

Different Molarities and Dissolution-Precipitation Duration Affect the Formation of Carbonate-Apatite Blocks for Bone Graft Material

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Abstract

Objective: To determine diametral tensile strength and water absorption ability of Carbonate-apatite blocks fabricated from gypsum precursors (CaSO_4) through a dissolution-precipitation method, with different solution molarities and immersion times. **Material and Methods:** Thirty-six CaSO_4 gypsum specimens with 6 mm diameter; 3 mm height (Group A for diametral tensile strength) and 36 specimens of 6 mm diameter; 3 mm thickness (Group B for water absorption ability) were used. Each group was divided into 4 group treatments of: 1) dissolution-precipitation in solution of 0.5 mol / L Na_2CO_3 + 0.5 mol / L Na_3PO_4 for 48 hours and 72 hours; 1 mol / L Na_2CO_3 + 1 mol / L Na_3PO_4 for 48 hours and 72 hours. The C-Ap blocks were then tested using ATR-FTIR spectrometer to identify the formation of C-Ap functional groups. Furthermore, Group A specimens were tested for diametral tensile strength using Universal Testing Machine, and Group B specimens were tested for water adsorption ability using an analytical balance by measuring initial and final weight after immersion in saline solution at 37°C for 24 hours. **Results:** The formation of carbonate-apatite (C-Ap) in groups with solution molarity of 1 mol/L for 48 hours and 72 hours resulted in a lower diametral tensile strength and water absorption ability than the groups with a 0.5 mol/L solution. **Conclusion:** Solution with different molarities and dissolution-precipitation duration affect the formation of carbonate-apatite blocks.

Keywords: Dental Materials; Calcium Sulfate; Tensile Strength; Adsorption.

Introduction

Bone grafting is defined as a method to repair bone [1]. The bone grafting material needs to have a similar structure and share the same mechanical properties with actual bone to promote cell growth and new bone formation. In addition to that, the similar structure and mechanical properties between bone grafting material and bones will allow for the material to slowly degrade and be replaced by the newly formed bone [2]. As a bone-replacement material, carbonate-apatite (C-Ap: $\text{Ca}_{10-a}(\text{PO}_4)_6-b(\text{CO}_3)_c(\text{OH})_{2-d}$) in the form of blocks has a strong tensile strength since the bone itself is composed of mostly C-Ap compared to hydroxyapatite (HA: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) [3,4]. Carbonate-apatite (C-Ap) is believed to have the capacity to induce osteoblastic cell differentiation and is considered to be highly osteoconductive. Carbonate-apatite shares similarities to the bone in regards that it could be resorbed by osteoclasts [5-7]. Although C-Ap is not stable at a sintering temperature because of the presence of CO_3^{2-} , fabrication of a pure C-Ap block is chemically possible when done in an aqueous solution through a dissolution-precipitation reaction using calcium carbonate (CaCO_3), calcium sulfate (CaSO_4), α -TCP (α -TCP: $\text{Ca}_3(\text{PO}_4)_2$), or dicalcium phosphate dihydrate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) as the precursor [6,8].

Gypsum (CaSO_4) could be used as the precursor due to its self-setting ability and its ability to be shaped freely at room temperature. To create a carbonate-apatite block using gypsum, a source of carbonates such as a phosphate and carbonate solution is needed [9]. Previous authors successfully created a porous C-Ap utilizing the dissolution-precipitation method at 100°C , by using a solution comprised of 0.5 mol/L of Na_2CO_3 and 0.5 mol/L of Na_3PO_4 from an anhydrous calcium sulfate block using porous polymethylmethacrylate (PMMA) [10]. Some authors fabricated a carbonate-apatite block using octacalcium phosphate as the precursor through a dissolution-precipitation method using 0.1-2 mol/L of NaHCO_3 for 3 days at 80°C . The experiment results showed a relatively high diametral tensile strength of 2-3 MPa for specimens immersed in a 0.5 mol/L and 1 mol/L solution [11]. Another study revealed the higher the temperature for the dissolution-precipitation method produced lower diametral tensile strength [9,12].

