

PACS numbers: 62.20.Qp, 62.23.Pq, 68.37.Hk, 81.07.Pr, 83.80.Ab, 87.85.jj, 87.85.Rs

Chitosan-Modified Alumina–Zirconia–Carbonate Apatite Nanoparticles-Filled Dental Restorative Composite Materials: Characterization and Mechanical Properties

Andrie Harmaji¹, Novita Dwi Saputri^{1,2}, and Bambang Sunendar^{1,3}

¹*Advanced Materials Processing Laboratory,
Institut Teknologi Bandung,
Bandung, Indonesia*

²*Department of Dentistry,
Jendral Soedirman University,
Purwokerto, Indonesia*

³*Department of Engineering Physics,
Institut Teknologi Bandung,
Bandung, Indonesia*

In the field of restorative dentistry, composite resin is quickly overtaking other materials in terms of usage. It resolves the issue of the toxic nature brought on by the amalgams' mercury content. The mechanical properties of resin-based dental restorative composites can be enhanced with the addition of filler material. This study examines the effects of chitosan addition to dental composites with alumina, zirconia, and carbonate apatite nanoparticles on their characterization and hardness. The composite for direct restoration is prepared by mixing UDMA–TEGDMA–DMAEMA–HEMA as the matrix and alumina–zirconia–carbonate apatite as the filler in the presence of chitosan as a coupling agent. The weight ratio of filler to matrix used is 70:30. The effects of various concentrations of chitosan are varied at 2%, 4%, and 6%. Samples are prepared by a synthesizing process to obtain alumina–zirconia–carbonate apatite nanoparticles. The diffractogram from XRD shows the formation of $t\text{-ZrO}_2$ and dahllite. SEM images reveals that the particle sizes of each sample with chitosan at 2%, 4%, and 6% are of 87.5 nm, 112.5 nm, and 150 nm, respectively. The hardness values of each sample with the same chitosan concentration are of 51.3 VHN, 28.24 VHN, and 25.48 VHN, respectively. Dental composites with less chitosan concentration promote a smaller size of alumina–zirconia–carbonate apatite nanoparticles and higher mechanical properties in dental restorative composites.

У сфері реставраційної стоматології композитна смола швидко обганяє

інші матеріали за рівнем використання. Це вирішує проблему токсичної природи, викликані вмістом ртуті в амальгамах. Механічні властивості стоматологічних реставраційних композитів на основі смоли можуть бути посилені додаванням наповнювача. У цьому дослідженні вивчається вплив додавання хітозану до стоматологічних композитів з наночастинками глинозему, двоокису цирконію та карбонатного апатиту на їхні характеристики та твердість. Композит для прямої реставрації готують шляхом змішування диметакрилатних мономерів UDMA-TEGDMA-DMAEMA-HEMA як матриці та глинозему-двоокису цирконію-карбонатного апатиту як наповнювача в присутності хітозану як сполучного агента. Вагове співвідношення наповнювача до використаної матриці становить 70:30. Ефекти різних концентрацій хітозану варіюються на 2%, 4% і 6%. Зразки готують за допомогою синтезувального процесу для одержання наночастинок глинозему-цирконію-карбонатного апатиту. Дифрактограма з рентгенівської дифракції показує утворення $t\text{-ZrO}_2$ та далліту. Зображення сканівної електронної мікроскопії показують, що розміри частинок кожного зразка з хітозаном на 2%, 4% і 6% становлять 87,5 нм, 112,5 нм і 150 нм відповідно. Значення твердості кожного зразка з однаковою концентрацією хітозану становлять 51,3 VHN, 28,24 VHN і 25,48 VHN (у числах твердості за Віккерсом) відповідно. Стоматологічні композити з меншою концентрацією хітозану сприяють меншому розміру наночастинок оксиду глинозему-двоокису цирконію-карбонатного апатиту та вищим механічним властивостям стоматологічних реставраційних композитів.

Key words: chitosan, alumina, zirconia, carbonate apatite, dental restorative composite.

