Fabrication and Physicochemical Properties of a Novel Gel-Like Liquid Chitosan-Carbonated Hydroxyapatite from Asian Moon Scallop (*Amusium Pleuronectes*) for Periodontal Application

Rizki Amalina[1,2], Retno Ardhani[3], Yusril Yusuf[4], Heni Susilowati[5]*

1. Student of Doctoral Program, Faculty of Dentistry, Universitas Gadjah Mada, Indonesia.
2. Department of Oral Biology, Faculty of Dentistry, Universitas Islam Sultan Agung, Indonesia.
3. Department of Dental Biomedical Sciences, Faculty of Dentistry, Universitas Gadjah Mada, Indonesia.
4. Department of Physics, Faculty of Mathematics and Natural Science, Universitas Gadjah Mada, Indonesia.
5. Department of Oral Biology, Faculty of Dentistry, Universitas Gadjah Mada, Indonesia.

Abstract

Asian moon scallop (*Amusium pleuronectes*) is known as indigenous species and serves as an alternative good source of calcium for bone grafts. Bone graft for periodontal application need to consider the effects of microbial load in the periodontal site defect. Chitosan is added to increase its antimicrobial properties. Gel-like liquid chitosan/carbonated hydroxyapatite (CS/CHA) synthesized as a material for regenerating narrow and irregular periodontal defects.

The aim of this study was to synthesize and evaluate the physicochemical properties of gel-like liquid CS/CHA from Asian moon scallop for periodontal regeneration. CHA was synthesized using a co-precipitation method. CS/CHA gel-like liquids were fabricated using a mixing method with various CHA concentrations. The CS/CHA gel-like liquids' physicochemical properties were evaluated using Fourier-transform infrared spectroscopy (FTIR), without X-ray diffraction (XRD), pH meter, viscometer, Atomic Absorption Spectrophotometry and Scanning Electron Microscopy–Energy Dispersive X-ray spectroscopy (SEM–EDX). The results of CHA characterization showed that CHA successfully synthesized with optimal physicochemical properties.

The characterization of gel-like liquid CS/CHA confirmed that FTIR spectra showed intermolecular bonds, the highest XRD peak in the gel-like liquid is the CS/CHA 5% gel, CHA crystal spreads in the chitosan gel-like liquid, has a good injectability and the pH is neutral. In conclusion, CS/CHA 5% meets the requirements as an injectable material that can be applied for periodontal regeneration.

Experimental article (J Int Dent Med Res 2023; 16(2): 588-593)

Keywords: Asian moon scallop, CS/CHA gel-like liquid, periodontal application.

Received date: 26 February 2023 **Accept date:** 29 March 2023

Introduction

The management of periodontal disease with intrabony defects is regenerative treatment using bone grafts[1]. Bone graft is defined as an inorganic and organic synthetic biomaterial that can be used to treat bone defects[2]. Bone grafts affect the formation of new bone through the induction of host cells and create an extracellular matrix (ECM) to form the bone microenvironment[3]. The use of grafts can also act as a scaffold in the process of bone formation. The expected biological functions of bone grafts include osteogenesis, osteoinduction (growth of bone by surrounding cells recruited by the graft material), and osteoconduction (growth of bone on the surface of the material)[4].

Hydroxyapatite (HA) is the most popular bioceramic in bone tissue engineering. Most synthetic HA have high crystallinity, in contrast to bone apatite, which contains carbonates and has low crystallinity. The high crystallinity of synthetic HA makes it difficult to absorb, so the bone remodeling process does not work properly[5]. Due to this deficiency, the HA component is substituted with carbonate ions to produce carbonated hydroxyapatite (CHA). Previous studies have stated that CHA is bioactive, biocompatible, bioresorbable, supports the differentiation of bone marrow cells, and supports the growth of bone tissue[6,7]. CHA compounds are found to be more soluble in vivo than HA and can increase local concentrations of calcium and

Corresponding author:
Heni Susilowati, DDS., Ph.D.
Jl. Denta 1, Sekip Utara, Yogyakarta, Indonesia 55281;
E-mail: henisusilowati@ugm.ac.id

phosphate ions, which are important for new bone formation[8].

