29,30). However, the powdered form of carbonate apatite cannot be used to treat bone defects, as it leads to inflammatory reactions and the formation of crystals (10,31). Therefore, it is mainly applied in the form of porous granules or blocks, which are obtained from pre-synthesized powdered nanocarbonate apatite, or by phase transformations of precursors with a bulk structure (11,31).

AIM

The aim of this review article is to investigate the most commonly used methods for obtaining synthetic carbonate apatite for bone repair in the last ten years .

MATERIALS AND METHODS

An electronic search was conducted in PubMed, Google Scholar, and ResearchGate. The keywords „carbonate apatite“, „carbonated hydroxyapatite“, „carbonate hydroxyapatite“, „bone“ and „regeneration“ were used. The inclusion criteria were: articles discussing methods for obtaining synthetic carbonate apatite for bone regeneration purposes and studies published within the last 10 years.

RESULTS AND DISCUSSION

Literature data indicated that there were two main types of methods for obtaining carbonate apatite, which were based on wet chemical precipitation and dissolution-precipitation reactions.

1. Wet chemical precipitation reactions

Table 1 presents starting reagents and reaction conditions for obtaining carbonate apatite by chemical precipitation.

Nanosized CO_3Ap was synthesized by adding an aqueous solution of diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) to a solution containing

Table 1. Reagents and reaction conditions for the preparation of synthetic carbonate apatite by wet chemical precipitation reactions.

Source of Calcium Ions	Source of Phosphate Ions	Source of Carbonate Ions	Time	Temperature	Reference
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	$(NH_4)_2CO_3$	2 h	37°C	(32)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	$(NH_4)_2CO_3$	3 h	37°C	(33)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	$NaHCO_3$	10 days	RT	(34)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	$NaHCO_3$	10 min	80°C	(35)
$Ca(NO_3)_2.4H_2O$	NaH_2PO_4	$NaHCO_3$	2 h	60°C	(36-38)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	NH_4HCO_3	24 h	180°C	(39)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	NH_4HCO_3	30 min	90°C	(40)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	NH_4HCO_3	-	RT	(41, 42)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	NH_4HCO_3	-	RT	(43)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	Na_2CO_3	-	-	(44, 45)
$Ca(NO_3)_2.4H_2O$	$(NH_4)_2HPO_4$	Urea	12 h	70°C	(46)
$Ca(OH)_2$	$(NH_4)_2HPO_4$	$CaCO_3$	2 days	-	(47)
$CaCl_2$	H_3PO_4	Na_2CO_3	4 h	80°C	(48)
CaO (snail shells)	$(NH_4)_2HPO_4$	$NaHCO_3$	24h	90°C	(49-51)
CaO (abalone shells)	$(NH_4)_2HPO_4$	NH_4HCO_3	24h	90°C	(52-55)
CaO (oyster shells)	$(NH_4)_2HPO_4$	NH_4HCO_3	-	RT	(56, 57)

RT: room temperature

calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) and diammonium carbonate ($(NH_4)_2CO_3$) (32, 33). The reaction was carried out by stirring the resulting suspension for 3 hours at a temperature of 37°C. Microspheres were produced from the obtained powders, which were studied in vitro and in vivo and proved to be effective for in vivo local delivery of minocycline (an antibiotic from the tetracycline group) (32) and rhBMP-2 (a cytokine that plays an essential role in skeletal development) (33).

Schardosim et al. (34) synthesized CO_3Ap by mixing aqueous solutions of $Ca(NO_3)_2.4H_2O$, $(NH_4)_2HPO_4$, and sodium hydrogen carbonate ($NaHCO_3$). The resulting suspension was stored in a sealed flask for 10 days to mature nanocrystalline apatite, then filtered and freeze-dried. The synthesized powder is used for the production of three-dimensional porous composite scaffolds that have been investigated for potential application as bone substitutes.

Carbonate substituted hydroxyapatite powders with a Ca/P ratio in the range of 1.62–1.87 were obtained under the influence of ultrasound (US) or microwave (MW) radiation. The synthesized powders were found to be biocompatible, non-toxic, and exhibit osteoconductive properties. Moreover, the use of US and MW irradiation significantly shortens the time to obtain B type CO_3Ap, similar to biological hydroxyapatite in structure and composition (35).

To ensure the purity of the final product, some researchers conducted the synthesis of CO_3Ap in ultrapure water through which nitrogen was bubbled before adding the starting reagents ($NaHCO_3$, $Ca(NO_3)_2.4H_2O$ and NaH_2PO_4) (36–38). In this way, the carbon dioxide dissolved in the water was removed, which helped to control the amount of carbonate ions in the interactions.

Carbonate apatite powders with carbonate contents of 8, 12, and 16 wt% were synthesized by a hydrothermal method (39). The aqueous stock solutions of $(NH_4)_2HPO_4$, NH_4HCO_3, and $Ca(NO_3)_2.4H_2O$ were mixed and stirred at pH 9–11, which was controlled by adding ammonium hydroxide solution. The resulting suspension was transferred to an autoclave and remained in an oven for 24 hours at 180 °C. The precipitate formed was washed and freeze-dried.

Copete et al. (40) used the same starting solutions but conducted the reaction at 90°C in an argon atmosphere for 30 min. In addition, they subjected the dried precipitate to heat treatment at 400°C for 2 hours to remove synthesis residues.