Ключові слова: хітозан, глинозем, діоксид цирконію, карбонатний апатит, стоматологічний реставраційний композит.

(Received 9 March, 2023)

1. INTRODUCTION

Dental caries is a disease that is still a major problem for oral health caused by cariogenic bacteria, primarily *Streptococcus mutans* [1]. This is a form of tooth decay and commonly called cavities are characterized by the demineralization of hard tissue such as bone, cementum, dentin, and enamel, as well as organic matter [2]. Dental caries can cause damage to tooth structure and decrease aesthetics; therefore, restorations are needed as an effort to repair and restore tooth function and aesthetics. The restoration consists of direct and indirect methods, with the latter are expected to have better longevity than former [3]. Although there are many different types of materials used for dental restorations, amalgam and composite fillings are the most frequently used [4]. Composite resins are typically made up of Bis-

GMA and other dimethacrylate monomers (TEGMA, UDMA, and HDDMA), a filler material, and, in most cases, a photoinitiator. For a long time, amalgam was the most frequently used material; however, composite fillings are becoming more and more popular due to their attractiveness and durability [5]. Following the addition of filler material, the composite resins can be customized to match the exact shade as the dental tissue [6]. It has the advantage of having a more natural appearance than amalgam, gold, or glass ionomer. Science and technology that continues to develop rapidly has made people more selective in choosing direct restorative materials. While composite fillings are the most popular material, up until now Indonesia has not been able to process the filler raw materials; so, the price of dental restorative composites is still relatively expensive because they have to be imported from other countries, even though Indonesia is rich in natural resources that can be used as filler materials [7, 8, 9].

Alumina and zirconia has been used as fillers for dental restorative composites [10, 11]. Alumina is an oxide ceramic that has very high strength, is hard, and is resistant to high temperatures. Zirconia is an oxide ceramic that has excellent physical, mechanical, and biological properties as a dental material. Pure zirconia cannot produce good stability; therefore, alumina is used as a zirconia stabilizer. The use of alumina together with zirconia can produce stable composite materials. Carbonate apatite can also be added as a filler to improve the mechanical properties of the composite [12, 13]. It has been reported that this material can also be used for coating of ceramic dental implant [14, 15, 16]. The value of hardness can be increased because it contains calcium phosphate, a widely used material in medical applications such as bone implants because its bioactivity and biocompatibility. The combination of the three alumina-zirconia-carbonate apatite materials is expected to have the advantages of each of these material properties to produce fillers with hardness that meet standards as direct dental restorative composite materials.

The coupling agents such are needed for dental restorative composite to increase the bond between matrix and filler, since resin is an organic material and the proposed filler are inorganic material. Chitosan can be used as coupling agents. Several fields such as health and industry use the material because it has non-toxic, biocompatible and biodegradable properties. Chitosan can encase the surface of the material, so that the particle size does not increase and agglomerate. The particles can be evenly distributed leads to increase of mechanical properties.

In this research, it was examined how the hardness and characterization of dental restorative composite materials containing alumina-zirconia-carbonate apatite with the variations of chitosan concentration.

2. EXPERIMENTAL

2.1. Synthesis of Alumina–Zirconia

Synthesis of alumina and zirconia was carried out by the sol gel method. First, 9.9042 g of $ZrCl_4$ was dissolved in 425 ml of aqua dm and stirred using a magnetic stirrer for 10 minutes. Next, 10.616 grams $Al(NO_3)_3$ was added to the $ZrCl_4$ solution and stirred using a magnetic stirrer for 10 minutes. The solution is heated until a black crust appears using the oven followed by grinding with a mortar. The crust was calcinated using a furnace at $900^\circ C$ for 2 hours.