Indonesia imports synthetic HA at a fairly expensive price[9]. The utilization of native Indonesian clam shell biogenic materials for the synthesis of CHA as indigenous species could be an alternative and at the same time help the environment become more sustainable by reducing shellfish waste products[10]. Previous study stated that calcium carbonate from cockle shells can be formed into scaffold for bone repair[11]. Another study stated that nacre has bioactive properties and compatible for bone scaffold[12]. Asian moon scallop *(A. pleuronectes*) is a species of shellfish from the *Pectinidae* family that is commonly found in tropical seas, such as Indonesia. This clam shell contains 96.15% calcium oxide (CaO), a good source of calcium for bioceramic synthesis (Figure 1)[9].

Figure 1. Asian moon scallop from Central Java.

Chitosan is a natural cationic polysaccharide that is biocompatible. Chitosan-based biocomposite scaffolds are non-toxic, have good osteoconductivity properties, and support cell proliferation and adhesion, thereby increasing bone regeneration[10]. In a previous study, chitosan particles succesfully inhibit the growth of both periodonthopathogenic bacteria, *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans*) and *Porphyromonas gingivalis* and modulated inflammatory responses in human gingival fibroblasts[13].

Granular bone grafts used in clinical practice are difficult to fill the entire bone defect[14]. An injectable gel-like liquid bone graft is relatively easy to apply to the defect site and distributes homogeneously, so it has the potential for use in bone tissue engineering applications[15]. Gel-like liquids containing CHA can enhance bone remodeling ability by providing the required levels of phosphate and calcium in the tissue environment during new bone formation. Phosphate and calcium released into the local environment can stimulate osteoblastic proliferation[16]. A goal for periodontal tissue engineering is to restore oral soft and hard tissues functionally and aesthetic. Reproducible methods for periodontal regeneration will need to consider occlusal load/biomechanical of the newly regenerated tissues, effects of microbial load and contamination of wounds, wound stability to reconstitute the original periodontal topography, appropriate cellular signals to recruit and direct cell populations to recapitulate the tissue-regenerative response in the proper conformation, cost-effective, and provide maximal function and esthetics.[17].

Materials and methods

The ethical approval of this study was obtained from the Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta no. 0021 / KKEP / FKG-UGM / EC / 2022. The materials for synthesizing CHA were NH_4HPO_4 and $NaHCO_3$ purchased from Merck (USA). The source of calcium (CaO) was the Asian moon scallop identified in Biology Laboratory, Universitas Negeri Semarang No. 209/UN/37.1.4.5/TU/2021, as in our previous work in our lab[9]. Chitosan medium molecular weight was purchased from Sigma-Aldrich (USA). Acetic acid (100%) was purchased from Merck (Germany).

Synthesis of CHA from Asian moon scallops

CaO powder from Asian moon scallop shell harvested form central java sea was mixed with distilled water at 350 rpm at 37°C until the solution formed calcium hydroxide ($Ca(OH)_2$). Then, $(NH_4)_2HPO_4$ was mixed with distilled water at 350 rpm at 37°C. The pH was controlled by adding NH_4OH 25% into the $(NH_4)_2HPO_4$ solution while stirring until the $(NH_4)_2HPO_4$ solution was formed in the alkaline phase. NH_4HCO_3 was mixed with distilled water at 350 rpm at 37°C. Then, the NH_4HCO_3 solution was added dropwise to the $(NH_4)_2HPO_4$ solution at 37°C while stirring until the carbonate-phosphate solution was formed. The carbonate-phosphate

solution was added dropwise to the $Ca(OH)_2$ solution. The solution was stirred at 350 rpm, while the pH of the mixture was kept above 9. The solution was subjected to an aging treatment and filtered to obtain the sample precipitation. After the filtering process, each sample was purified using distilled water at 4000 rpm. The sample was dried in an oven at a temperature of 100°C until a dry CHA compound was formed. Finally, CHA was calcined at a temperature of 1000°C for 2 h.