Some researchers (41–43) suggested a method for CO_3Ap synthesis by dissolving $Ca(NO_3)_2{\cdot}4H_2O$ in acetone and adding it to an aqueous solution of $(NH_4)_2HPO_4$ and NH_4HCO_3. An advantage of the proposed technology was that heating of the reaction mixture was unnecessary and the reaction was performed at room temperature.

Usually, the production of carbonate apatite is carried out at lower temperatures, since carbonate content starts to decrease at about 400°C (11). It has been reported that this can be solved by sintering synthetic CO_3Ap in a carbon dioxide atmosphere at a temperature of 780°C (44,45).

Anwar et al. (46) applied urea as a source of carbonate ions for the synthesis of carbonate substituted hydroxyapatite nanorods. The classic aqueous solutions of $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$ were used as sources of calcium and phosphate ions, and the reaction was carried out at a low temperature for a relatively short time.

Hydroxyapatite with 6 vol% carbonate content was synthesized by dropwise addition of an aqueous solution of $(NH_4)_2HPO_4$ to a solution of $CaCO_3$ and $Ca(OH)_2$ (47). In vitro studies have demonstrated that the obtained CO_3Ap was suitable for local delivery of curcumin, which would contribute to a combined anti-cancer, anti-inflammatory, and bone-regenerative effect.

The influence of different organic modifiers (citric acid, acetic acid, glutamic acid, and gallic acid) on the nucleation and crystallinity of CO_3Ap obtained by mixing aqueous solutions of calcium chloride (CaCl), phosphoric acid (H_3PO_4), and sodium carbonate (Na_2CO_3) was studied (48). Using different modifiers resulted in powders with different crystal particle sizes and morphologies.

The use of biological waste products for the production of synthetic CO_3Ap has also been suggested. For example, the shells of abalone, shrimps, snails, and other molluscs usually contain more than 90% calcium carbonate, which is a suitable source of calcium for carrying out chemical reactions in solution.

For its extraction, the shells are ground and subjected to heat treatment. Thus, the calcium carbonate breaks down to calcium oxide, which when mixed with water forms a calcium hydroxide solution. It, in turn, reacts with phosphate and carbonate solutions at room temperature, resulting in CO_3Ap (49–57).

The above-discussed reactions were carried out by classical chemical reactions in aqueous solution, accompanied by precipitation of the desired product. Usually, they are easy to perform, do not require expensive equipment and reagents, are carried out at relatively low temperatures, and allow for relatively fast obtaining of the products. On the other hand, the course of these interactions depends on a number of conditions, such as: concentration of starting reagents, pH and temperature of the solution, speed of stirring, etc. In addition, the resulting sludge is dried to a powdery state, which requires additional processing to obtain a porous structure with a certain shape and size.

2. Dissolution-precipitation reactions

Methods for the production of CO_3Ap bone scaffolds by dissolution-precipitation reactions mainly consist of soaking calcium precursors with a bulk structure in an aqueous solution containing phosphate and/or carbonate ions. The most commonly used precursors are granules or blocks of calcium carbonate ($CaCO_3$) (59–72), calcium sulfate hemihydrate ($CaSO_4.1/2H_2O$) or anhydrous calcium sulfate ($CaSO_4$) (74–83), alpha tricalcium phosphate (α-TCP), beta tricalcium phosphate (β-TCP) and calcium hydrogen phosphate dihydrate ($CaHPO_4.2H_2O$) (90,91) (Table 2).

Recently, Ishikawa and Hayashi (58) have pointed out that the simultaneous presence of calcium and carbonate ions, low solubility in water, and easy preparation of chemically pure blocks defined calcium carbonate as one of the ideal precursors for the production of bone substitutes from CO_3Ap. The authors used calcium carbonate powder to obtain porous honeycomb granules through an extrusion molding method. At the next stage, the granules were subjected to phosphatization by soaking them in a solution

Table 2. Precursors, soaking solutions and reaction conditions for the preparation of carbonate apatite by dissolution-precipitation reactions.

Precursor	Soaking Solution	Reaction Temperature	Soaking Time	Reference
$CaCO_3$	Na_2HPO_4	80°C	5–30 days	(59-72)
$CaCO_3$	Na_2HPO_4	60˚C	14 days	(73)
$CaSO_4.1/2H_2O$	1) Na_2CO_3 2) Na_2HPO_4	60˚C 90˚C	3 days; 7 days	(74, 75)
$CaSO_4.1/2H_2O$	$Na_3PO_4 + Na_2CO_3$	100˚C	1, 3, 5 days	(76)
$CaSO_4.1/2H_2O$	1) $Na_2CO_3 + NaHCO_3$; 2) Na_2HPO_4	90˚C; 80˚C	24 h; 3 weeks	(77)
$CaSO_4.1/2H_2O$	1) Na_2CO_3; 2) Na_2HPO_4	4˚C; 120˚C	7 days; 14 days	(78)
$CaSO_4.1/2H_2O$	1) $NaHCO_3$ 2) Na_2HPO_4	60˚C; 80˚C	24 h; 7 days	(79)
$CaSO_4$	1) $NaHCO_3 + Na_2CO_3$ 2) Na_2HPO_4	40˚C; 80˚C	4 days; 7 days	(80–82)
$CaSO_4.1/2H_2O$	$Na_2CO_3 + Na_3PO_4$	100˚C	48 or 72 h	(83)
α-TCP	$NaHCO_3 + Na_2HPO_4$	80˚C	7 days	(84)
β-TCP	$NaHCO_3$	200˚C	6 days	(85, 86)
β-TCP	Na_2CO_3	200˚C	5 days	(87, 88)
$CaHPO_4.2H_2O$	$NaHCO_3$ or Na_2CO_3	80°C	up to 14 days	(89)
$CaHPO_4.2H_2O$	Na_2CO_3	80˚C	14 days	(90)
$CaHPO_4.2H_2O$	$NaHCO_3$	80˚C	3 days	(91)

of disodium hydrogen phosphate (Na_2HPO_4), during which the calcium carbonate was transformed into CO_3Ap (59–64).