To facilitate the cell growth and differentiation, it is imperative to understand the water absorption ability of a bone grafting material because it is related with water permeability and is an important parameter that defines the characteristics and their potential clinical use [13,14].

This study evaluated the mechanical properties and absorption ability of carbonate-apatite (C-Ap) blocks fabricated using calcium sulfate hemihydrate as the precursor using the dissolution-precipitation method in a Na_2CO_3 and Na_3PO_4 solution with different molarities.

Material and Methods

Fabrication of Gypsum Blocks

Gypsum blocks were created by mixing calcium hemihydrate powder with distilled water with a ratio of 0.50. The gypsum mixture was then poured into a mold (6 mm in diameter and 3 mm thick) and set aside for 24 hours at room temperature. In total, 76 specimens of calcium sulfate dihydrate were included in this study.

Dissolution-Precipitation Solution

To create a 0.5 mol/L Na_2CO_3 solution, 13.25 grams of Na_2CO_3 powder was mixed with 250 mL of distilled water and stirred until the powder has dissolved. To make the 0.5 mol/L Na_3PO_4 solution, 47.5 grams of Na_3PO_4 powder was mixed with 250 mL of distilled water, and then stirred on top of a magnetic stirrer until the powder has dissolved. Lastly, to create a 1 mol/L of Na_2CO_3 solution, 26.5 grams of Na_2CO_3 powder was

mixed with 250 mL of distilled water, while a 1 mol/L Na_3PO_4 solution was created by mixing 95 grams of Na_3PO_4 powder with 250 mL distilled water. All solutions were kept inside a PET bottle at room temperature.

Formation of Carbonate-apatite Blocks

The calcium sulphate dihydrate blocks were divided into 4 groups and immersed in different solutions and for different times, which were: Group 1 (dissolution-precipitation reaction in a 0.5 mol/L Na_2CO_3 + 0.5 mol/L Na_3PO_4 solution for 48 hours at 100°C), Group 2 (dissolution-precipitation reaction in a 1 mol/L Na_2CO_3 + 1 mol/L Na_3PO_4 solution for 48 hours at 100°C), Group 3 (dissolution-precipitation reaction in a 0.5 mol/L Na_2CO_3 + 0.5 mol/L Na_3PO_4 solution for 72 hours at 100°C), and Group 4 (dissolution-precipitation reaction in a 1 mol/L Na_2CO_3 + 1 mol/L Na_3PO_4 solution for 72 hours at 100°C). After the dissolution-precipitation reaction has ended, the gypsum blocks are dried using an oven (100°C) for 24 hours. Nine specimens from each group were subjected to an absorption ability test, while 1 specimen from each group was used for an FTIR analysis. The carbonate-apatite (C-Ap) reference wavenumbers were based on a previous report [13].

Characterization using ATR-FTIR Spectrometer

One specimen from each group (Groups 1 to 4) was pulverized into a powder using mortar and pestle. The powder (15-20 mg) was then put on a crystal table and leveled. Afterward, the powder's characterization is done using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with the wavelength of 800-1800 cm^{-1} to see the functional group compounds that have formed. The ATR-FTIR reference wavenumbers have been adopted from previous studies [15,16].

Diametral Tensile Strength (DTS) Test

Four specimen groups of C-Ap blocks fabricated by dissolution-precipitation method (n=9) were subjected to a diametral tensile strength test using a Universal Testing Machine (AGS-5kNX Shimadzu, Japan) with 0.5 mm/minute crosshead speed and 5kN weight.

Water Absorption Ability Test

The specimen was weighed using an analytical balance (Shimadzu AX 200, Shimadzu Corp, Kyoto, Japan) to measure its initial weight, and then immersed in a saline solution (37°C) for 24 hours. After that, the specimen was dried using a filter paper and once again weighed using an analytical balance to measure the weight after immersion (final weight). Water absorption percentage was calculated using the formula [17,18]:

$$W_a \% = \frac{W_t - W_0}{W_0} \times 100\%$$

Where, W_a = water sorption ratio; W_t = specimen weight in time (t) and W_0 = initial weight of the specimen in a dry condition.

