Conservative Dentistry Journal Vol. 13 No. 1 January-June 2023; 24-28

Research Report

The effect addition of epigallocatechin-3-gallate (EGCG) in nano hydroxyapatite on surface porosity as a candidate pulp capping material

Kun Ismiyatin[1], Nanik Zubaidah[1], Indira Moza Azzaria[2]
[1]Departement of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: *Deep caries, cavity preparation and use of burs or other dental instruments often result in pulpal perforation. In the case of an exposed pulp, regenerative pulp tissue treatment aims to regenerate normal tissue and maintain pulp vitality by inducing pulp regeneration using the right material, so that a good percentage and size of material porosity is needed to help cell regeneration by supporting cell proliferation and attachment, stimulating remineralization and differentiation of odontoblast-like cells. Nano-HA has the ability to produce dentine bridges continuously, has porous properties that allow cell growth, improves mechanical properties, but is not anti-inflammatory so that EGCG is added which has the advantage of being an antioxidant, anti-inflammatory which can optimize pulp tissue regeneration and also acts as an antimicrobial by reduce the growth of bacteria in the oral cavity and can trigger the proliferation and differentiation of human dental pulp cells.* ***Purpose:*** *This study aims to prove the difference in surface porosity of nano hydroxyapatite added with EGCG compared to nano hydroxyapatite and aquadest.* ***Methods:*** *This study used a laboratory experimental study with a posttest-only control group design. The research sample consisted of 32 samples which were divided into 2 groups, the treatment group (nano HA - EGCG) and the control group (nano HA - aquadest). Each research group was subjected to freeze drying and SEM tests.* ***Results:*** *There was a significant difference in the percentage of surface porosity between the nano hydroxyapatite added with EGCG compared to nano hydroxyapatite and aquadest.on the results of the Independent T-test ($p<0.05$).* ***Conclusion:*** *The addition of EGCG to nano hydroxyapatite has a higher porosity percentage compared to nano hydroxyapatite with aquadest.*

Keywords: *dentistry; EGCG; nano hydroxyapatite; porosity; SEM*

Correspondence: Kun Ismiyatin, Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga. Jl. Mayjend. Prof. Dr. Moestopo No. 47, Surabaya 60132, Indonesia. E-mail: kun-is@fkg.unair.ac.id

INTRODUCTION

Pulp tissue is a connective tissue that contains nerve fibers and blood vessels, which play a role in dentinogenetic and also protect teeth.[1,2] Deep dental caries preparation, tooth trauma, and iatrogenic reasons are some of the causes of exposure of the dental pulp which can lead to loss of pulp vitality. In cases of exposed pulp, regenerative pulp tissue treatment is carried out to maintain pulp vitality through pulp capping treatment, regenerating exposed pulp by stimulating the formation of reparative dentin.[3] Maintaining pulp vitality is important because it maintains homeostasis, durability and helps to save many teeth due to fractures due to failed endodontic treatment. One of the approaches in the treatment of pulp dentine complex regeneration is vital pulp therapy. Pulp exposure can stimulate regeneration due to the presence of mesenchymal cell progenitors and mild inflammation induces dental pulp protection mechanisms.[4]

Calcium hydroxide or $Ca(OH)_2$ is the gold standard for treatment in maintaining pulp vitality with strong antibacterial properties that can induce the formation of dentine bridges but has some disadvantages with the formation of tunnel defects which allow bacteria to enter the pulp tissue.[5,6] Based on research conducted by Gandolfi et al., 2015, calcium hydroxide as the gold standard shows a porosity value of 7.49%-9.04%.[7] Mineral Trioxide Aggregate (MTA) shows a porosity range between 39.3-49.47%, and porosity of bioceramic cement made from calcium silicate was 22.9%. MTA that have a fairly high porosity are associated with a large enough amount of Ca release to encourage ion exchange. The higher the porosity of the material, the easier and more abundant the release of ions will occur.[7]

In recent years, nanotechnology has begun to develop rapidly and be applied in the field of dentistry, where nanoparticles have material properties that are influenced by particle size, porosity and increased surface area

DOI: https://doi.org/10.20473/cdj.v13i1.2023.24-28

which will affect interactions with other materials so as to enable an increase in the strength of a material.[8,9] Nano hydroxyapatite with a chemical structure of $Ca_{10}(PO_4)_6(OH)_2$ has hydroxyapatite crystal sizes ranging from 20-50 nm.[10] Nano-HA has properties similar to bone tissue in the body, is biocompatible, non-toxic and does not cause inflammation,[11] and the size of the nanoparticle significantly increases the surface area so that it has many sites for protein binding.[12] The use of nano-HA synthesized from egg shells was chosen because it has good mechanical properties with high osteoinductivity and osteoconductivity so that it can increase the ability to form dentin bridges.[13]