Characterization of CHA from Asian moon scallops

The CHA was characterized by X-ray diffractometry (XRD) (PAN analytical Type X'Pert Pro, Tokyo, Japan), Fourier-transform infrared (FTIR) (Thermo Nicolet iS10, Tokyo, Japan), and scanning electron microscopy–energy dispersive X-ray (SEM–EDX) (JEOL JSM-6510LA-1400, Tokyo, Japan). XRD was used to determine the crystallinity of the CHA. The XRD pattern was recorded in the range of 2θ: 10–80° using Cu–Kα radiation at λ = 0,154 nm. The obtained crystaline phases were compared against the Joint Committee on Powder Diffraction Standards (JCPDS). FTIR was used for the identification of the functional groups of CHA. SEM was used to evaluate the morphology of the CHA particles, and EDX was used to specify the element compositions of the CHA powders.

Synthesis of CS/CHA gel-like liquid

The CS was dissolved in a 1% (v/v) acetic acid solution at a concentration of 2% (w/v) and stirred at ambient temperature for 24 h. The CHA powder was dispersed in distilled water for 1 h. The amount of CHA powder varied at 0, 2.5, 5, and 7.5 wt%. The CHA solution and CS solution were then mixed at a ratio of 80:20 (v/v). The solution was sonicated for 1 h to obtain a homogenous, well-dispersed solution and to remove bubbles from the solution. The mixed solution was stirred at ambient temperature for the next 24 h. The pH of CS/CHA gel-like liquid was neutralized using 25% β-glycerophosphate (β-GP).

Characterization of CS/CHA gel-like liquids

The CHA/CS gel-like liquid was characterized by XRD, FTIR, and SEM–EDX. XRD was used to determine the crystallinity of the CHA in the gel-like liquid CS/CHA. The XRD pattern was recorded in the range of 2θ: 10–80° using Cu–Kα radiation at λ = 0.154 nm. The obtained crystalline phases were compared against the JCPDS.

FTIR was used for the identification of functional groups of CS/CHA gel-like liquid. SEM was used to evaluate the spread of CHA in chitosan gel-like liquids, and EDX was used to specify the element compositions of the CHA. Gel-like liquid pH evaluation was held three times using a pH meter. A kinematic viscosity test was carried out using a viscometer ASTM D 445 (Stanhope-Seta, UK).

The calcium content in CHA/CS gel-like liquids was characterized by Atomic Absorption Spectrophotometry. Qualitative injectability testing of gel evaluated the flow rates from syringes 22 G and 23 G.

Results

Physicochemical properties of CHA

Based on the XRD analysis (Figure 2), the pattern formed was CHA. CHA characteristic peaks were identified (JCPDS No. 19-0272), respectively. They formed three characteristic peaks at diffraction angles of 31.6°, 32.8°, and 33.96° with diffraction planes (112), (300), and (202), respectively. Lattice parameter was *a* 9.77226 Å and *c* 5.462 Å. The microstrain was 0.00756, crystallinity degree was 86%, and particle size was 16.65 ± 0.02 nm.