The conversion of $CaCO_3$ granules to CO_3Ap via a dissolution-precipitation reaction was found to be accelerated by the addition of carbonate ions to the phosphate solution (65). Soaking the starting granules in an aqueous solution containing Na_2HPO_4, Na_2CO_3, and $NaHCO_3$ reduced the time for obtaining CO_3Ap granules and led to an increased carbonate content in the final product.

Carbonate apatite blocks with a continuous pore architecture were obtained from calcite powder and polymethyl methacrylate (PMMA) raw materials (66). Initially, calcite-PMMA granules were obtained and subjected to heat treatment to remove PMMA and form porous structures. Next, the calcite granules were soaked in Na_2HPO_4 until the calcium carbonate was transformed into CO_3Ap.

Shimabukuro et al. (67) conducted in vivo and in vitro experiments and demonstrated that the addition of silver phosphate to the CO_3Ap granules was an effective strategy for the treatment of fracture-related infections. The authors obtained the investigated granules by pressing mixtures of $CaCO_3$ and Ag_3PO_4 and soaking the obtained tablets in a phosphate solution.

Wang et al. (68) investigated the effect that the pH of the phosphate solution had on the composition and architecture of CO_3Ap granules obtained from $CaCO_3$ by a dissolution-precipitation reaction. The authors found that the rate of $CaCO_3$ transformation was much higher at pH 6.0 and pH 7.0 than at pH 8.0 and pH 8.9.

Tanaka et al. (69) obtained fully interconnected porous CO_3Ap blocks by hydrate expansion of CaO granules at 100% CO_2 humidity and phosphatization of the resulting porous $CaCO_3$ blocks in aqueous solution of Na_2HPO_4.

Powdered calcium hydroxide has been successfully used as a starting material for CO_3Ap production (70–73). For this purpose, powdered calcium hydroxide was compacted into tablets, which were carbonized with CO_2. The resulting blocks of $CaCO_3$ were crushed into granules of certain sizes and immersed in a solution of disodium hydrogen phosphate (Na_2HPO_4). In this, the dissolution-precipitation reaction took place and $CaCO_3$ was transformed into CO_3Ap.

An interesting precursor for the production of porous CO_3Ap blocks by dissolution-precipitation reactions is calcium sulfate hemihydrate. It allows easy molding, hardens rapidly to calcium sulfate dihydrate in the presence of a minimal amount of water, and enables the production of blocks with interconnected pores (74). A disadvantage of calcium sulfate in this type of conversion is the presence of sulfate ions that can remain in the apatite structure (58).

Carbonate apatite blocks with interconnected pores were produced by sequential dissolution-precipitation reactions of calcium sulfate dihydrate blocks obtained by solidification of calcium sulfate hemihydrate (74). Later, CO_3Ap blocks obtained by an analogous technology were studied in vivo, by implanting them in a rabbit femur (75). After analyzing the results of histological studies, the authors concluded that the introduction of interconnected pores was an effective approach for rapid bone regeneration.

The porosity of CO_3Ap blocks can also be ensured by the addition of porogenic agents to calcium sulfate hemihydrate. As such, PMMA granules (76), spherical phenol resin (77), or microfibers (78) were used. The blocks obtained after solidification were subjected to heat treatment to remove the porogen, during which porous blocks of calcium sulfate were formed. The latter, according to the already described technology, were transformed to CO_3Ap through dissolution-precipitation reactions.

The application of calcium sulfate hemihydrate as a substrate for the preparation of 3D printed carbonate apatite scaffolds with controlled porosity has been reported (79). For this purpose, a suitable binder was added to the starting powder and through 3D printing, blocks were obtained and subjected to heat treatment. The formed porous blocks of calcium sulfate were soaked in aqueous solutions of $NaHCO_3$ and Na_2HPO_4 until CO_3Ap was obtained.

Calcium sulfate powder has been used as a starting material to produce honeycomb carbonate apatite granules (80–82). For this purpose, calcium sulfate granules were initially obtained by extrusion, after which they were soaked in an aqueous solution of $NaHCO_3$ and Na_2CO_3 for four days. The obtained

granules of calcium carbonate were subjected to phosphorylation in a solution of Na_2HPO_4 for seven days, during which they were transformed to CO_3Ap.

Eriwati et al. (83) reported the preparation of carbonate-apatite blocks by a dissolution-precipitation reaction of calcium sulfate dihydrate in an aqueous solution containing 1 mol/L Na_2CO_3 and 1 mol/L Na_3PO_4 for 48 and 72 hours at a temperature of 100°C.

Spheres of α-TCP have been used as starting material to produce interconnected porous CO3Ap blocks (84). The basis of the proposed technology was a dissolution-precipitation reaction in an aqueous solution containing 1 mol/L $NaHCO_3$ and 0.1 mol/L Na_2HPO_4. The tissue response to the obtained scaffolds was evaluated in a rabbit tibial bone defect. The results showed excellent tissue response and good osteoconductivity of the CO_3Ap blocks.