Several natural ingredients are proposed as alternative ingredients in pulp regenerative treatment, EGCG or catechins from green tea will be added as a combination ingredient because they have advantages in the health sector, such as good antioxidants, anti-inflammatory which can optimize pulp tissue regeneration and also acts as an antimicrobial by reduce the growth of bacteria in the oral cavity and can trigger the proliferation and differentiation of human dental pulp cells.[14,15] The addition of EGCG can improve the physical properties of materials such as porosity. In addition, the addition of EGCG was shown to make the nano-HA surface morphology smoother and flatter.[21] This has an impact on reducing inflammation that occurs because on a rough surface, macrophages will spread more easily so that they can d ecrease inflammation. While the addition of nano-HA is proven to increase the compressive strength and shear bond strength so that the combination of nano-HA and EGCG is also expected to provide advantages in mechanical properties.[11]

In tissue regeneration, a good material must have biocompatibility, mechanical and physical properties that are important for material strength.[4] One of the physical properties of tissue regeneration treatment materials is the porosity of the material.[16] Porosity is the pore size on the surface of the material examined using the SEM tool. Pores are needed for the formation of body tissues because they can help the migration and proliferation of body cells.[17] Porosity properties are necessary for bone cell regeneration, increasing cell infiltration, cell migration, vascularization, transfer of nutrients and oxygen.[18] The porosity of a material also determines the amount of leakage, therapeutic yield, adsorption, permeability, strength, and density of the pulp regeneration material.[19,20] Based on the background of the problems above, a study was carried out to prove the difference in surface porosity by nano hydroxyapatite added with EGCG compared to nano hydroxyapatite with aquadest.

MATERIALS AND METHODS

The ethical clearance which was prepared as a condition for conducting research has received approval from the Research Ethics Commission of the Faculty of Dental Medicine, Airlangga University (No. 014/HRECC.FODM/I/2023). This type of research is a laboratory experiment with a post-test only control group design. The data used is primary data which is directly collected directly from research conducted by researchers.

Nano hydroxyapatite (PT. Pertiwi Parahita Technology, Bogor, Indonesia) was added as much as 0.1 gram to all samples of each group. The total number of samples used in the study were 32 samples which were divided into 2 groups, the control group was nano hydroxyapatite dissolved in aquadest (w/p) 0.1 g : 0.2 ml. While the treatment group consisted of nano hydroxyapatite samples added with EGCG solution (w/p) 0.1 g : 0.2 ml. The EGCG solution was obtained by diluting 0.12 mg of EGCG (Sigma Aldrich) powder with 100 ml of sterile aquadest to obtain a concentration of 0.012 mg/ml or 10 µmol/L.[15]

Each group consists of 16 cylindrical samples with a diameter of 5 mm and a height of 2 mm (according to American Standard Testing and Materials (ASTM) E384). The sample mold is made of square acrylic and a cylindrical hole is placed in the center of the sample mold. The sample mold has a base and cover made of square acrylic. The sample is printed into the sample mold, then pressed so that the sample surface is flat. After setting, the samples were freeze dried at 48°C for 2x24 hours.

The porosity test of the material was carried out using a Scanning Electron Microscope (SEM) with 5000x and 50000x magnification. The SEM characterization results were then analyzed using ImageJ software and the average porosity percentage value was obtained for each sample. Research data were analyzed using the SPSS (Statistical Package for Social Science) application. Data were tested for normality using the Shapiro-Wilk test and homogeneity was tested using the Levene's test. Data that is normally distributed and homogeneous, then tested for differences in data variance using an independent T-test.

RESULTS

The results of the surface porosity test for nano hydroxyapatite and EGCG using SEM and analyzed with ImageJ software (Table 1) showed that the treatment group had a higher average result than the control group. Surface porosity values of nano hydroxyapatite and EGCG were then tested

Table 1. The mean and standard deviation of surface porosity in all groups

Groups	N	Surface Porosity (%) $\bar{x} \pm SD$	P
Nano-HA + aq	16	29.05 ± 5.15	0.000*
Nano-HA + EGCG	16	48.41 ± 3.95	

Note: * significant surface porosity at α=0.05 (Independent T-test)

for normality using Shapiro-Wilk. The results of the surface porosity normality test for nano hydroxyapatite and EGCG were normally distributed ($p>0.05$). The results of the homogeneity test with Levene's test showed that the data was homogeneous ($p>0.05$). Furthermore, the data was tested using the Independent T-test. Independent T-test for surface porosity of nano hydroxyapatite and EGCG (Table 1) showed that there was a significant difference between groups ($p<0.05$).

