Chiang Mai Dental Journal

Original Article

The Development of Bioceramic Root Canal Sealer

Sureeporn Oransakunwong[1], Thanakorn Wasanapiarnpong[2], Busayarat Santiwong[3], Pairoj Linsuwanont[1]
[1]Department of Operative Dentistry, Faculty of Dentistry, Chulalongkorn University, Thailand
[2]Department of Materials Science, Faculty of Science, Chulalongkorn University, Thailand
[3]Department of Pediatric Dentistry, Faculty of Dentistry, Chulalongkorn University, Thailand

Received: November 2, 2021 • Revised: January 4, 2022 • Accepted: January 21, 2021

Corresponding Author: ***Associate Professor Dr. Pairoj Linsuwanont,*** *Department of Operative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand.(E-mail:* ***linspairoj@gmail.com****)*

Abstract

Objectives: This study evaluated the physical properties of cockle shell derived bioceramic sealer (Biosealer) and compared it with commercial bioceramic sealer (iRoot SP).

Methods: Cockle shell derived tricalcium silicate powder was manufactured. Various additives were mixed with tricalcium silicate powder to modify the physical properties of Biosealer. According to a modified ISO 6876:2012 standard, the flowability, setting time, film thickness, solubility, and radiopacity of the Biosealer and iRoot SP were investigated.

Results: Biosealer exhibited acceptable flowability, setting time, film thickness, and radiopacity according to ISO 6876:2012 requirements. There was no significant difference between the physical properties of Biosealer and iRoot SP, except for the setting time ($p<0.05$).

Conclusions: Biosealer possessed good physical properties and was comparable to iRoot SP.

Keywords: bioceramic, cockle shell, physical properties, root canal sealer, tricalcium silicate

Introduction

Bioceramic sealer has been used widely in endodontics for their good physicochemical and biological properties.(1) *In vitro* and *in vivo* studies illustrated its good sealing ability, antibacterial activity and biological advantages.(2-4) Clinically, root canal treatments using a single-cone technique with bioceramic sealer exhibited a high clinical success rate.(5,6)

To date, bioceramic root canal sealer was marketed in both premixed (iRoot SP, Ceraseal, Endoseal MTA) and powder-liquid (Bioroot RCS, Proroot ES) formulations. Similarly, both contain calcium silicate as the main component, requiring water to enhance setting via a hydration reaction. Zirconium oxide, calcium phosphate, calcium hydroxide, and other additives are added to improve the physical properties of the sealer.(1)

Cockle shells are made of more than 95% calcium carbonate.(7) When processed, cockle shells provide calcium which can be the basis of various biomaterials for clinical use. In medical applications, cockle shell derived nanoparticles were used to create drug delivery scaffolds and bone grafts.(8) *In vitro* studies show cockle shell derived calcium carbonate nanoparticles possess minimal toxicity and high biocompatibility.(9) For dental applications, cockle shell derived calcite nanoparticles have been proposed for use as dentin desensitizing agent.(10) Also, a study on cockle shell derived hydroxyapatite paste as a remineralization agent has been conducted.(11) Moreover, cockle shell derived hydroxyapatite-tricalcium phosphate was formulated for use as a bone scaffold.(12)

Theoretically, calcium carbonate reacts with the silica at a temperature of 1450°C to yield calcium silicate(13), to be used as the main component of a bioceramic sealer. To acquire acceptable physical properties, additives such

as a radiopacifier, plasticizer, and medium were added to adjust the properties of bioceramic sealer.

This study evaluated the physical properties of cockle shell derived bioceramic sealer (Biosealer) and compared it with a commercial bioceramic sealer (iRoot SP).

Materials and Methods

To prepare cockle shell derived tricalcium silicate powder (C-C_3S), the cockle shells were boiled in distilled water for 1 hour and were boiled in 5wt% acetic acid solution for another 1 hour. Then, they were boiled in distilled water for 1 hour twice. After that, they were dried, and milled into powder. The cockle shell powder and Zircon were mixed in a weight ratio of 2.2 to 1 and then dried. After that, the mixed raw powder was uniaxial hydraulic pressed at 10 MPa to form pellets sized 35 mm in diameter and 10 mm thick. The pellets were placed in an alumina crucible and fired at 1450°C for 2 hours. Next, they were rapidly cooled by a blower fan and grounded in an alumina mortar. The powder was milled and dried in an oven at 50°C overnight to finish C-C_3S synthesis.