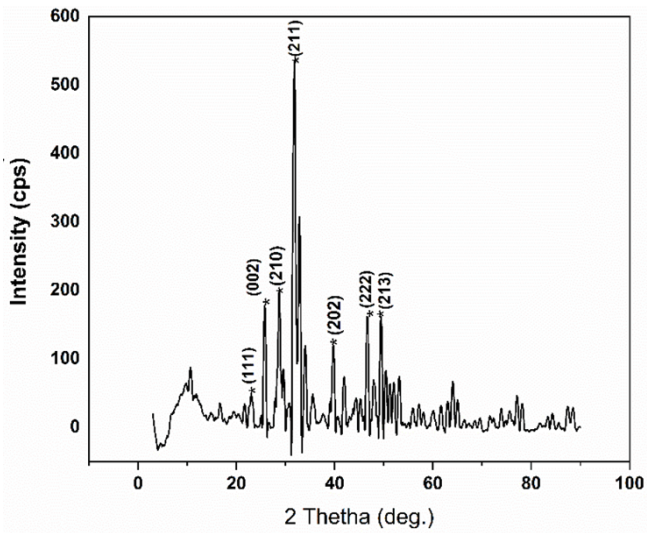

Figure 2. Diffractogram of CHA from the Asian moon scallop.

The SEM image magnification 5000 showed that CHA crystal morphology is hexagonal-shaped and has uniform size (Figure

3). CHA particle size was evaluated using image-J applications, obtained ±0.5µm. EDX analysis showed a carbon content of 4,35% and a Ca:P ratio of 1,7.

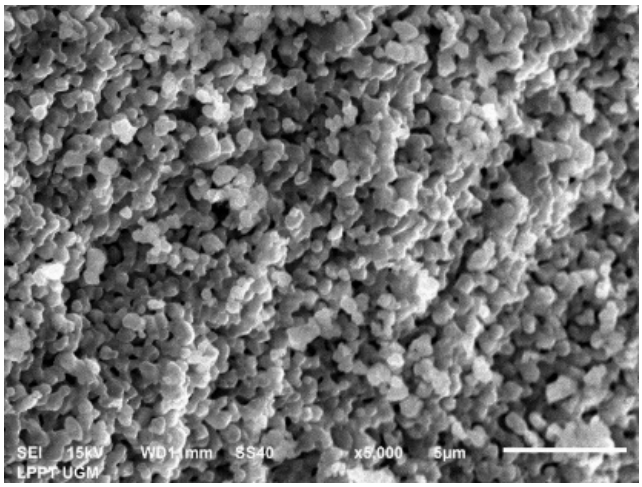

Figure 3. SEM image of CHA Asian moon scallop.

The FTIR spectrum of the CHA Asian moon scallop showed that CO_3^{2-} functional group was identified at 1455 cm^{-1}, which indicated that the carbonate ion was substituted into the HA lattice structure and supported the EDX data. The functional group of PO_4^{3-} stretching was identified at 1023 cm^{-1}. H_2O absorption and OH^- mode were observed at 3571 cm^{-1} (Figure 4).

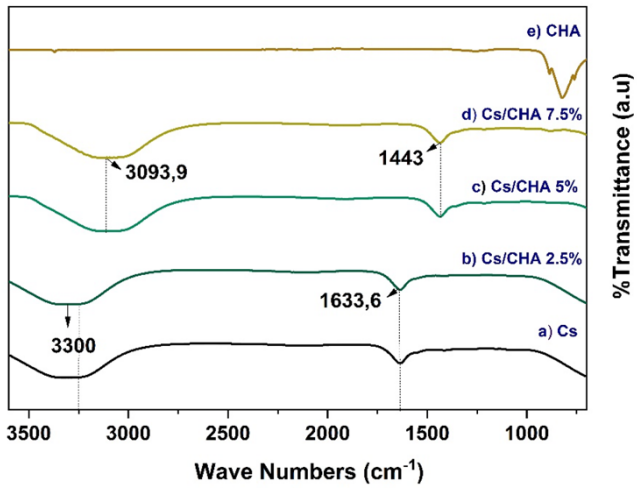

Figure 4. FTIR spectra of various groups.

Physicochemical properties of CS/CHA gel-like liquids

Homogeneous gel-like liquid CS/CHA was successfully obtained. The gel was divided into four groups: CS, CS/CHA 2,5%, CS/CHA 5%, and dan CS/CHA 7,5%. After 24h, CS/CHA 7,5% showed precipitation problem.