The SEM image results show the particle morphology in the control sample group with a magnification of 5,000x (Figure 1a) and in the treatment sample group with a magnification of 5,000x (Figure 2a). It was found that the surface of the samples in the control and treatment groups had unequal (irregular) particle shapes, with rounded edges (spherical-shape). In the control group the distance between the particles is rather tenuous with a rough and lumpy surface. Observations were also made on sample pore size and obtained different results between the two study sample groups. The results of observing the pore size in the treatment sample group had a more increased pore size compared to the control sample group. The pore size range obtained from the control sample group (nanoHA-aquadest) was between 0.15-3.62 µm with an average of 1.67 µm (Figure 1b). Whereas in the treatment sample group (nanoHA-EGCG) the pore size range was between 0.25-4.58 µm with an average of 2.31 µm (Figure 2b). The pore size results that can be found in both sample

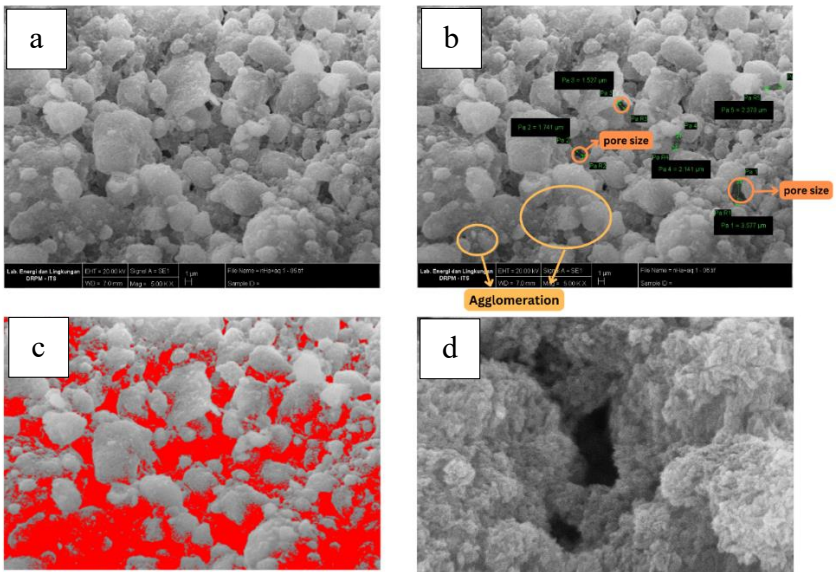

Figure 1. The surface and pore morphology of the sample using SEM (a) nanoHA-aquadest 5000x magnification; (b) Measurement pore size nanoHA-aquadest; (c) nanoHA-aquadest porosity measurement; (d) nanoHA-aquadest 50000x magnification.

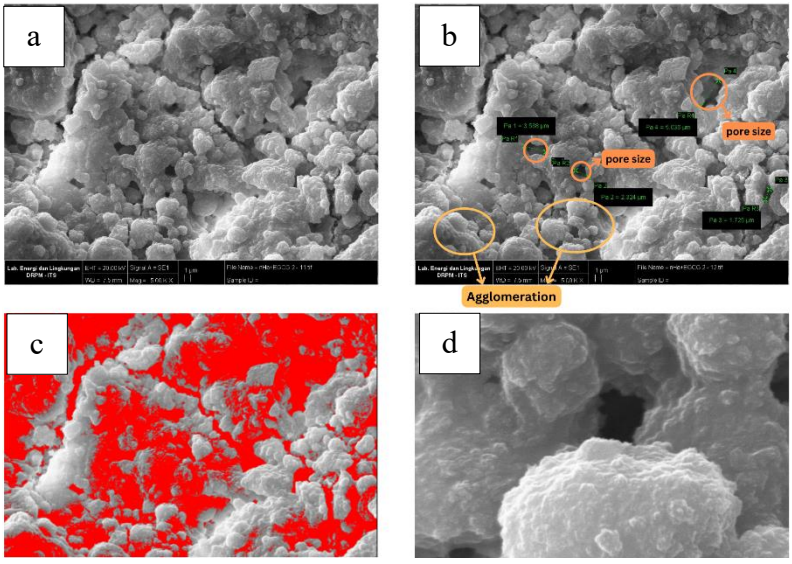

Figure 2. The surface and pore morphology of the sample using SEM (a) nanoHA-EGCG 5000x magnification; (b) Measurement pore size nanoHA-EGCG; (c) nanoHA-EGCG porosity measurement; (d) nanoHA-EGCG 50000x magnification.