2.2. Synthesis of Carbonate Apatite

The procedure for synthesizing apatite carbonate powder is carried out in by dissolving 2.3615 g of calcium nitrate ($Ca(NO_3)_2$) in 500 ml of aqua dm for 10 minutes and mixed using a magnetic stirrer. Ammonia solution was dissolved to raise the pH to around 9–11 using a pipette. Di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$ as much as 0.7923 g was put into 100 ml of aqua dm. Sodium hydrogen carbonate ($NaHCO_3$) of 0.50406 gr was added to 100 ml of aqua dm. The $(NH_4)_2HPO_4$ solution was added to the $(Ca(NO_3)_2)$ followed by $NaHCO_3$ solution. The solution was covered using aluminium foil and left for 24 hours until a white precipitate formed. The white precipitate was centrifuged using a centrifugation tube for 15 minutes then transferred into a petri dish and then placed in the oven to form a paste or ointment. The paste is put into the combustion boat and calcined into powder using a furnace with a temperature of 700° for 2 hours.

2.3. Synthesis of Chitosan 2%, 4%, and 6% Solution

Synthesis of chitosan solution begins with combining 2 ml of acetic acid with 98 ml of aqua dm while stirring using a magnetic stirrer. Chitosan powder of 2 grams, 4 grams and 6 grams was dissolved in each measuring cup until homogeneous to produce chitosan 2%, 4%, and 6%, respectively.

2.4. Synthesis of Dental Restorative Composites

The dental restorative composites are consisted of matrix and filler. The matrix consisted of 17% UDMA, 6% TEGDMA, 5% DMAEMA, 10% HEMA, and 2% Champorquinone. The filler consisted of alumina–zirconia and carbonate apatite of 50:50 ratio. The filler and

matrix were mixed with ratio of 70:30. The mixture were transferred into a petri dish until homogeneous and put in an oven with a temperature below 80° to form a powder followed by grinding with a mortar. It was then mixed with 75 ml chitosan solution with the concentration of 2% (C-2%), 4% (C-4%), and 6% (C-6%) and casted into a mould with a diameter of 6 mm and a height of 3 mm conformed to American Dental Association (ADA) standards. The composite mixture is inserted layer by layer every 1 mm into the mould after which it is levelled using a plastic instrument. The composite was irradiated with light curing unit (LCU) for 40 seconds every 1–2 mm of the composite layer until the entire mould was filled. After hardened, the sample is released from the mould. Vickers microhardness testing machine is used.

2.5. Mechanical Properties and Characterization of Dental Restorative Composite

The alumina–zirconia and carbonate apatite powder were characterized using x-ray diffraction (XRD). The hardened sample of dental restorative composites were tested its hardness using micro-Vickers hardness tester to analyse the effect of chitosan concentration to hardness of composite. The instrument used is micro-Vickers hardness tester with 100 g load. Cylindrical sample was put in object table right below indenter, and the load is applied. Resulting indentation can be seen under microscope was a rectangular, which has diagonal value (d). The corresponding unit of HV is then the kilogram-force per square millimetre (kgf/mm²) or HV number:

$$HV = 1.854/d^2 .$$

Both XRD and hardness test were conducted at Solid Oxide System Laboratory, Faculty of Mining and Petroleum Engineering, Institut Teknologi Bandung. For SEM characterization, the composite was coated with carbon to form conductive layer followed by vacuum treatment since it is not a conductive material. Sample was then transferred into holder and exposed by electron. The characterization was conducted at Basic Science Center (BSC) A, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung.

3. RESULTS AND DISCUSSION

3.1. XRD Analysis

The x-ray diffraction (XRD) analysis was performed to identify the

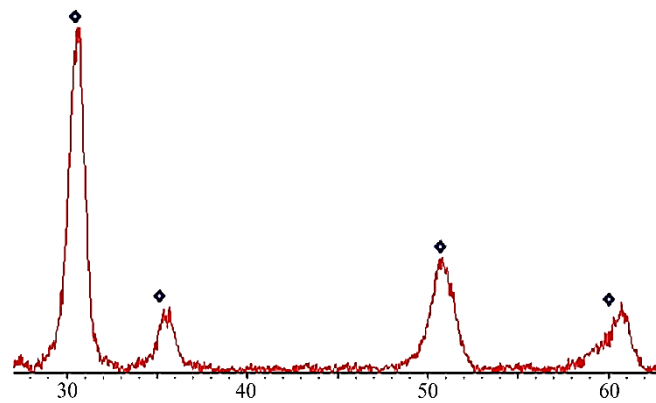


Fig. 1. XRD of alumina-zirconia (note: ◊ = *t*-ZrO₂).