The chemical composition and particle size of C-C_3S were determined by X-ray diffractometer (Bruker AXS Model D8 Discover, Karlsruhe, Germany) and dynamic laser scattering particle size analyzer (Mastersizer 2000, Malvern, UK).

Formulation of Biosealer

Biosealer consisted of two parts: a powder and a liquid. The powder consisted of C-C_3S, zirconium oxide (Inframat, Connecticus, US), ethylene-vinyl laurate-vinyl chloride terpolymer (Vinnapas 8031 H, Wacker, Germany), and microsilica (Labchem, Ajax Finechem, Australia). The liquid consisted of propylene glycol (Unilab, Ajax Finechem, Australia) and silicone oil (Labchem Ajax Finechem, Australia). The powder and liquid were put into a plastic capsule and mixed by amalgamator at 4,000 rpm. Lastly, it was loaded into a plastic syringe and be ready for use.

Evaluation of physical properties following a modified ISO 6876:2012

Each test was conducted on 6 samples for each group.

Flow

Using the prepared syringe, 0.05 ml of sealer was placed on the center of a glass plate. After 180 seconds, a second plate was placed atop the sealer with an additional mass on the plate, totaling 120 grams of load. After 10 minutes, the weight was removed. The diameter of the compressed sealer was measured and recorded as the flow value.

Setting time

The gypsum molds (10 mm in diameter and 1 mm in height) were filled with sealer and stored in a controlled environment at 37°C and 95% relative humidity (RH) for 24 hours. A Gilmore-type indenter measuring 2 mm in diameter was placed vertically on the surface of the sealer along with 100 grams of load. The setting time was recorded when the indentations ceased to be visible.

Film thickness

The combined thickness of the two glass plates was measured using a micrometer. A portion of sealer was deposited onto the center of one glass plate. Then, the other glass plate was placed centrally on top of the sealer. After 180 seconds, a load of 150 newtons was applied vertically on the top plate. After 10 minutes, the combined thickness of the two glass plates with the sealer film was measured. Film thickness was calculated by taking the difference between the thickness of the plates with and without sealer.

Solubility

Two grams of sealer was mixed with 0.02g of water and placed into molds 20 mm in diameter and 2 mm in height. The samples were stored in a controlled environment at 37°C and 95% RH for 7 days. The samples were then weighed before being placed into beaker A. Fifty milliliters of water were poured into beaker A, and then returned to the controlled environment for 24 hours. The water from beaker A was poured together with the specimens through filter paper into beaker B. Beaker B was then placed in an oven at 110°C until the water evaporated. The difference between the original and final weight of beaker B was the amount of sealer dissolved from the specimens. The solubility value is the dissolved sealer mass expressed as percentage of its initial mass.

Radiopacity

A specimen 10 mm in diameter and 1 mm in height was positioned in the center of a digital imaging plate adjacent to an aluminum step wedge. Both were then radiated with X-rays at 65 kilovolts at a target-film distance of 300 mm for 0.25 seconds. The density of the specimen images was compared with that of the aluminum step wedge using

densitometric analysis function (Trophy DICOM 6.3.0.0 program).

Statistical analysis

Statistical analysis was performed using IBM SPSS software version 22 (IBM Corporation, Armonk, NY, USA). Data were subjected to independent t-test to determine significant differences between groups ($p<0.05$).

Results

The chemical composition of C-C_3S

X-ray diffractometer showed the prepared powder's crystalline structure contained 55% tricalcium silicate (Ca_3SiO_5) and 40% calcium zirconate ($CaZrO_3$), as shown in Figure 1. Median particle size was 5.32 microns, as measured by dynamic laser scattering particle size analyzer.

The physical properties of the sealers

The physical properties of iRoot SP and Biosealer are shown in Table 1. Both materials exhibited comparable physical properties, except the observation of a longer setting time for Biosealer ($p<0.05$).