DOI: https://doi.org/10.20473/cdj.v13i1.2023.24-28

groups belong to macropores, namely pores with a size of > 50 nm. Meanwhile, the pore size in the microporous or mesoporous groups was not observed. This can occur due to several reasons, one of which is the limited microstructure observation using SEM tools. The use of SEM tools in observing the microstructure can show the surface of porous materials, but to see very small pore sizes with a clearer picture this tool still cannot give good results. Equipment with higher magnification capabilities such as Field Emission Scanning Electron Microscopy (FE-SEM) or Transmission Electron Microscopy (TEM) with magnification capabilities reaching.[22]

Porosity measurements were analyzed using ImageJ software from the results of SEM images, results in both groups showed different porosity percentage values, measurements in the control group (Figure 1c), surface porosity measurements of nano-Ha added with EGCG (Figure 2c). As for the description of the SEM results in the treatment group, it can be seen that the distance between particles is somewhat denser than in the control sample group. At 50,000x magnification (Figure 2d) it appears that the surface of the nano-HA mixed with EGCG has a network of interconnected pores and a smoother surface compared to the control sample group (Figure 1d) the surface appears slightly rough, and there is accumulation or agglomeration on the both groups but not as much as in the control sample group.

DISCUSSION

In the development of pulp tissue regeneration, the use of nano-hydroxyapatite in medical applications has been modified by cross-linking with other materials as in this study the addition of EGCG was carried out to increase the proliferation and differentiation of pulp-dentin cells thereby accelerating the process of tissue regeneration. Observations were made using SEM to obtain pore size results and surface percentage of regenerated pulp material. The results showed that the sample adding EGCG to nano HA affected the resulting porosity with a higher percentage of porosity than the control group. This is due to the release of Ca which is quite large and encourages ion exchange so that strong intermolecular bonds are formed and the internal network of water-filled pores provides a larger surface area.[7] The smaller the particle size, the greater the surface area, so that the bond between hydroxyapatite and the surrounding tissue when applied will also increase.[23] The addition of EGCG to nano HA causes an increase in cellular behavior and pore interconnectivity so that the resulting pore size increases. The interconnected pores are essential for transporting nutrients and oxygen to cells.[11]

The strength of intermolecular forces has a considerable influence on the properties of a compound, in this study, namely porosity. Intermolecular forces are the attractive forces of attraction between molecules with one another. The stronger the intermolecular forces of a compound, the stronger the intermolecular attractions so that the viscosity also increases.[24] This is in accordance with research, where the addition of EGCG to nano hydroxyapatite has a high viscosity. EGCG has the chemical formula $C_{22}H_{18}O_{11}$ and nano hydroxyapatite has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Both of these compounds have a large relative mass so they have many electrons and tend to form dipole. High polarizability causes van der Waals forces. In addition, the two compounds also have hydrogen bonds which are the strongest intermolecular forces so that the viscosity is also large, the viscosity of the solution becomes thick and causes low porosity.

EGCG contains phenolic acids in the form of gallic acid which is an acidic compound and nano hydroxyapatite which is a basic compound so that the combination of the two will produce an acid-base reaction.[25] Increasing the EGCG ratio will cause more gallic acid in EGCG to bind to nano hydroxyapatite. More acid-base reactions cause more salt and water products so that the viscosity decreases. Decreasing viscosity will increase the percentage of porosity of a material because the porous surface is filled with salt and water products resulting from acid-base reactions. In addition, the porosity percentage results from the addition of EGCG to nano hydroxyapatite show to be in the same range as MTA material so that this material can potentially be a good candidate for pulp capping.