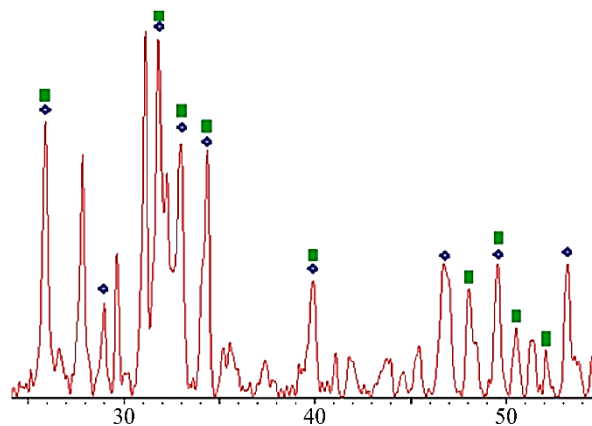


Fig. 2. XRD of carbonate apatite (note: ◊ = HAp, ■ = CO₃Ap).

resulting compound of alumina-zirconia and carbonate apatite. Figure 1 shows the appearance of tetragonal phase zirconia (*t*-ZrO₂; JCPDS #27-0997) with the highest peak observed at 30.50°, 50.90°, and 60.90°, respectively. Figure 2 shows the appearances of some hydroxyapatite (HAp; JCPDS #74-0565) overlapped with dahllite (CO₃Ap; JCPDS #02-1145).

Zirconia, unlike alumina, is a metastable ceramic with monoclinic, tetragonal, and cubic phases. Tetragonal phase zirconia has exceptional mechanical properties, including high initial hardness and fracture resistance [17]. Transformation toughening creates a transformation zone around developing cracks, slowing crack growth and producing few chips and fractures in the frame material as long as the tetragonal phase is present in zirconia. The tetragonal form is preferred in dentistry because it has the best mechanical

properties, such as high fracture toughness [18].

Dahllite is more closely related to biological apatite than hydroxyapatite and a number of studies have been done on the bulk properties of carbonated apatite [19]. This mineral can also be formed in kidney stones if one person consumed high amount of tricitrates that also increase the pH of urine. Dahllite is a form of calcium phosphate that is a naturally occurring derivative of apatite, which closely related to the inorganic constituents of bones, dental enamel, and dentin. The formation of hydroxyapatite was also visible in the synthesized carbonate apatite powder. This calcium phosphate mineral, which makes up about 65% of bone and makes an appealing choice for a synthetic bone composite that aids in natural bone regeneration and bone replacement materials [20]. The hydroxyapatite crystal arrangements can differ in terms of quantity, size, and shape. These configurations support the bone's structural capacity, hardness, and functionality. Aside from bone, hydroxyapatite is also the main component of enamel and one of the most studied biomaterials in the medical and dental fields [21].

3.2. Vickers Microhardness Analysis

Figure 3 represents the mean hardness values of C-2%, C-4%, and C-6%, which are of 51.3 *VHN*, 28.24 *VHN*, and 25.48 *VHN*, respectively. The hardness value of C-2% has reached the hardness value as a composite restoration, namely, of 30–100 *VHN*, but could not reach the dentin hardness standard of 60 *VHN* [22, 23]. Carbonate apatite is more appropriate to use compared to apatite because it not only contains calcium and phosphate but also carbonate, which is one of the enamel compounds, so that it can improve the mechanical properties of the composite material. The hardness value can also be affected by the