Group	Kinematic viscosity (mm^2/s)	Calcium content (ppm)	pH before β-GP addition	pH after β-GP addition	Ca/P ratio
CS/CHA 2,5%	97,79	1.203,89±11,18	5,47	6.83	0,114
CS/CHA 5%	81,19	1000,05±19,43	5,5	6,9	0,113
CS/CHA 7,5%	132,2	1.266,377±19,34	5,55	6.98	0,285

Table 1. CS/CHA gel viscosity, calcium content, pH and Ca/P ratio.

FTIR spectra analysis of CS/CHA gel showed functional groups of PO_4^{3-}, OH^-, and CO_3^{2-}. At CS/CHA, 5% and 7,5% have similar spectra, while CS and CS/CHA 2,5% have similar spectra (Figure 4). The pH test for all samples (CS gel, CS/CHA gel, and CS/CHA gel after β-GP addition) showed that the pH is ±4,4, ±5,47–5,55, and 6,8–7,18, respectively. The kinematic viscosity result showed the highest viscosity in CS/CHA 7,5% gel. Calcium content evaluation showed the highest result in the CS/CHA 7,5% (Table 1).

Qualitative injectability testing showed that all groups of gel can flow from syringe 22G and 23G (Table 2). The SEM images magnification 3000x revealed that the surface of the chitosan matrix was covered by spherical CHA particles (Figure 5). Ca/P ratio from EDX analysis showed at the table 1. XRD analysis showed that the addition of CHA to CS gel affects the physical properties of CHA crystals. Based on the data, CS/CHA 5% gel has the highest crystallinity compared to other groups (Figure 6).

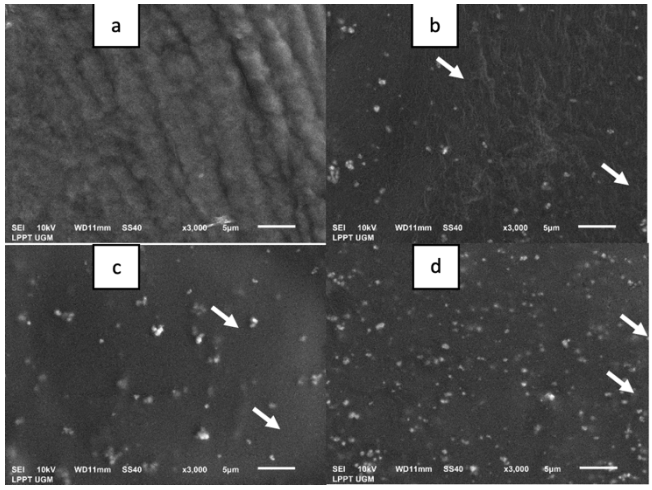

Figure 5. (a) SEM image of CS gel, (b) SEM image of CS/CHA 2,5% gel, (c) SEM image of

CS/CHA 5% gel and (d) SEM image of CS/CHA 7,5% gel.

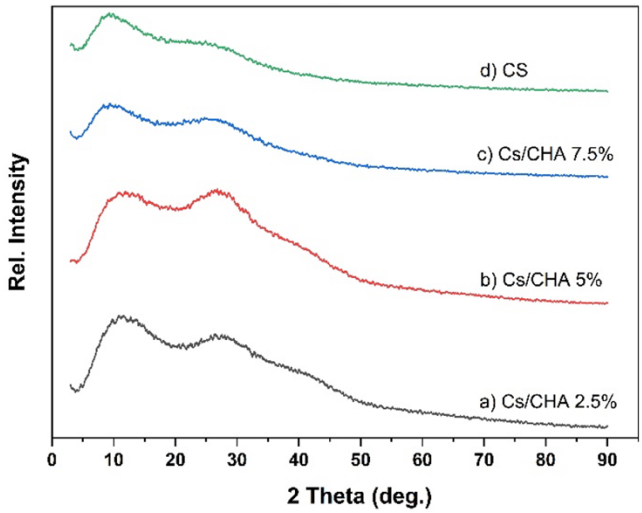

Figure 6. XRD Spectra of CS/CHA Gel.