Data Analysis

Data from the DTS test and water absorption ability test were analyzed using SPSS software (IBM Corp., Armonk, NY, USA). The normality test using the Shapiro-Wilk test showed a normal distribution of data ($p \geq 0.05$); therefore, the data were analyzed using the One-Way ANOVA statistical test followed by a Post-hoc Tamhane test. A homogeneity test using the Levene-static test showed that the data was heterogenous ($p < 0.05$).

Results

Specimen fabrication before and after conducting the dissolution-precipitation reaction showed that the specimens did not change its shape and size for every group. They still held their cylindrical shape with a diameter of 6 mm and a thickness of 3 mm (Figure 1).

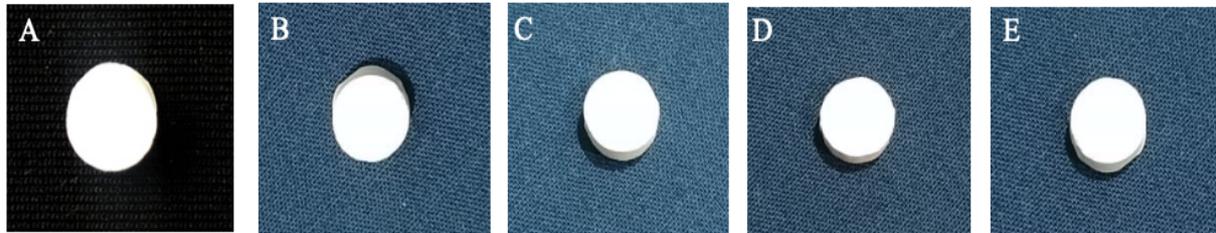


Figure 1. (A) Before dissolution-precipitation reaction; (B) After dissolution-precipitation reaction with 0.5mol/L Na_2CO_3 + 0.5mol/L Na_3PO_4 solution at 100°C for 48 hours; (C) After dissolution-precipitation with 0.5mol/L Na_2CO_3 + 0.5mol/L Na_3PO_4 solution at 100°C for 72 hours; (D) After dissolution-precipitation with 1 mol/L Na_2CO_3 + 1 mol/L Na_3PO_4 solution at 100°C for 48 hours; (E) After dissolution-precipitation with 1 mol/L Na_2CO_3 + 1 mol/L Na_3PO_4 solution at 100°C for 72 hours.

ATR-FTIR absorption pattern of the carbonate-apatite (C-Ap) blocks that were formed from calcium sulfate dihydrate by dissolution-precipitation with 0.5 mol/L Na_2CO_3 and 0.5 mol/L Na_3PO_4 solution, as well as 1 mol/L Na_2CO_3 and 1 mol/L Na_3PO_4 solution, with an immersion time of 48 and 72 hours at 100°C (Figure 2).

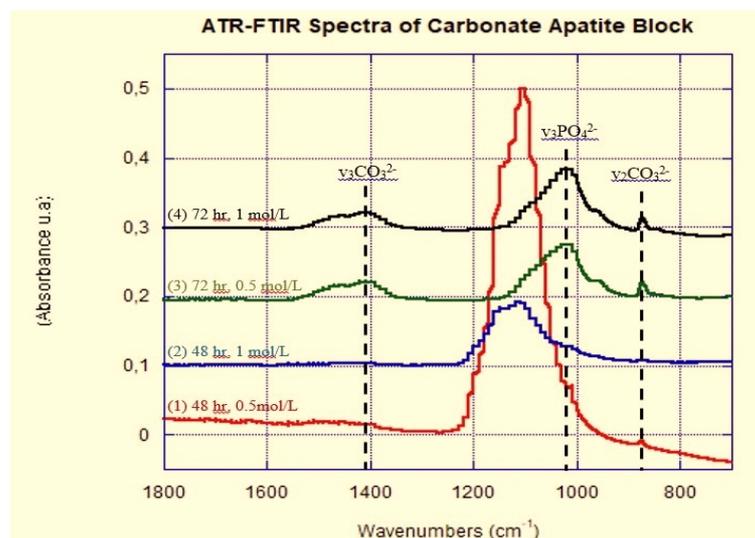


Figure 2. FTIR absorption pattern of carbonate-apatite (C-Ap) blocks created through dissolution-precipitation reaction.