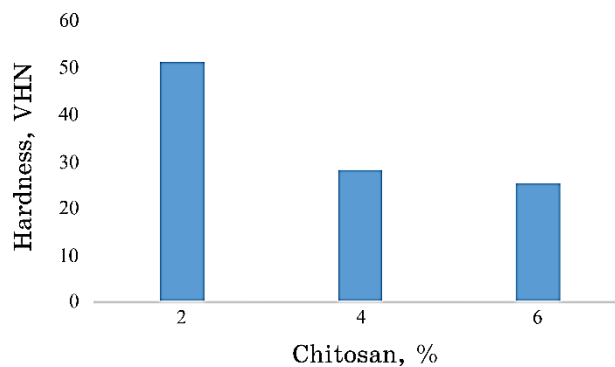


Fig. 3. Microhardness Vickers value of composite with chitosan variations.

concentration of chitosan used as a coupling agent. Chitosan with a low viscosity is more effectively used as a coupling agent than chitosan with a high viscosity. High concentrations of chitosan increase the viscosity of the mixture, which leads to inhomogeneous mixing of the composite and more distance between each filler particle. The high viscosity of a solution cannot be used clinically in the oral cavity because it cannot form homogeneous bonds. An inhomogeneous composite mixture can be susceptible to the formation of cavities, cause the composite to crack easily when exposed to pressure, and affect the hardness value. The viscosities of C-4% and C-6% were high, since they contained more chitosan powder compared, which caused the mixing process between filler and matrix to be not as good as with C-2%. Higher concentration of chitosan also makes stronger bond that occurs in chitosan solution itself rather than the bond between chitosan and composite.

3.3. SEM Analysis

Figure 4 shows the SEM results of the composite with chitosan 2% addition (C-2%) magnified by $\times 1000$ and $\times 10000$, respectively. Based on the figure, it can be seen that the particles are distributed evenly, though agglomeration is still found. Agglomeration causes the formation of gaps between composite particles. The average particle size formed is $0.0875 \mu\text{m}$ or 87.5 nm .

Figure 5 shows the SEM results of the composite with chitosan 4% addition (C-4%) magnified by $\times 1000$ and $\times 10000$, respectively. Based on the figure, it can be seen that the distribution of the particles formed is less homogeneous and quite a lot of agglomeration is formed. The average particle size formed is $0.1125 \mu\text{m}$ or 112.5 nm .

Figure 6 shows the SEM results of composite with chitosan 6% addition (C-6%) magnified by $\times 1000$ and $\times 10000$, respectively. Based on the figure, it can be seen that the distribution of particles is less homogeneous and there are still many agglomerations. The average particle size formed is $0.15 \mu\text{m}$ or 150 nm .

The SEM results showed that different chitosan concentrations had different effects on the distribution and size of the formed nanoparticles. The C-2% with the lowest concentration had a smaller particle size and a more homogeneous particle distribution. Meanwhile, composite with chitosan concentrations of 4% and 6% produced larger particle sizes and a less homogeneous particle distribution. A good composite material can be identified by its small particle size, even distribution, and lack of agglomeration [24]. The C-2% has a mean particle size of 87.5 nm and less agglomerate compared to the C-4% and C-6%. Agglomeration, which causes the particles to not be dispersed properly, occurs during the mixing process between the filler and the matrix.

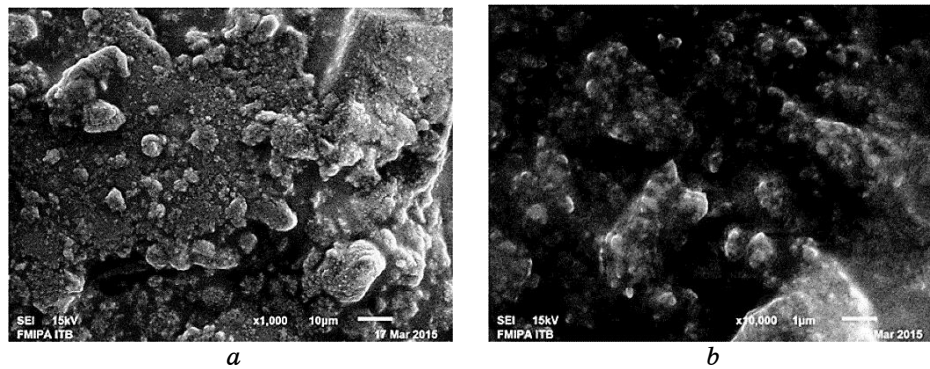


Fig. 4. SEM results of C-2% magnified by $\times 1000$ (left) and $\times 10000$ (right).