Darus et al. (85,86) used porous blocks of β-TCP as a precursor to obtain CO_3Ap scaffolds. The transformation was carried out by soaking the starting blocks in a solution of $NaHCO_3$ or Na_2CO_3 for 2, 4, 6, 8, and 10 days at 200°C. The reactions were carried out in a hydrothermal vessel, with optimal results obtained when using an aqueous solution of $NaHCO_3$ for 6 days.

Freeze-casted β-TCP blocks have been used to fabricate CO_3Ap scaffolds (87,88). Aqueous Na_2CO_3 solution was used as a source of carbonate ions, in which the blocks were soaked for 5 days at 200°C. The obtained CO_3Ap scaffolds were characterized by lower crystallinity and more than 11 wt.% carbonate content, which, according to the authors, was a prerequisite for maintaining high cell adhesion and cell proliferation.

Calcium hydrogen phosphate dihydrate (DCPD, $CaHPO_4.2H_2O$) was successfully used as a precursor for the production of CO_3Ap scaffolds for bone regeneration (89–91). When DCPD blocks were soaked in a solution of $NaHCO_3$ or Na_2CO_3, a dissolution-precipitation reaction took place and the blocks were transformed into CO_3Ap.

Dissolution-precipitation reactions allow for the production of porous granules and blocks of carbonate apatite without the need for preliminary synthesis in a powder state. The precursors are readily available and easily formed, but their complete transformation is time-consuming (typically several days), even at temperatures of 200°C.

CONCLUSION

The disadvantages of the considered technologies require new methods that allow for rapid and efficient production of bone scaffolds from carbonate apatite with predetermined properties. A good alternative to conventional methods is 3D printing, which provides rapid serial production of scaffolds with set geometric dimensions and porosity. The ability to control the shape, size, and interconnectivity of the pores is essential to achieve successful results in the application of synthetic bone substitutes.

REFERENCES

1. Pandit A, Indurkar A, Locs J, Haugen HJ, Loca D. Calcium Phosphates: A Key to Next-Generation In Vitro Bone Modeling. Adv. Healthcare Mater. 2024; 2401307. doi: 10.1002/adhm.202401307.
2. Hou X, Zhang L, Zhou Z, Luo X, Wang T, Zhao X, et al. Calcium phosphate-based biomaterials for bone repair. J Funct Biomater. 2022;13(4):187. doi: 10.3390/jfb13040187.
3. Dorozhkin S. Calcium orthophosphate (CaPO4)-based bioceramics: Preparation, properties, and applications. Coatings. 2022;12(10):1380. doi: 10.3390/coatings12101380.
4. Chen X, Li H, Ma Y, Jiang Y. Calcium phosphate-based nanomaterials: preparation, multifunction, and application for bone tissue engineering. Molecules. 2023;28(12):4790. doi: 10.3390/molecules28124790.
5. Radulescu D, Vasile O, Andronescu E, Ficai A. Latest research of doped hydroxyapatite for bone tissue engineering. Int J Mol Sci. 2023;24(17):13157. doi: 10.3390/ijms241713157.
6. Kareem R, Bulut N, Kaygili O. Hydroxyapatite biomaterials: A comprehensive review of their properties, structures, medical applications, and fabrication methods. J Chem Rev. 2024;6(1):1-26. doi: 10.48309/jcr.2024.415051.1253.
7. Kharissova O, Nikolaev A, Kharisov B, Dorozhkin S, López I, Méndez Y, et al. Enzymatic synthesis of calcium phosphates: A review. Nano-Struct Nano-Objects. 2024;39:101214. doi: 10.1016/j.nanoso.2024.101214.
8. Tolmacheva N, Bhattacharyya A, Noh I. Calcium phosphate biomaterials for 3D bioprinting in

bone tissue engineering. Biomimetics (Basel). 2024;9(2):95. doi: 10.3390/biomimetics9020095.

9. Min K, Kim D, Kim K, Seo J, Pack S. Biomimetic Scaffolds of Calcium-Based Materials for Bone Regeneration. Biomimetics (Basel). 2024;9(9):511. doi: 10.3390/biomimetics9090511.

10. Rahyussalim A, Supriadi S, Marsetio A, Pribadi P, Suharno B. The potential of carbonate apatite as an alternative bone substitute material. Med J Indones. 2019;28:92–97. doi: 10.13181/mji.v28i1.2681.

11. Ishikawa K. Carbonate apatite bone replacement: learn from the bone. J Ceram Soc Jpn 2019;127(9):595-601. doi: 10.2109/jcersj2.19042.

12. Ressler A, Žužić A, Ivanišević I, Kamboj N, Ivanković H. Ionic substituted hydroxyapatite for bone regeneration applications: A review. Open Ceram. 2021; 6:100122. doi: 10.1016/j.oceram.2021.100122.

13. Kono T, Sakae T, Nakada H, Kaneda T, Okada H. Confusion between carbonate apatite and biological apatite (carbonated hydroxyapatite) in bone and teeth. Minerals. 2022;12(2):170. doi: 10.3390/min12020170.

14. Yotsova R, Peev S. Carbonate apatite as a bone substitute material. A review. Scr Sci Med Dentalis. 2023; 9(1):33-42. doi: 10.14748/ssmd.v9i1.8776.

15. Adekanmi D, Garcia C, Lopez-Badillo C, Carbonate Hydroxyapatite - A Multifunctional Bioceramics with Non-Medical Applications. Eng Chem. 2024;7:1-24. doi: 10.4028/p-518pjS.

16. Aoki K, Ideta H, Komatsu Y, Tanaka A, Kito M, Okamoto M, et al. Bone-Regeneration Therapy Using Biodegradable Scaffolds: Calcium Phosphate Bioceramics and Biodegradable Polymers. Bioeng. 2024;11:180. doi: 10.3390/bioengineering11020180.