ATR-FTIR wavenumbers for specimen Groups 1, 2, 3, and 4 created through dissolution-precipitation reaction for 48 and 72 hours using solutions with different molarities, as well as reference wave numbers for carbonate-apatite (C-Ap) and CaSO_4 are listed in Table 1.

Carbonate-apatite seemed to have formed in Group 3 (48 hours of dissolution-precipitation reaction with 1 mol/L Na_2CO_3 + 1 mol/L Na_3PO_4 solution), and Group 4 (72 hours of dissolution-precipitation reaction with 1 mol/L Na_2CO_3 + 1 mol/L Na_3PO_4 solution), according to the reference wavenumbers of carbonate-apatite (C-Ap) due to the formation of peaks $\text{V}_2\text{CO}_3^{2-}$, $\text{V}_3\text{PO}_4^{3-}$, and $\text{V}_3\text{CO}_3^{2-}$.

Table 1. ATR-FTIR wavenumber from carbonate-apatite block fabrication process.

Vibrational	Wavenumber				C-Ap	CaSO ₄
	ATR-FTIR					
	Group 1 (0.5 mol/L 48 hr)	Group 2 (0.5 mol/L 72 hr)	Group 3 (1 mol/L 48 hr)	Group 4 (1 mol/L 72 hr)		
V ₂ CO ₃ ²⁻ (cm ⁻¹)	873	-	872	872	873	
V ₃ PO ₄ ³⁻ (cm ⁻¹)	-	-	1020	1020	960-1200	
V ₃ CO ₃ ²⁻ (cm ⁻¹)	-	-	1417	1410	1410-1565	
CaSO ₄ (cm ⁻¹)	1104	1098	-	-		1092-1115

The tensile strength comparison of carbonate-apatite (C-Ap) blocks formed through the dissolution-precipitation method for 48 hours and 72 hours with different solution molarities was presented in Figure 3.

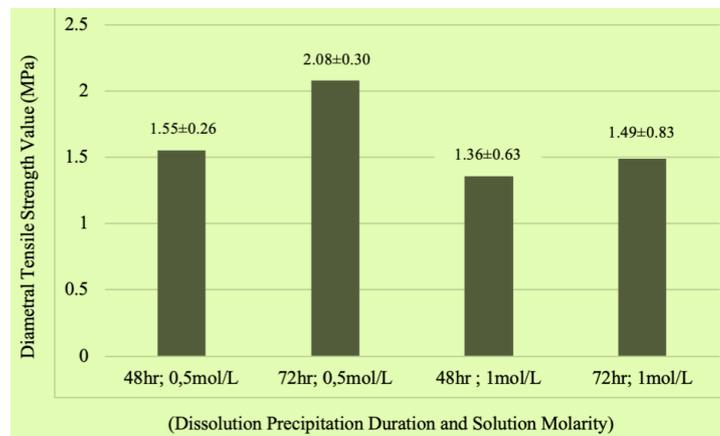


Figure 3. Diametral tensile strength (DTS) of carbonate apatite block. Mean values of DTS of carbonate-apatite blocks fabricated through dissolution-precipitation reaction.

Tukey HSD Post-Hoc test showed that DTS of carbonate-apatite blocks formed with calcium sulfate hemihydrate through a dissolution-precipitation reaction for 48 hours (0.5 mol/L) and 72 hours (0.5 mol/L) has a statistically significant difference with the 48 hours (1 mol/L) and 72 hours (1 mol/L) specimen groups.