In addition to having biocompatible properties and having the ability to induce the formation of reparative dentin, pulp capping has other requirements both physically and mechanically. The mechanical properties of the pulp capping can affect its resistance to fracture during placement of the final restorative material or when supporting the overlying restoration over time.[26] Mechanical properties include tensile strength, compressive strength, shear strength and flexural strength. One of the mechanical properties that must be possessed is compressive strength.[27] There are many factors that affect the compressive strength of a material, namely porosity, solubility, water content, and particle size, as well as acid-base reactions. The more water content, the porosity will increase so that the compressive strength will decrease.

However, in this study no observation was made of the effect of increasing porosity on mechanical properties such as the amount of compressive strength, so the results cannot be known. But there is a possibility that the addition of EGCG to nano hydroxyapatite has an increased compressive strength value like that of MTA. This is because the addition of EGCG is proven to cause the surface morphology of nano-HA to become smoother and spread evenly. This affects the decrease in inflammation that occurs because on a rough surface, macrophages will be easier to spread so that it can decrease inflammation. While the addition of nano-HA is proven to increase compressive strength and shear-bond strength so that the combination of nano-HA and EGCG is also expected to provide advantages in mechanical properties.[11]

This study proves that the effect of nano hydroxyapatite added with EGCG can cause an increase in the percentage of surface porosity of the material for pulp regeneration.

DOI: https://doi.org/10.20473/cdj.v13i1.2023.24-28

The surface porosity of the nano hydroxyapatite added with EGCG was higher than that of nano hydroxyapatite and aquadest. Further studies about nano hydroxyapatite added with EGCG materials should be performed to explore the potential in dental pulp tissue regeneration treatment and it can support pulp vital therapy treatment in clinical application.