Sample	Syringe needle sizes	
	22G	23G
CS CHA 2,5%	**	**
CS CHA 5%	***	***
CS CHA 7,5%	***	***

Table 2. Injectability rating of different concentrations of CHA in the CS/CHA gel.
****a good drop flow,
***a drop flow with slight pressure,
** a drop flow with moderate pressure,
* a drop flow with high pressure.

Discussion

Based on the CHA characterization data, the composition of the carbonate ion elements in CHA met the target of carbonate ions in natural bone[18]. Related to the composition of carbonate elements in the sample, the composition of carbonate ions in natural bones ranged from 2 to 8 wt.%[19]. Its Ca : P ratio is also similar to the natural bone ratio 1,73[20]. The chitosan matrix showed up in the SEM image had some particulates on the surface which is considered due to sodium salts from neutralising agent. Its particulates is nano spherical CHA particles which are considered to provide active surface properties, similar to previous study[21]. Nanostructured morphology of CHA powder is important as it may improve osteoblast adhesion. Previous study stated that calcium particles in the matrix improved osteoblastic cell differentiation[22]. The XRD peak around 32° corresponds to the plane (112), which overlapped with the peak of the plane (211) as a typical peak of carbonated hydroxyapatite[19].

This study shows that all characteristic functional groups of CHA have been formed. FTIR spectra confirmed that the carbonate ion has been substituted into the HA lattice structure, similar to previous research[23]. The presence of CHA from Asian moon scallop as a bioceramic material can play an important role in the regeneration of bone defect[18].

Gel-like liquid formulation for oral cavity application requires specific characteristics, including neutral pH, provide ideal conditions and to be physiologically acceptable as the biomaterials used in tissue regeneration. Neutral pH is needed to avoid irritating the oral cavity and to determine the safety of the gel-like liquid[22,24]. The addition of β-GP into CS/CHA gel-like liquid works as a physical crosslinking agent and neutralizes the pH[25]. From SEM analysis, the presence of spherical particles indicates that the CHA has been well homogenized in the gel[26]. Based on FTIR data, CS/CHA 5% and 7,5% gel showed CHA functional group spectra and both gels showed similar spectra. The interaction of chitosan and CHA showed the absence of a new peak. It can be seen that there were no residual compounds or polymers resulting from chemical interactions between chitosan and CHA components. This indicates that CHA did not change the structure of chitosan and can be combined into synergistic material[27].

Based on XRD data, CS/CHA 5% gel has sufficient crystallinity. Increased CHA concentration leads to increased kinematic viscosity. Qualitative injectability test showed that CS/CHA gel 5% had a good drop flow with slight pressure. The CS/CHA gel-like liquid is found to be suitable for injectability in bone regeneration applications (dental applications are 16–25 G). Injectable gel is recommended for periodontal application since they offer minimal invasive straight forward injection[21].

CS/CHA injectable gel-like liquid synthesized from Asian moon scallop was successfully obtained using the simple mixing method with CS:CHA composition 80:20. CS-based composites can be used as a local drug delivery system and have a good characteristic[28]. It is suitable to treat infected bone defects using biodegradable bone graft combined with an antimicrobial agent, such as chitosan[29].

Conclusions

The combination of chitosan/carbonated hydroxyapatite from Asian moon scallops is synergistic. Based on the physicochemical properties, pH and injectability test, CS/CHA 5% gel meets the requirements as an injectable material that can be applied for periodontal regeneration. However, further study is still needed to investigate its biocompatibility and biological properties in vitro and in vivo.

Acknowledgments

The authors would like to thank DIKTI for providing support through PDD grant no. 1880/UN1/DITLIT/Dit-Lit/PT.01.03/2022.