The absorption test showed a significant difference between the four groups after the dissolution-precipitation reaction has been conducted ($p < 0.01$). Figure 4 displayed the absorption ratio for carbonate-apatite after immersion in saline solution.

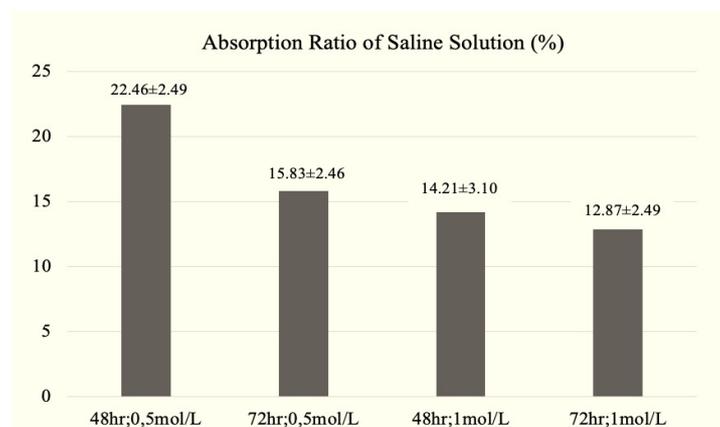


Figure 4. Absorption values of Carbonate-apatite Blocks (C-Ap) after submersion in saline solution.

Discussion

In this study, there were no changes in the shape of calcium sulfate dihydrate specimens before and after a dissolution-precipitation reaction. This indicates the precursor material, which is gypsum or calcium sulphate dihydrate, has good dimensional stability; hence the specimen shape did not change even after undergoing a dissolution-precipitation reaction during submersion [18,19].

The FTIR analysis showed that different molarities of a solution and the time of dissolution-precipitation reaction affects the formation of carbonate-apatite. Previous studies showed the functional group compound CO_3^{2-} [20]. Theoretically, a solution with higher molarity has a larger amount of reactant particles than a solution with lower molarity. The more reactant particles present will accelerate the reaction rate. The reaction plays an important part in transforming the composition that forms carbonate-apatite [21].

The present study demonstrated that the dissolution-precipitation reaction method can be successfully used to form carbonate-apatite in the gypsum specimens and is qualified to be used as a bone grafting material. The material comprised of inorganic material (6-9% weight of carbonate in crystal apatite), resembling the composition of bone. A bone graft material with a high mechanical strength value is not an ideal bone graft material since it is difficult to be reabsorbed and replaced by newly formed bone [2,14]. In a past study, one of the criteria for a bone graft material is to have a diametral tensile strength value within the range of 1-2 MPa [9]. In this study, the diametral tensile strength (DTS) of the carbonate-apatite (C-Ap) that was successfully formed. Carbonite apatite created with a more concentrated solution has a lower diametral tensile strength compared to the ones that were created with a lower concentration (0.5 mol/L). An explanation for this is because the specimens in Groups 1 and 2 that were immersed in a solution with a lower concentration still contain calcium sulphate anhydrite (CaSO_4), making it have a higher diametral tensile strength. In short, the carbonate-apatite (C-Ap) blocks that were fabricated in this study using a calcium sulfate hemihydrate precursor through a dissolution-precipitation reaction in a 1 mol/L Na_2CO_3 and 1 mol/L Na_3PO_4 solution for 48 hours and 72 hours at 100°C has an acceptable diametral tensile strength that could be used as a bone graft material.

The water adsorption ability test done in this study showed a change in adsorption values of C-Ap as the concentration (molarity) and dissolution-precipitation reaction time increases. As a result of the dissolution-precipitation reaction in the specimens of Group 1 and Group 2, the loss of water molecules in the calcium sulfate anhydrite could cause water to penetrate back into the CaSO_4 block once the two groups are immersed once again in saline solution. In Group 3 and Group 4 where C-Ap has already formed due to inducing a dissolution-precipitation reaction using a 1mol/L solution for 48 and 72 hours, the adsorption value of these two groups (14.21% and 12.87%) is lower compared to Groups 1 and 2 (22.45% and 15.83%). So far, it is not yet known whether the lower absorption ability of Groups 3 and 4 is caused by the difference in concentration (molarity) or the time length of the dissolution-precipitation reaction in this study. However, bone grafts will undergo absorption at different rates because of differences in composition, particle size, crystallinity, porosity, and fabrication methods. Ideally, the absorption rate of a bone graft material must be proportional to the new bone formation rate [22].