17. Nunn M, Rudick C, Nikaido M, Miyamoto T. A systematic review of a novel alloplast carbonate apatite granules. Front Dent Med 2024;5:1418039. doi: 10.3389/fdmed.2024.1418039.

18. Yotsova R, Peev S. Biological Properties and Medical Applications of Carbonate Apatite: A Systematic Review. Pharmaceutics. 2024;16(2):291. doi: 10.3390/pharmaceutics16020291.

19. Nowicki D, Skakle J, Gibson I. Faster synthesis of A-type carbonated hydroxyapatite powders prepared by high-temperature reaction. Adv Powder Technol. 2020;31(8):3318-27. doi: 10.1016/j.apt.2020.06.022.

20. Siddiqi S, Azhar U. Chapter 6. Carbonate substituted hydroxyapatite. In: Khan AS, Chaudhary AA, editors. Handbook of Ionic Substituted Hydroxyapatites. Elsevier; 2020. pp. 149-73.

21. Pasteris J. A mineralogical view of apatitic biomaterials. Am Min. 2016;101:2594–610. doi: 10.2138/am-2016-5732.

22. Yotsova R, Peev S, Kolarov R. Immediate implant placement using customized healing abutments as a method of hard and soft tissue preservation. A review article. Scr Sci Med Dent. 2022;8(2):7-13. doi: 10.14748/ssmd.v8i2.8518.

23. Yankov G. Socket Preservation and Guided Bone Regeneration: Prerequisites for Successful Implant Dentistry. Cureus. 2023;15(11):e48785.doi: 10.7759/cureus.48785.

24. Yotsova R, Peev S, Kolarov R. Application of platelet-rich plasma for alveolar ridge preservation. A review article. Scr Sci Med Dent. 2022;8(1):18-37. doi: 10.14748/ssmd.v8i1.8493.

25. Yotsova R, Peev S, Georgiev T. Alveolar ridge preservation using free gingival grafts. A review article. Scr Sci Med Dent. 2021;7(2):24-30. doi: 10.14748/ssmd.v7i2.8178.

26. Yotsova R, Peev S, Georgiev T. Alveolar ridge preservation using dense polytetrafluoroethylene membranes. A review article. Scr Sci Med Dent. 2021;7(2):31-8. doi: 10.14748/ssmd.v7i2.8216.

27. Yotsova R, Peev S, Ivanova N. Bone histomorphometry as a diagnostic tool. A review article. Scr Sci Med Dent. 2023; 9(1):7-23. doi: 10.14748/ssmd.v9i1.8602.

28. Papanchev G, Yotsova R. Odontogenic maxillary sinusitis. Etiological, clinical, diagnostic and therapeutic aspects. Systematic review. J Union Sci Varna. Med Ecol Series. 2017;22(2):71-6. doi: 10.14748/isuvsme.v22i2.5529.

29. Šupová M. Substituted hydroxyapatites for biomedical applications: A review. Ceram Int. 2015; 41(8):9203-31. doi: 10.1016/j.ceramint.2015.03.316.

30. Fleet M. Carbonated Hydroxyapatite: Materials, Synthesis, and Applications. Pan Stanford Publishing Pte. Ltd; 2015.

31. Ishikawa K. Carbonate Apatite Bone Replacement. In: Antoniac IV, editor. Handbook of Bioceramics and Biocomposites. Springer International Publishing Switzerland; 2016. pp. 2013-232. doi: 10.1007/978-3-319-12460-5_8.

32. Calasans-Maia M, Barboza Junior C, Soriano-Souza C, Alves A, Uzeda M, Martinez-Zelaya V, et al. Microspheres of alginate encapsulated minocycline-loaded nanocrystalline carbonated hydroxyapatite: therapeutic potential and effects on bone regeneration. Int J Nanomedicine. 2019;14:4559-71. doi: 10.2147/IJN.S201631.

33. Schneider Werner Vianna T, Sartoretto SC, Neves Novellino Alves AT, Figueiredo de Brito Resende R, de Almeida Barros Mourão CF, de Albuquerque Calasans-Maia J, et al. Nanostructured Carbonated Hydroxyapatite Associated to rhBMP-2 Improves Bone Repair in Rat Calvaria. J Funct Biomater. 2020;11(4):87. doi: 10.3390/jfb11040087.

34. Schardosim M, Soulié J, Poquillon D, Cazalbou S, Duployer B, Tenailleau C, Rey C, Hübler R, Combes C. Freeze-casting for PLGA/carbonated apatite composite scaffolds: Structure and properties. Mater Sci Eng. 2017;77:731-8. doi: 10.1016/j.msec.2017.03.302.

35. Stanislavov A, Sukhodub L, Sukhodub L, Kuznetsov V, Bychkov K, Kravchenko M. Structural features of hydroxyapatite and carbonated apatite formed under the influence of ultrasound and microwave radiation and their effect on the bioactivity of the nanomaterials. Ultrason Sonochem. 2018;42:84-96. doi: 10.1016/j.ultsonch.2017.11.011.

36. Deymier A, Nair A, Depalle B, Qin Z, Arcot K, Drouet C, et al. Protein-free formation of bone-like apatite: New insights into the key role of carbonation. Biomaterials. 2017;127:75-88. doi: 10.1016/j.biomaterials.2017.02.029.