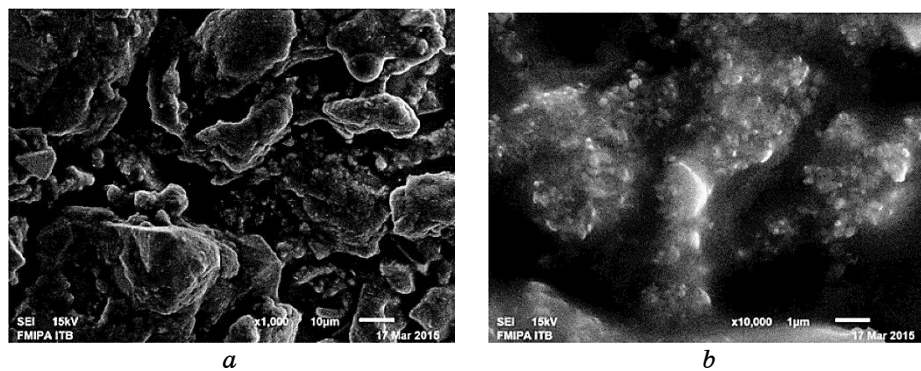


Fig. 5. SEM results of C-4% magnified by $\times 1000$ (left) and $\times 10000$ (right).

There is a visually large agglomeration in samples C-4% and C-6%, and the particle distribution is uneven because there are many cavities. This affects the mechanical properties of the composite with a lower hardness value due to the higher chitosan concentration, and their particle sizes were larger compared to C-2%. The concentration of chitosan in this study has an effect related to the number of amine groups present in the solution.

The bond formed by dissolving chitosan powder, which has a positively charged amine group, and acetic acid can cause the solution to have bioadhesive properties so that it can bond with negatively charged filler materials. The bond formed between the nanoparticle filler and chitosan causes the nanoparticles' surfaces to be enveloped by chitosan so that the particle size remains small. Chitosan 2% emerged as the best coupling agent because the bonds formed between matrix, filler, and chitosan produce a homogeneous mixture with only a small formation of agglomerates.

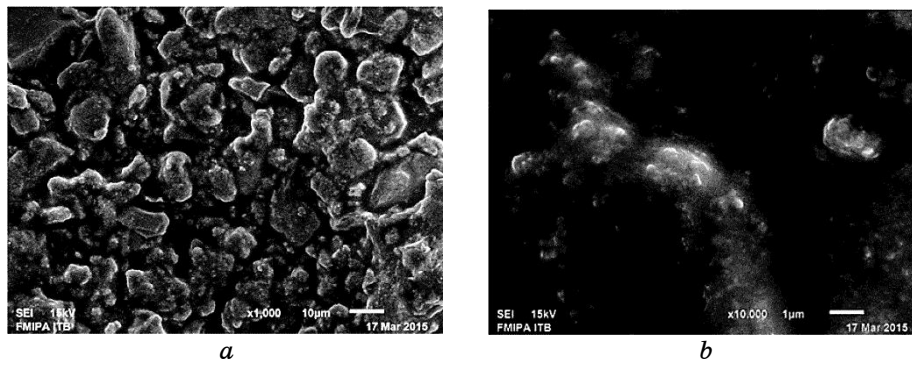


Fig. 6. SEM results of C-6% magnified by $\times 1000$ (left) and $\times 10000$ (right).