Reports have stated that synthetic bone grafts, such as calcium sulfate, have a reabsorption rate of 4-12 weeks. Meanwhile, coralline hydroxyapatite (contains calcium carbonate) that could be absorbed into the circulatory system of cells or tissues has a slower reabsorption rate of 6 months to 10 years. It has an ultimate compressive strength similar to trabecular bone, which is 0.22 to 10.44 MPa or a mean of 3.9 MPa (± 2.7) [23,24]. Understanding the absorption properties warrant further study.

Conclusion

Fabrication of carbonate-apatite (C-Ap) is possible using a dissolution-precipitation reaction, with a 1 mol/L Na_2CO_3 and 1 mol/L Na_3PO_4 solution for 48 and 72 hours at a temperature of 100°C. The diametral tensile strength and absorption value of the carbonate-apatite block specimens are influenced by differences in solution concentration (molarity) and the dissolution-precipitation time.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

References

- [1] Kumar P, Vinitha B, Fathima G. Bone grafts in dentistry. *J Pharm Bioallied Sci* 2013; 5(Suppl 1):125-7. <https://doi.org/10.4103/0975-7406.113312>
- [2] Cama G. Calcium Phosphate Cements For Bone Regeneration. In: Dubruel P, Vlierberghe SV (Editors). *Biomaterials for Bone Regeneration: Novel Techniques and Applications*. Burlington: Elsevier; 2014. p. 3–25. <https://doi.org/10.1533/9780857098104.1.3>
- [3] Fleet ME. *Carbonated Hydroxyapatite: Materials, Synthesis, and Applications*. Singapore: Pan Stanford Publishing Pte; 2015.
- [4] LeGeros RZ, Ben-Nissan B. Introduction to Synthetic and Biologic Apatites. In: Ben-Nissan B. *Advances in Calcium Phosphate Biomaterials*. Berlin: Springer; 2014.
- [5] Ishikawa K, Miyamoto Y, Tsuchiya A, Hayashi K, Tsuru K, Ohe G. Physical and histological comparison of hydroxyapatite, carbonate-apatite, and B-Tricalcium phosphate bone substitutes. *Materials* 2018; 11(10):1993. <https://doi.org/10.3390/ma11101993>
- [6] Ishikawa K. Bone substitute fabrication based on dissolution-precipitation reactions. *Materials* 2010; 3(2):1138-55. <https://doi.org/10.3390/ma3021138>
- [7] Nagai H, Kobayashi-Fujioka M, Fujisawa K, Ohe G, Takamaru N, Hara K, et al. Effects of low crystalline carbonate apatite on proliferation and osteoblastic differentiation of human bone marrow cells. *J Mater Sci Mater Med* 2015; 26(2):99. <https://doi.org/10.1007/s10856-015-5431-5>
- [8] Ishikawa K, Matsuya S, Lin X, Lei Z, Yuasa T, Miyamoto Y. Fabrication of low crystalline b-type carbonate apatite block from low crystalline calcite block. *J Ceram Soc Jpn* 2010; 118(1377):341-4. <https://doi.org/10.2109/jcersj2.118.341>
- [9] Nomura S, Tsuru K, Maruta M, Matsuya S, Takahashi I, Ishikawa K. Fabrication of carbonate apatite blocks from set gypsum based on dissolution-precipitation reaction in phosphate-carbonate mixed solution. *Dent Mater J* 2014; 33(2):166-72. <https://doi.org/10.4012/dmj.2013-192>
- [10] Arsista D, Eriwati YK, Triaminingsih S, Sunarso S. Fabrication of porous carbonate apatite based on the dissolution-precipitation of calcium sulfate hemihydrate. *Dentino Jur Ked Gigi* 2019; 4(2):205-9.
- [11] Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: a review. *Bioact Mater* 2017; 2(4):224-47. <https://doi.org/10.1016/j.bioactmat.2017.05.007>
- [12] Ishikawa K. Carbonate Apatite Bone Replacement. In: Antoniac IV. *Handbook of Bioceramics and Biocomposites*. Cham: Springer International Publishing; 2016. p. 213-232.