Declaration of Interest

The authors report no conflicts of interest.

References

1. Sanz M, Herrera D, Kebschull M, et al. Treatment of stage I–III periodontitis—The EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2020;47(S22):4-60. doi:10.1111/jcpe.13290
2. Kheirallah M, Almeshaly H. Present Strategies for Critical Bone Defects Regeneration Oral Health Case Reports Present Strategies for Critical Bone Defects Regeneration. *Oral Heal Case Rep*. 2016;2(3). doi:10.4172/2471-8726.1000127
3. Yang T, Xie P, Wu Z, et al. The Injectable Woven Bone-Like Hydrogel to Perform Alveolar Ridge Preservation with Adapted Remodeling Performance After Tooth Extraction. *Front Bioeng Biotechnol*. 2020;8(February):1-15. doi:10.3389/fbioe.2020.00119
4. Shimauchi H, Nemoto E, Ishihata H, Shimomura M. Possible functional scaffolds for periodontal regeneration. *Jpn Dent Sci Rev*. 2013;49(4):118-130. doi:10.1016/j.jdsr.2013.05.001
5. Dewi AH, Ana ID. The use of hydroxyapatite bone substitute grafting for alveolar ridge preservation, sinus augmentation, and periodontal bone defect: A systematic review. *Heliyon*. 2018;4(10):e00884. doi:10.1016/j.heliyon.2018.e00884
6. Januariyasa IK, Yusuf Y. Porous carbonated hydroxyapatite-based scaffold using simple gas foaming method. *J Asian Ceram Soc*. 2020;8(3):634-641. doi:10.1080/21870764.2020.1770938
7. Rahyussalim AJ, Supriadi S, Marsetio AF, Pribadi PM, Suharno B. The potential of carbonate apatite as an alternative bone substitute material. *Med J Indones*. 2019;28(1):92-97.
8. Juhasz JA, Best SM, Bonfield W. Preparation of novel bioactive nano-calcium phosphate — hydrogel composites. *Sci Technol Adv Mater*. 2010;11(1):1-7. doi:10.1088/1468-6996/11/1/014103
9. Syafaat FY, Yusuf Y. Influence of Ca/P Concentration on Hydroxyapatite (HAp) from Asian Moon Scallop Shell (Amusium pleuronectes). *Int J Nanoelectron Mater*. 2019;12(3):357-362.
10. Aguilar A, Zein N, Harmouch E, et al. Application of Chitosan in Bone and Dental Engineering. *Molecules*. 2019;24:1-17. doi:10.1201/9780429265297
11. Mahmood SK, Zakaria MZAB, Razak ISBA, et al. Preparation and characterization of cockle shell aragonite nanocomposite porous 3D scaffolds for bone repair. *Biochem Biophys Reports*. 2017;10(April):237-251. doi:10.1016/j.bbrep.2017.04.008
12. De Almeida AC, Da Silva ARP, Filho AN, De Carvalho MD, Cardoso AV. Nacre compared to aragonite as a bone substitute: Evaluation of bioactivity and biocompatibility. *Mater Res*. 2015;18(2):395-403. doi:10.1590/1516-1439.339614
13. Lee BS, Lee CC, Wang YP, et al. Controlled-release of tetracycline and lovastatin by poly(D,L-lactide-co-glycolide acid)-chitosan nanoparticles enhances periodontal regeneration in dogs. *Int J Nanomedicine*. 2016;11:285-297. doi:10.2147/IJN.S94270
14. Kaneko A, Marukawa E, Harada H. Hydroxyapatite Nanoparticles as Injectable Bone Substitute Material in a Vertical Bone Augmentation Model. *In Vivo (Brooklyn)*. 2020;34:1053-1061. doi:10.21873/invivo.11875