37. Wang S, Zhang J, Ma J, Yang H, Shao X, Su M, et al. Applying Pb2+ to probe the dissolution of carbonated hydroxylapatite by enterobacter sp.: a new insight into the bioerosion of tooth mineral. J Biomed Mater Res B Appl Biomater. 2021;109(8):1230-8. doi: 10.1002/jbm.b.34784.

38. Wang S, Shao C, Zhao X, Guo Y, Song H, Shen L, et al. Application of three-dimension printing nano-carbonated-hydroxylapatite to the repair of defects in rabbit bone. Int J Nanomedicine. 2024;19:1667-81. doi: 10.2147/IJN.S439775.

39. Li S, Yu W, Zhang W, Zhang G, Yu L, Lu E. Evaluation of highly carbonated hydroxyapatite bioceramic implant coatings with hierarchical micro-/nanorod topography optimized for osseointegration. Intl J Nanomedicine 2018;13:3643-59. doi: 10.2147/IJN.S159989.

40. Copete H, López E, Baudin C. Synthesis and characterization of B-type carbonated hydroxyapatite materials: Effect of carbonate content on mechanical strength and in vitro degradation. Bol Soc Esp Cerám Vidr. 2024;63(4):255-67. doi: 10.1016/j.bsecv.2023.12.001.

41. Safarzadeh M, Ramesh S, Tan C, Chandran H, Noor A, Krishnasamy S, et al. Effect of multi-ions doping on the properties of carbonated hydroxyapatite bioceramic. Ceram Int. 2019;45(3):3473-7. doi: 10.1016/j.ceramint.2018.11.003.

42. Safarzadeh M, Ramesh S, Tan C, Chandran H, Ching Y, Noor A, et al. Sintering behaviour of carbonated hydroxyapatite prepared at different carbonate and phosphate ratios. Bol Soc Esp Cerám Vidr. 2020;59(2):73-80. doi: 10.1016/j.bsecv.2019.08.001.

43. Ezekiel I, Kasim S, Ismail Y, Noor A. Nanoemulsion synthesis of carbonated hydroxyapatite nanopowders: Effect of variant CO32−/PO43− molar ratios on phase, morphology, and bioactivity. Ceram Int. 2018;44(11):13082-9. doi: 10.1016/j.ceramint.2018.04.128.

44. Nakamura M, Hiratai R, Hentunen T, Salonen J, Yamashita K. Hydroxyapatite with high carbonate substitutions promotes osteoclast resorption through osteocyte-like cells. ACS Biomaterials Sci Eng. 2016;2(2):259-67. doi: 10.1021/acsbiomaterials.5b00509.

45. Bergara-Muguruza L, Mäkelä K, Yrjälä T, Salonen J, Yamashita K, Nakamura M. Surface electric fields increase human osteoclast resorption through improved wettability on carbonate-incorporated apatite. ACS Appl Mater Interfaces. 2021;13(49):58270-8. doi: 10.1021/acsami.1c14358.

46. Anwar A, Asghar M, Kanwal Q, Kazmi M, Sadiqa A. Low temperature synthesis and characterization of carbonated hydroxyapatite nanocrystals. J Mol Struct. 2016;1117:283-6. doi: 10.1016/j.molstruc.2016.03.061.

47. Verma A, Kumar T, Madhumathi K, Rubaiya Y, Ramalingan M, Doble M. Curcumin releasing eggshell derived carbonated apatite nanocarriers for combined anti-cancer, anti-inflammatory and bone regenerative therapy. J Nanosci Nanotechnol. 2019;19(11):6872-80. doi: 10.1166/jnn.2019.16640.

48. Aneem T, Saha S, Jahan R, Wong S, Li X, Arafat M. Effects of organic modifiers and temperature on the synthesis of biomimetic carbonated

hydroxyapatite. Ceram Int. 2019;45(18):24717-26. doi: 10.1016/j.ceramint.2019.08.211.

49. Januariyasa I, Yusuf Y. Synthesis of carbonated hydroxyapatite derived from snail shells (Pilla ampulacea): effect of carbonate precursor to the crystallographic properties. IOP Conf Ser Mater Sci Eng. 2019;546(4):042015. doi: 10.1088/1757-899X/546/4/042015.

50. Januariyasa I, Yusuf Y. Porous carbonated hydroxyapatite-based scaffold using simple gas foaming method. J Asian Ceram Soc. 2020;8(3):634-41. doi: 10.1080/21870764.2020.1770938.

51. Anggraini R, Yusuf Y. The effect of stirring time on the characteristics of carbonated hydroxyapatite from pearl shells (Pinctada maxima). IOP Conf Ser Mater Sci Eng. 2019;546(4):042002. doi: 10.1080/21870764.2020.1770938.

52. Permatasari H, Yusuf Y. Characteristics of carbonated hydroxyapatite based on abalone mussel shells (Halioitis asinina) synthesized by precipitation method with aging time variations. IOP Conf Ser Mater Sci Eng. 2019;546(4):042031. doi: 10.1088/1757-899X/546/4/042031.

53. Permatasari H, Supii A, Suparta G, Yusuf Y. Characteristics of abalone mussel shells (Halioitis asinina) with calcination temperature variations as a basic material for synthesis of carbonated hydroxyapatite. Key Eng Mater. 2019;818:31-6. doi:10.4028/www.scientific.net/KEM.818.31.

54. Permatasari H, Sari M, Aminatun, Suciati T, Dahlan K, Yusuf Y. Nano-carbonated hydroxyapatite precipitation from abalone shell (Haliotis asinina) waste as the bioceramics candidate for bone tissue engineering. Nanomater Nanotechnol. 2021;11:18479804211032851. doi: 10.1177/18479804211032851.