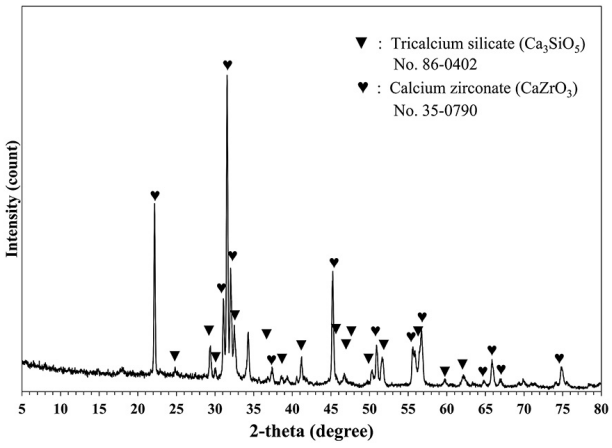

Figure 1: The x-ray diffractogram of C-C3S powder showed peaks of tricalcium silicate (▼), and calcium zirconate (♥). The quantitative phase analysis based on Rietveld structure refinement found that C-C_3S contained 55% tricalcium silicate (Ca_3SiO_5), and 40% calcium zirconate ($CaZrO_3$).

Table 1: The physical properties of the sealers.

Physical properties	iRoot SP	Biosealer
Flow (mm)	24.58±0.92[a]	24.83±1.17[a]
Setting time (hour)	7±0.15[a]	29±0.08[b]
Film thickness (μm)	23.33±3.33[a]	25.67±4.08[a]
Solubility (% by weight)	5.85±0.75[a]	4.68±1.06[a]
Radiopacity (mm of Aluminum thickness)	7.61±0.26[a]	7.25±0.34[a]

The different superscript letters in the same row indicated statistically significant differences between groups according to independent *t*-test ($p<0.05$).

Discussion

Tricalcium silicate can be prepared using many methods, including the solid-state reaction technique, as used in this study.[13-15] Cockle shell derived calcium carbonate ($CaCO_3$) and zircon ($ZrSiO_4$) were used as substrates for tricalcium silicate powder manufacture, according to the following equation: $4CaCO_3 + ZrSiO_4 \rightarrow Ca_3SiO_5 + CaZrO_3 + 4CO_2$. The cockle shell was used as source material because it is an abundant and cheap source of calcium carbonate, which can be source of calcium. It is also reprocessing of waste material. XRD analysis showed C-C_3S contained tricalcium silicate as the main phase (55%). During the manufacturing process, zircon was added to the cockle shell powder as a source of silica. And calcium zirconate which detected by the XRD analysis could act as a radiopacifier.

Root canal sealer should provide the following properties: good flowability, adequate setting time for clinical use, sufficient radiopacity, dimensional stability and biocompatibility.[16] To modify the physical properties of our developed Biosealer, additives were combined with the C-C_3S. Zirconium oxide was used to increase radiopacity. Study showed that zirconium oxide exhibited good biocompatibility, and it did not alter physicochemical properties of the sealer.[17] Moreover, it did not discolor tooth dentin.[18] Propylene glycol was added to improve flowability and handling property, as demonstrated in previous studies.[19,20] Ethylene-vinyl laurate-vinyl chloride terpolymer acted as a binder to improve the handling property and sealer adhesion.[21] Microsilica provides a more uniform distribution of cement. It affects material hydration via the pozzolan reaction and enhances cement durability.[22] Lastly, silicone oil is an antifoaming agent and reduces sealer pores.

The ability of the root canal sealer to reach the irregular areas of complicated root canal systems can be enhanced by improving its flowability. In this study, the flow value of iRoot SP was consistent with other studies.[23-25] Both iRoot SP and Biosealer showed a flowability of approximately 24 mm, which fulfils the requirements of ISO 6876:2012. The literature showed that bioceramic sealer exhibited higher flowability than the commonly used AH plus.[23-25]