The mechanical properties of the composite are also affected by the bond formed between the matrix and the filler used during the mixing process. UDMA as the primary matrix material has a high viscosity, which makes it difficult to apply clinically, so it requires an additional matrix with a lower viscosity to be used in the oral cavity. These include triethyleneglycoldimethacrylate (TEGDMA) and dimethylamineethylmethacrylate (DMAEMA). In this study, the light curing method was used for composite polymerization. The weakness of this method is that it causes shrinkage to the composite. Observation of the distance between the beam and exposed surface and the thickness of each composite layer during the irradiation process can reduce shrinkage possibilities in the composite. High-intensity curing should be used cautiously in clinical work due to its conflicting effects on hardness and crosslinking density in flowable composites [25].

4. CONCLUSION

This study concludes that tetragonal-phase zirconia was formed after the mixing of alumina and zirconia using the sol-gel method, while the dahllite and hydroxyapatite phases appeared in the synthesized carbonate apatite. The lower concentration of chitosan resulted in smaller nanoparticle fillers. The composite with decreasing chitosan concentration produced a higher hardness value. Chitosan affects the microstructural behaviour of composites. The composite with the addition of 2%, 4%, and 6% chitosan resulted in nanoparticles with sizes of 87.5 nm, 112.5 nm, and 150 nm, respectively. The best effect of chitosan on the hardness value of composites was achieved at a concentration of 2%, which has an average hardness value of 51.3 *VHN*. The hardness value already meets the hardness

standard as a composite restoration for use in dentistry of 30–90 *VHN*. For future research, it is necessary to carry out further research on dental restorative composites with another polymerization method such as self-curing as an alternative to reduce shrinkage generated by the light curing method.

REFERENCES

1. M. Rathee and A. Sapra, *Dental Caries* (Treasure Island, FL: StatPearls Publishing LLC: 2023); <https://www.ncbi.nlm.nih.gov/books/NBK551699/>
2. E. A. Abou Neel, A. Aljabo, A. Strange, S. Ibrahim, M. Coathup, A. M. Young, L. Bozec, and V. Mudera, *International Journal of Nanomedicine*, **11**: 4743 (2016); <https://doi.org/10.2147/IJN.S107624>
3. N. J. M. Opdam, R. Frankenberger, and P. Magne, *Oper Dent*, **41**: S27 (2016); <https://doi.org/10.2341/15-126-LIT>
4. H. V. Worthington, S. Khangura, K. Seal, M. Mierzwinski-Urban, A. Veitz-Keenan, P. Sahrman, P. R. Schmidlin, D. Davis, Z. Ihezor-Ejiofor, and M. G. Rasines Alcaraz, *Cochrane Database of Systematic Reviews*, **8** (2021); <https://doi.org/10.1002/14651858.CD005620.PUB3/INFORMATION/EN>
5. K. Cho, G. Rajan, P. Farrar, L. Prentice, and B. G. Prusty, *Composites Part B: Engineering*, **230**: 109495 (2022); <https://doi.org/10.1016/J.COMPOSITESB.2021.109495>
6. P. Mourouzis, E. A. Koulaouzidou, G. Palaghias, and M. Helvatjoglu-Antoniades, *Journal of Applied Biomaterials & Functional Materials*, **13**, No. 3: 259 (2015); <https://doi.org/10.5301/jabfm.5000228>
7. Y. Faza, A. Harmaji, V. Takarini, Z. Hasratiningsih, and A. Cahyanto, *Key Engineering Materials*, **829**: 182 (2019); <https://doi.org/10.4028/www.scientific.net/KEM.829.182>
8. B. Sunendar, A. Fathina, A. Harmaji, D. F. Mardhian, L. Asri, and H. B. Widodo, *AIP Conference Proceedings* (2017), p. 1887; <https://doi.org/10.1063/1.5003503>
9. E. Febrina, A. Evelyn, A. Harmaji, and B. Sunendar, *Dental Journal*, **56**, No. 1: 30 (2023); <https://doi.org/10.20473/j.djmk.v56.i1.p30-35>
10. J. C. Souza, J. B. Silva, A. Aladim, O. Carvalho, R. M. Nascimento, F. S. Silva, A. E. Martinelli, and B. Henriques, *The Open Dentistry Journal*, **10**: 58 (2016); <https://doi.org/10.2174/1874210601610010058>
11. G. Guo, Y. Fan, J. F. Zhang, J. L. Hagan, and X. Xu, *Dental Materials: Official Publication of the Academy of Dental Materials*, **28**, No. 4: 360 (2012); <https://doi.org/10.1016/j.dental.2011.11.006>
12. M. N. Zakaria, A. Cahyanto, and A. El-Ghannam, *Key Engineering Materials*, **720**: 147 (2017); <https://doi.org/10.4028/WWW.SCIENTIFIC.NET/KEM.720.147>
13. Y. D. Rakhmatia, Y. Ayukawa, A. Furuhashi, and K. Koyano, *Materials*, **11**, No. 7: 1201 (2018); <https://doi.org/10.3390/ma11071201>
14. A. Yumeisa, L. Damayanti, T. Sumarsongko, A. Harmaji, and A. Cahyanto, *Key Engineering Materials*, **829**: 145 (2019); <https://doi.org/10.4028/www.scientific.net/KEM.829.145>
15. F. Erfan, R. Rikmasari, L. Damayanti, A. Harmaji, and A. Cahyanto, *Key*