55. Sari M, Hening P, Ana I, Yusuf Y. Porous structure of bioceramics carbonated hydroxyapatite-based honeycomb scaffold for bone tissue engineering. Mater Today Commun. 2021;26:102135. doi: 10.1016/j.mtcomm.2021.102135.

56. Almukarrama, Yusuf Y. (, June). Development carbonated hydroxyapatite powders from oyster shells (Crassostrea gigas) by sintering time controlling. IOP Conf Ser Mater Sci Eng. 2019;546(4):042001. doi: 10.1088/1757-899X/546/4/042001.

57. Wati R, Yusuf Y. Effect of sintering temperature on carbonated hydroxyapatite derived from common cockle shells (Cerastodermaedule): Composition and crystal characteristics. Key Eng Mater. 2019;818:37-43. doi: 10.4028/www.scientific.net/KEM.818.37.

58. Ishikawa K, Hayashi K. Carbonate apatite artificial bone. Sci Technol Adv Mater. 2021;22(1):683-94. doi: 10.1080/14686996.2021.1947120.

59. Hayashi K, Ishikawa K. Effects of nanopores on the mechanical strength, osteoclastogenesis, and osteogenesis in honeycomb scaffolds. J Mater ChemB. 2020;8(37):8536-45. doi: 10.1039/D0TB01498B.

60. Hayashi K, Ishikawa K. Honeycomb scaffolds fabricated using extrusion molding and the sphere-packing theory for bone regeneration. ACS Appl Bio Mater. 2020;4(1):721-30. doi: 10.1021/acsabm.0c01279.

61. Hayashi K, Tsuchiya A, Shimabukuro M, Ishikawa K. Multiscale porous scaffolds constructed of carbonate apatite honeycomb granules for bone regeneration. Mater Design. 2022;215:110468. doi: 10.1016/j.matdes.2022.110468.

62. Hayashi K, Yanagisawa T, Shimabukuro M, Kishida R, Ishikawa K. Granular honeycomb scaffolds composed of carbonate apatite for simultaneous intra-and inter-granular osteogenesis and angiogenesis. Mater Today Bio. 2022;14:100247. doi: 10.1016/j.mtbio.2022.100247.

63. Hayashi K, Yanagisawa T, Kishida R, Tsuchiya A, Ishikawa K. Gear-shaped carbonate apatite granules with a hexagonal macropore for rapid bone regeneration. Comput Struct Biotechnol J. 2023;21:2514-23. doi: 10.1016/j.csbj.2023.03.053.

64. Shimabukuro M, Hayashi K, Kishida R, Tsuchiya A, Ishikawa K. Surface functionalization with copper endows carbonate apatite honeycomb scaffold with antibacterial, proangiogenic, and pro-osteogenic activities. Biomater Adv. 2022;135:212751. doi: 10.1016/j.bioadv.2022.212751.

65. Shimabukuro M, Hayashi K, Kishida R, Tsuchiya A, Ishikawa K. Effects of carbonate ions in phosphate solution on the fabrication of carbonate apatite through a dissolution–precipitation reaction. Ceram Int. 2022;48(1):1032-7. doi: 10.1016/j.ceramint.2021.09.188.

66. Putri T, Hayashi K, Ishikawa K. Fabrication of three-dimensional interconnected porous blocks

composed of robust carbonate apatite frameworks. Ceram Int. 2020;46(12):20045-9. doi: 10.1016/j.ceramint.2021.03.324.

67. Shimabukuro M, Hayashi K, Kishida R, Tsuchiya A, Ishikawa K. No-observed-effect level of silver phosphate in carbonate apatite artificial bone on initial bone regeneration. ACS Infect Dis. 2021;8(1):159-69. doi: 10.1021/acsinfecdis.1c00480.

68. Wang Z, Shimabukuro M, Kishida R, Yokoi T, Kawashita M. Effects of pH on the microarchitecture of carbonate apatite granules fabricated through a dissolution–precipitation reaction. Front Bioeng Biotechnol. 2024;12:1396275. doi: 10.3389/fbioe.2024.1396275.

69. Tanaka K, Tsuchiya A, Ogino Y, Koyano K, Ishikawa K. Fabrication and histological evaluation of a fully interconnected porous CO3Ap block formed by hydrate expansion of CaO granules. ACS Appl Bio Mater. 2020; 3(12):8872-8. doi: 10.1021/acsabm.0c01176.

70. Hayashi K, Munar M, Ishikawa K. Effects of macropore size in carbonate apatite honeycomb scaffolds on bone regeneration. Mater Sci Eng C. 2020;111:110848. doi: 10.1016/j.msec.2020.110848.

71. Mano T, Akita K, Fukuda N, Kamada K, Kurio N, Ishikawa K, et al. Histological comparison of three apatitic bone substitutes with different carbonate contents in alveolar bone defects in a beagle mandible with simultaneous implant installation. J Biomed Mater Res Part B: Appl Biomater. 2020;108(4):1450-9. doi: 10.1002/jbm.b.34492.

72. Yanagisawa T, Hayashi K, Tsuchiya A, Kishida R, Ishikawa K. In vivo trial of bioresorbable mesh cages contained bone graft granules in rabbit femoral bone defects. Sci Rep. 2024;14(1):12449. doi: 10.1038/s41598-024-63067-y.