15. Ren B, Chen X, Du S, et al. Injectable polysaccharide hydrogel embedded with hydroxyapatite and calcium carbonate for drug delivery and bone tissue engineering. *Int J Biol Macromol*. 2018;118(June):1257-1266. doi:10.1016/j.ijbiomac.2018.06.200
16. Alhasyimi AA, Suparwitri S, Christnawati C. Effect of Carbonate Apatite Hydrogel-Advanced Platelet-Rich Fibrin Injection on Osteoblastogenesis during Orthodontic Relapse in Rabbits. *Eur J Dent*. Published online 2020:1-8. doi:10.1055/s-0040-1721234
17. Larsson L, Decker AM, Nibali L, Pilipchuk SP, Berglundh T, Giannobile W V. Regenerative Medicine for Periodontal and Peri-implant Diseases. *J Dent Res*. 2016;95(3):255-266. doi:10.1177/0022034515618887
18. N J, SM L, MG M. Hydrothermal synthesis and characterization of cellulose-carbonated hydroxyapatite nanocomposites in NaOH-urea aqueous solution. *Sci Adv Mater*. 2010;2:210–214.
19. Landi E, Celotti G, Logroscino G, Tampieri A. Carbonated hydroxyapatite as bone substitute. *J Eur Ceram Soc*. 2003;3:2931–2937.
20. Sari M, Hening P, Chotimah, Ana ID, Yusuf Y. Porous structure of bioceramics carbonated hydroxyapatite-based honeycomb scaffold for bone tissue engineering. *Mater Today Commun*. 2021;26(January):102135. doi:10.1016/j.mtcomm.2021.102135
21. Kocak FZ, Talari ACS, Yar M, Rehman IU. In-situ forming pH and thermosensitive injectable hydrogels to stimulate angiogenesis: Potential candidates for fast bone regeneration applications. *Int J Mol Sci*. 2020;21(5). doi:10.3390/ijms21051633
22. Latifi SM, Tang C, Donahue HJ. Fabrication and Characterization of Chitosan Based Injectable Thermosensitive Hydrogels Containing Silica/Calcium Phosphate Nanocomposite Particles. *J Biomater Nanobiotechnol*. 2021;12(03):34-48. doi:10.4236/jbnb.2021.123004
23. Permatasari HA, 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;11:1-9. doi:10.1177/18479804211032851
24. Anindyajati TP, Lastianny SP, Yogianti F, Murdiastuti K. Effect of collagen-chitosan hydrogel formula combined with platelet-rich plasma (A study of ph, viscocity, and swelling test). *Maj Kedokt Gigi Indones*. 2020;6(3):123-129.
25. Yan XZ, Van Den Beucken JJJP, Cai X, Yu N, Jansen JA, Yang F. Periodontal tissue regeneration using enzymatically solidified chitosan hydrogels with or without cell loading. *Tissue Eng - Part A*. 2015;21(5-6):1066-1076. doi:10.1089/ten.tea.2014.0319
26. Nisa A, Sari M, Yusuf Y. Fabrication and characterization of HA-oyster shell based on biopolymer - propolis as an agent of dental enamel remineralization material. *Mater Res Express*. 2022;9(11). doi:10.1088/2053-1591/aca31c
27. Herawati D, Babgei AM, Anwaristi AY, Supriatno. Synergistic Test of Chitosan-Ozonated Olive Oil and Chitosan-Coenzyme Q10 as. *J Int Dent Med Res*. 2022;15(1):7-14.
28. Haugen HJ, Basu P, Sukul M, Mano JF, Reseland JE. Injectable biomaterials for dental tissue regeneration. *Int J Mol Sci*. 2020;21(10). doi:10.3390/ijms21103442
29. Wang D, Liu Y, Liu Y, et al. A dual functional bone-defect-filling material with sequential antibacterial and osteoinductive properties for infected bone defect repair. *J Biomed Mater Res - Part A*. 2019;107(10):2360-2370. doi:10.1002/jbm.a.36744.