In this study, the film thickness of iRoot SP was complied with other reports.[23] There was no statistically significant difference between iRoot SP and Biosealer. However, film thickness might not be as important an issue in hydraulic condensation (Single cone technique) because root canal sealer need not be as thin as possible. Reported observed setting times of iRoot SP were in the range of 2.7-22.3 hours.[23,25,26] This variation could be partly from the difference of the experimental set-up, such as definitions for setting time, different experimental models and etc.[23,25,26] In this study, iRoot SP had a setting time of 7 hours which was comparable to other studies.[26] Biosealer exhibited a longer setting time than iRoot SP. Possible reasons for this may be due to the larger particle size of the Biosealer, and/or the different additives used in the tested sealers. Both sealers provide plenty of time for clinicians to complete the root canal obturation. The laboratory setting-time testing method may not be identical to clinical situations. Bioceramic sealer, when encountering the moisture inside the root canal, will gradually complete the hydration reaction. Therefore, other factors such as the amount of fluid inside the root canal should be taken into consideration.

The evaluation of the quality of root canal obturation is partly based on radiographic interpretation. Theoretically, root canal filling materials should be more radiopaque than root dentin. The radiopacity of iRoot SP were reported with great variation and ranged between 3.8-10.8 mm of aluminum.[24-26] In this study, the radiopacity of both sealers was about 7 mm of aluminum, making the root canal distinguishable from root filling material.

The bioceramic sealers' solubility values were reported diversely due to variations in test methods. Most studies reported more than 3% solubility for bioceramic sealers[27], which is greater than allowed for in ISO 6876:2012. In this study, the solubility of iRoot SP and Biosealer were 5.85, and 4.68 respectively. High sealer solubility can compromise the sealing ability of root canal filling material. However, when using bioceramic sealer, due to its ability to form hydroxyapatite on its exposed surface when contacting tissue fluid[28], the porosities and voids could be reduced over time.[29]

In general, Biosealer and iRoot SP exhibited acceptable physical properties as required by ISO 6876:2012. However, other aspects such as dimensional stability and the ability to penetrate radicular dentinal tubules deserve further investigation. Prior to clinical use, cell cytotoxicity, tissue biocompatibility, animal trials, and clinical trials need to be conducted.

Conclusions

The local-made cockle shell derived bioceramic sealer (Biosealer) exhibited good physical properties which were comparable to commercial bioceramic sealer (iRoot SP).

Acknowledgments

We acknowledge the Dental materials R&D center of Chulalongkorn university, for providing us the experimental apparatus. This work was supported by The Chulalongkorn Academic Advancement into Its 2nd Century Project.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Wang Z. Bioceramic materials in endodontics. Endod Topics. 2015;32(1):3-30.
2. Asawaworarit W, Pinyosopon T, Kijsamanmith K. Comparison of apical sealing ability of bioceramic sealer and epoxy resin-based sealer using the fluid filtration technique and scanning electron microscopy. J Dent Sci. 2020;15(2): 186-92.
3. Camps J, Jeanneau C, El Ayachi I, Laurent P, About I. Bioactivity of a calcium silicate-based endodontic cement (BioRoot RCS): interactions with human periodontal ligament cells *in vitro*. J Endod. 2015;41(9):1469-73.
4. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. J Endod. 2009;35(7):1051-5.
5. Chybowski EA, Glickman GN, Patel Y, Fleury A, Solomon E, He J. Clinical outcome of non-surgical root canal treatment using a single-cone technique with endosequence bioceramic sealer: a retrospective analysis. J Endod. 2018;44(6):941-5.