73. Fujioka-Kobayashi M, Tsuru K, Nagai H, Fujisawa K, Kudoh T, Ohe G, et al. Fabrication and evaluation of carbonate apatite-coated calcium carbonate bone substitutes for bone tissue engineering. J Tissue Eng Regen Med. 2018;12(10):2077-87. doi: 10.1002/term.2742.

74. Kishida R, Elsheikh M, Hayashi K, Tsuchiya A, Ishikawa K. Fabrication of highly interconnected porous carbonate apatite blocks based on the setting reaction of calcium sulfate hemihydrate granules. Ceram Int. 2021;47(14):19856-63. doi: 10.1016/j.ceramint.2021.03.324.

75. Elsheikh M, Kishida R, Hayashi K, Tsuchiya A, Shimabukuro M, Ishikawa K. Effects of pore interconnectivity on bone regeneration in carbonate apatite blocks. Regen Biomater. 2022;9:rbac010. doi: 10.1093/rb/rbac010.

76. Arsista D, Eriwati Y, Triaminingsih S, Sunarso S. Fabrication of porous carbonate apatite based on the dissolution-precipitation of calcium sulfate hemihydrate. Dentino (Online). 2019;4(2):205-9. doi: 10.20527/dentino.v4i2.7055.

77. Sakemi Y, Hayashi K, Tsuchiya A, Nakashima Y, Ishikawa K. Fabrication and histological evaluation of porous carbonate apatite block from gypsum block containing spherical phenol resin as a porogen. Mater. 2019;12(23):3997. doi: 10.3390/ma12233997.

78. Akita K, Fukuda N, Kamada K, Kudoh K, Kurio N, Tsuru K, Ishikawa K, Miyamoto Y. Fabrication of porous carbonate apatite granules using microfiber and its histological evaluations in rabbit calvarial bone defects. J Biomed Mater Res Part A. 2020;108(3):709-21. doi: 10.1002/jbm.a.36850.

79. Hayashi K, Kato N, Kato M, Ishikawa K. Impacts of channel direction on bone tissue engineering in 3D-printed carbonate apatite scaffolds. Mater Design. 2021;204:109686. doi: 10.1016/j.matdes.2021.109686.

80. Hayashi K, Kishida R, Tsuchiya A, Ishikawa K. Honeycomb blocks composed of carbonate apatite, β-tricalcium phosphate, and hydroxyapatite for bone regeneration: effects of composition on biological responses. Mater Today Bio. 2019;4:100031. doi: 10.1016/j.mtbio.2019.100031.

81. Hayashi K, Kishida R, Tsuchiya A, Ishikawa K. Carbonate apatite micro-honeycombed blocks generate bone marrow-like tissues as well as bone. Adv Biosys. 2019;3(12):1900140. doi: 10.1002/adbi.201900140.

82. Kudoh K, Fukuda N, Akita K, Kudoh T, Takamaru N, Kurio N, et al. Reconstruction of rabbit mandibular bone defects using carbonate apatite honeycomb blocks with an interconnected porous structure. J Mater Sci Mater Med. 2022;34(1):2. doi: 10.1007/s10856-022-06710-2.

83. Eriwati Y, Yulfa R, Wijatmo I, Irawan B. Different molarities and dissolution-precipitation duration affect the formation of carbonate-apatite blocks for bone graft material. Pesqui Bras Odontopediatria Clín Integr. 2020;20:e5644. doi: 10.1590/pboci.2020.110.

84. Ishikawa K, Arifta T, Hayashi K, Tsuru K. Fabrication and evaluation of interconnected porous carbonate apatite from alpha tricalcium phosphate spheres. J Biomed Mater Res Part B Appl Biomater. 2019;107(2):269-77. doi: 10.1002/jbm.b.34117.

85. Darus F, Jaafar M, Ahmad N. Preparation of carbonate apatite scaffolds using different carbonate solution and soaking time. Process Appl Ceram. 2019;13(2):139-48. doi: 10.2298/PAC1902139D.

86. Darus F, Jaafar M. Enhancement of carbonate apatite scaffold properties with surface treatment and alginate and gelatine coating. J Porous Mater. 2020;27(3):831-42. doi: 10.1007/s10934-019-00848-1.

87. Mamat N, Jaafar M, Hamid Z. Fabrication of Carbonate Apatite Based on Hydrothermal Reaction Using Freeze-Casted β-TCP Precursor. Solid State Phenom. 2017;264:50-3. doi: 10.4028/www.scientific.net/SSP.264.50.

88. Mamat N, Jaafar M, Hamid Z, Yahaya B. Silane treatment of coated carbonate apatite scaffold affects bioactivity and cell viability. J Phys Conf Ser. 2019;1372(1):012054. doi: 10.1088/1742-6596/1372/1/012054.

89. Tsuru K, Yoshimoto A, Kanazawa M, Sugiura Y, Nakashima Y, Ishikawa K. Fabrication of carbonate apatite block through a dissolution–precipitation reaction using calcium hydrogen phosphate dihydrate block as a precursor. Mater. 2017;10(4):374. doi: 10.3390/ma10040374.

90. Kanazawa M, Tsuru K, Fukuda N, Sakemi Y, Nakashima Y, Ishikawa K. Evaluation of carbonate apatite blocks fabricated from dicalcium phosphate dihydrate blocks for reconstruction of rabbit femoral and tibial defects. J Mater Sci Mater Med. 2017;28:1-11. doi: 91. Sugiura Y, Ishikawa K. Fabrication of carbonate apatite blocks from octacalcium phosphate blocks through different phase conversion mode depending on carbonate concentration. J Solid State Chem 2018;267:85-91. doi: 10.1016/j.jssc.2018.08.008.