- [13] Ciardelli G, Gentile P, Chiono V, Mattioli-Belmonte M, Vozzi G, Barbani N, et al. Enzymatically crosslinked porous composite matrices for bone tissue regeneration. *J Biomed Mater Res A* 2010; 92(1):137-51. <https://doi.org/10.1002/jbm.a.32344>
- [14] Kane RJ, Roeder RK. Effects of hydroxyapatite reinforcement on the architecture and mechanical properties of freeze-dried collagen scaffolds. *J Mech Behav Biomed Mater* 2012; 7:41-9. <https://doi.org/10.1016/j.jmbbm.2011.09.010>
- [15] Ren F, Ding Y, Leng Y. Infrared spectroscopic characterization of carbonated apatite: A combined experimental and computational study. *J Biomed Mater Res A* 2014; 102(2):496-505. <https://doi.org/10.1002/jbm.a.34720>
- [16] 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. *Materials* 2017; 10(4):374. <https://doi.org/10.3390/ma10040374>
- [17] Hara K, Fujisawa K, Nagai H, Takamaru N, Ohe G, Tsuru K, et al. Fabrication and physical evaluation of gelatin-coated carbonate apatite foam. *Materials* 2016; 9(9):711. <https://doi.org/10.3390/ma9090711>
- [18] Kazemzadeh-Narbat M, Orang F, Solati-Hashjin M, Goudarzi A. Fabrication of porous hydroxyapatite-gelatin composite scaffolds for bone tissue engineering. *Iran Biomed* 2006; 10(4):215-23.
- [19] Grunenwald A, Keyser C, Sautereau AM, Crubézy E, Ludes B, Drouet C. Revisiting carbonate quantification in apatite (bio)minerals: a validated ftir methodology. *J Archaeol Sci* 2014; 49(1):134-41. <https://doi.org/10.1016/j.jas.2014.05.004>
- [20] Eriwati YK, Arsista D, Triaminingsih S, Sunarso. Effect of CaSO₄ dissolution-precipitation time on formation of porous carbonate apatite as bone replacement material. *J Biomim Biomater Biomed Eng* 2020; 44:83-90. <https://doi.org/10.4028/www.scientific.net/JBBBE.44.83>
- [21] Daitou F, Maruta M, Kawachi G, Tsuru K, Matsuya S, Terada Y, et al. Fabrication of carbonate apatite block based on internal dissolution-precipitation reaction of dicalcium phosphate and calcium carbonate. *Dent Mater J* 2010; 29(3):303-8. <https://doi.org/10.4012/dmj.2009-095>
- [22] Titsinnides S, Agrogiannis G, Karatzas T. Bone grafting materials in dentoalveolar reconstruction: a comprehensive review. *Jpn Dent Sci Rev* 2019; 55(1):26-32. <https://doi.org/10.1016/j.jdsr.2018.09.003>
- [23] Roberts TT, Rosenbaum AJ. Bone grafts, bone substitutes and orthobiologics: the bridge between basic science and clinical advancements in fracture healing. *Organogenesis* 2012; 8(4):114-24. <https://doi.org/10.4161/org.23306>
- [24] Misch CE, Qu Z, Bidez MW. Mechanical properties of trabecular bone in the human mandible: implications for dental implant treatment planning and surgical placement. *J Oral Maxillofac Surg* 1999; 57(6):700-6. [https://doi.org/10.1016/S0278-2391\(99\)90437-8](https://doi.org/10.1016/S0278-2391(99)90437-8)