- Engineering Materials*, **829**: 138 (2019);
<https://doi.org/10.4028/www.scientific.net/KEM.829.138>
16. W. Awalia, T. Sumarsongko, R. Rikmasari, A. Harmaji, and A. Cahyanto, *Key Engineering Materials*, **829**: 131 (2019);
<https://doi.org/10.4028/www.scientific.net/KEM.829.131>
 17. F. Kern, H. Reveron, J. Chevalier, and R. Gadow, *Journal of the Mechanical Behavior of Biomedical Materials*, **90**: 395 (2019);
<https://doi.org/10.1016/J.JMBBM.2018.11.001>
 18. H. Tanaka, T. Maeda, H. Narikiyo, and T. Morimoto, *Journal of Asian Ceramic Societies*, **7**, No. 4: 460 (2019);
<https://doi.org/10.1080/21870764.2019.1665767>
 19. G. Xu, I. A. Aksay, and J. T. Groves, *Journal of the American Chemical Society*, **123**, No. 10: 2196 (2001); <https://doi.org/10.1021/JA002537I>
 20. H. A. Siddiqui, K. L. Pickering, and M. R. Mucalo, *Materials (Basel, Switzerland)*, **11**, No. 10: 1813 (2018); <https://doi.org/10.3390/ma11101813>
 21. S. Balhuc, R. Campian, A. Labunet, M. Negucioiu, S. Buduru, and A. Kui, *Crystals*, **11**, No. 6: 674 (2021); <https://doi.org/10.3390/cryst11060674>
 22. P. Yılmaz Atalı, B. Doğu Kaya, A. Manav Özen, B. Tarçın, A. A. Şenol, E. Tüter Bayraktar, and B. Korkut, *Polymers*, **14**, No. 22: 4987 (2022);
<https://doi.org/10.3390/polym14224987>
 23. M. del P. Gutiérrez-Salazar, and J. Reyes-Gasga, *Materials Research*, **6**, No. 3: 367 (2003); <https://doi.org/10.1590/S1516-14392003000300011>
 24. S. S. Mirsasaani, M. Hemati, T. Tavasoli, E. S. Dehkord, G. T. Yazdi, and D. A. Poshtiri, *Nanobiomaterials in Clinical Dentistry*, Ch. 2: 17 (2013);
<https://doi.org/10.1016/B978-1-4557-3127-5.00002-7>
 25. M. Par, D. Marovic, T. Attin, Z. Tarle, and T. T. Tauböck, *Scientific Reports*, **10**, No. 1: 1 (2020); <https://doi.org/10.1038/s41598-020-67641-y>