REFERENCES

1. Sharma, L. A., & Love, R. M. Scaffolds for regeneration of the pulp-dentine complex. In Handbook of Tissue Engineering Scaffolds, 2019; 1: 459–78. Elsevier.
2. Tjäderhane, L., & Paju, S. Dentin-Pulp and Periodontal Anatomy and Physiology. In Essential Endodontology: Prevention and Treatment of Apical Periodontitis, Third Edition, 2019: 11–23.
3. Doumani, M. D., Arnous, W. A., Alsafadi, M. F., Alnazer, H. A., Alanazi, S. M., Alotaibi, K. S., & Al-Ammari, A. I. The vital pulp therapy of permanent teeth: A dental practitioner's perspective from Saudi Arabia. Journal of International Society of Preventive & Community Dentistry, 2020; 10(3): 300.
4. Morotomi, T., Washio, A., & Kitamura, C. Current and future options for dental pulp therapy. The Japanese dental science review, 2019; 55(1): 5–11.
5. Swarup, S. J., Rao, A., Boaz, K., Srikant, N., & Shenoy, R. Pulpal response to nano hydroxyapatite, mineral trioxide aggregate and calcium hydroxide when used as a direct pulp capping agent: an in vivo study. The Journal of clinical pediatric dentistry, 2014; 38(3): 201–06.
6. Dwiandhono I, Effendy R, Kunarti S. The thickness of odontoblast-like cell layer after induced by propolis extract and calcium hydroxide. Dent J (Majalah Kedokt Gigi), 2016; 49(1): 17–21.
7. Gandolfi, M. G., Siboni, F., Botero, T., Bossù, M., Riccitiello, F., & Prati, C. Calcium silicate and calcium hydroxide materials for pulp capping: biointeractivity, porosity, solubility and bioactivity of current formulations. Journal of applied biomaterials & functional materials, 2015; 13(1): 43–60.
8. Akbarianrad, N., Mohammadian, F., Nazari, A. A., & Nobar, R. Applications of nanotechnology in endodontic: A Review. Nanomed. J, 2018; 5(3): 121–6.
9. Pepla, E., Besharat, L. K., Palaia, G., Tenore, G., & Migliau, G. Nano-hydroxyapatite and its applications in preventive, restorative and regenerative dentistry: a review of literature. Annali Di Stomatologia, 2014; 5(3): 108-14.
10. Septiani T, Hidayati N, Mohadi R. Calcium Oxide from Pomacea canaliculata and Babylonia spirata Snails. Sci Technol Indonesia, 2017: 68-70.
11. Chu, C., Deng, J., Man, Y., and Qu, Y. Green Tea Extracts Epigallocatechin-3-gallate for Different Treatments. BioMed Research International. Hindawi, 2017, 1-9.
12. Alipour, M., Firouzi, N., Aghazadeh, Z., Samiei, M., Montazersaheb, S., Khoshfetrat, A. B., & Aghazadeh, M. The osteogenic differentiation of human dental pulp stem cells in alginate-gelatin/Nano-hydroxyapatite microcapsules. BMC biotechnology, 2021; 21(1): 6.
13. Horta, M. K., Moura, F. J., Aguilar, M. S., Westin, C. B., Campos, J. B., Peripolli, S. B., Ramos, V. S., Navarro, M. I., & Archanjo, B. S. Nanostructured hydroxyapatite from hen´s eggshells using sucrose as a template. Materials Research, 2020; 23(6).
14. Lee, S., Razqan, G. S. al, & Kwon, D. H. Antibacterial activity of epigallocatechin-3-gallate (EGCG) and its synergism with β-lactam antibiotics sensitizing carbapenem-associated multidrug resistant clinical isolates of Acinetobacter baumannii. Phytomedicine, 2017; 24: 49–55.
15. Kwon, Y., Kim, H., & Hwang, Y. Effects of Epigallocatechin Gallate, an Antibacterial Cross-linking Agent, on Proliferation and Differentiation of Human Dental Pulp Cells Cultured in Collagen Scaffolds. Journal of Endodontics, 2017; 43(2): 289–96.
16. Al-Sherbiny, I. M., Farid, M. H., Abu-Seida, A. M., Motawea, I. T., & Bastawy, H. A. Chemico-physical and mechanical evaluation of three calcium silicate-based pulp capping materials. The Saudi dental journal, 2021; 33(4): 207–14.
17. Milla, L. El, Indrani, D.J. & Irawan, B. Sintesis dan Uji Porositas scaffold Hidroksiapatit/Alginat. Odonto Dental Journal,2018; 5: 49–3.
18. Kim, T.R., Kim, M.S., Goh, T.S., Lee, J.S., Kim, Y.H., Yoon, S.Y. & Lee, C.S. Evaluation of structural and mechanical properties of porous artificial bone scaffolds fabricated via advanced TBA-based freeze-gel casting technique. Applied Sciences (Switzerland), 2019; 9(9): 1–17.
19. Kaur, M., Singh, H., Dhillon, J. S., Batra, M., & Saini, M. MTA versus Biodentine: Review of Literature with a Comparative Analysis. Journal of clinical and diagnostic research: JCDR, 2017; 11(8): ZG01–ZG05.
20. Sarna-Boś, K., Skic, K., Sobieszczański, J., Boguta, P., & Chałas, R. Contemporary Approach to the Porosity of Dental Materials and Methods of Its Measurement. International journal of molecular sciences, 2021; 22(16): 8903.
21. Kook, Y. J., Tian, J., Jeon, Y. S., Choi, M. J., Song, J. E., Park, C. H., Reis, R. L., & Khang, G. Nature-derived epigallocatechin gallate/duck's feet collagen/hydroxyapatite composite sponges for enhanced bone tissue regeneration. Journal of biomaterials science. Polymer edition, 2018; 29(7-9): 984–96.
22. Nurdin, A. Teknologi Proses Pembuatan Molecular Sieve TiZA Untuk Pemekatan Asam Nitrat. Jurnal Kimia dan Kemasan, 2017; 39(2): 55-60.
23. Wardani, S. C., Hapsari, D. N., & Fatima, F. Perbandingan Morfologi dan Rasio Ca/P Serbuk Hidroksiapatit dari Tulang Ikan Cakalang (Katsuwonus Pelamis) dengan Hidroksiapatit Sisik Ikan. E-Prodenta Journal of Dentistry, 2020; 4(2), 314-20.
24. Brown, T. E., LeMay, H. E., Bursten, B. E., Murphy, C., Woodward, P., & Stoltzfus, M. E. 2015. Chemistry: The Central Science (13th edition). New Jersey: Pearson Education, Inc.
25. Xu, Y. Q., Yu, P., & Zhou, W. Combined effect of pH and temperature on the stability and antioxidant capacity of epigallocatechin gallate (EGCG) in aqueous system. Journal of Food Engineering, 2019; 250: 46–4.
26. Nielsen, M. J., Casey, J. A., VanderWeele, R. A., & Vandewalle, K. S. Mechanical properties of new dental pulp-capping materials. General dentistry, 2016; 64(1): 44–8.
27. Ranjbar Omrani, L., Moradi, Z., Abbasi, M., Kharazifard, M. J., & Tabatabaei, S. N. Evaluation of Compressive Strength of Several Pulp Capping Materials. Journal of dentistry (Shiraz, Iran), 2021; 22(1): 41–7.