6. Zavattini A, Knight A, Foschi F, Mannocci F. Outcome of root canal treatments using a new calcium silicate root canal sealer: a non-randomized clinical trial. J Clin Med. 2020;9(3):782.
7. Mailafiya MM, Abubakar K, Danmaigoro A, Chiroma SM, Abdul Rahim EB, Mohd Moklas MA, *et al*. Cockle shell derived calcium carbonate (aragonite) nanoparticles: a dynamite to nanomedicine. Appl Sci. 2019;9(14):2897.
8. Ibiyeye KM, Zuki ABZ. Cockle shell derived aragonite CaCO3 nanoparticles for co-delivery of doxorubicin and thymoquinone eliminates cancer stem cells. Int J Mol Sci. 2020;21:1900.
9. Mailafiya MM, Mohd Moklas MA, Abubakar K, Danmaigoro A, Chiroma SM, Rahim EBA, *et al.* Cytotoxicity studies of curcumin loaded-cockle shell derived calcium carbonate nanoparticles. Nanosci Nanotechnol Asia. 2021;11(1): 35-41.
10. Tram NXT. Synthesis and characterization of calcite nano-particle derived from cockle shell for clinical application. ASEAN Eng J. 2020;10:49-54.
11. Nugroho JJ, Natsir N, Trilaksana AC, Rovani CA, Atlanta MM. The increse of tooth enamel surface hardness after application blood cockle shells (Anadara granosa) paste as remineralization agent. Int J Appl Pharm. 2019;11(4):26-9.
12. Ferdynanto RA, Dharmayanti PES, Dewi PTK, Prananingrum W. The effect of various concentrations of HA-TCP derived from cockle shell synthesis on scaffold porosity. DJMKG. 2018;51(3:)114-8.
13. Song J, Lee DW, Cho YH, Ok KM. Solid state synthesis, characterization, and nonlinear optical properties of a monoclinic tricalcium silicate, Ca3SiO5. B Korean Chem Soc. 2012;33(7):2423-6.
14. Chen CC, Ho CC, Lin SY, Ding SJ. Green synthesis of calcium silicate bioceramic powders. Ceram. 2015;41(4): 5445-53.
15. Wu M, Wang T, Wang YY, Li F, Zhou M, Wu X. A novel and facile route for synthesis of fine tricalcium silicate powders. Mater Lett. 2018;227:187-90.
16. Grossman LI, Oliet S, Del Rio CE. Endodontic practice. 10th ed. Philadelphia: Lea & Febiger; 1981.
17. Camilleri J, Cutajar A, Mallia B. Hydration characteristics of zirconium oxide replaced Portland cement for use as a root-end filling material. Dent Mater. 2011;27(8):845-54.
18. Marciano MA, Duarte MA ,Camilleri J. Dental discoloration caused by bismuth oxide in MTA in the presence of sodium hypochlorite. Clin Oral Investig. 2015;19(9):2201-9.
19. Duarte MA, Alves de Aguiar K, Zeferino MA, Vivan RR, Ordinola-Zapata R, Tanomaru-Filho M, *et al*. Evaluation of the propylene glycol association on some physical and chemical properties of mineral trioxide aggregate. Int Endod J. 2012;45(6):565-70.
20. Marciano MA, Guimaraes BM, Amoroso-Silva P, Camilleri M, Duarte MA. Physical and chemical properties and subcutaneous implantation of mineral trioxide aggregate mixed with propylene glycol. J Endod. 2016;42(3):474-9.
21. Tarannum N, Pooja K, Khan R. Preparation and applications of hydrophobic multicomponent based redispersible polymer powder: a review. Constr Build Mater. 2020; 247.
22. Hewlett P, Liska M. Lea's Chemistry of Cement and Concrete. 5th ed. Burlington: Elsevier; 2019.
23. Zhou HM, Shen Y, Zheng W, Zheng YF, Haapasalo M. Physical properties of 5 root canal sealers. J Endod. 2013;39(10):1281-6.
24. Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. J Endod. 2012;38(6):842-5.
25. Tanomaru-Filho M, Torres FFE, Chavez-Andrade GM, Ribeiro-Siqueira DC, Givini G. Physicochemical properties and volumetric change of silicone/bioactive glass and calcium silicate-based endodontic sealers. J Endod. 2017;43(12):2097-101.
26. Xuereb M, Vella P, Damidot D, Sammut CV, Camilleri J. In situ assessment of the setting of tricalcium silicate-based sealers using a dentin pressure model. J Endod. 2015; 41(1):111-24.
27. Silva E, Cardoso ML, Rodrigues JP, De-Deus G, Fidalgo TKS. Solubility of bioceramic - and epoxy resin-based root canal sealers: a systematic review and meta-analysis. Aust Endod J. 2021.
28. Poggio C, Dagna A, Ceci M, Meravini MV, Colombo M, Pietrocola G. Solubility and pH of bioceramic root canal sealers: a comparative study. J Clin Exp Dent. 2017;9: e1189-e94.
29. Milanovic I, Milovanovic P, Antonijevic D, Dzeletovic B, Djuric M, Miletic V. Immediate and long-term porosity of calcium silicate-based sealers. J Endod. 2020;46(4):515-23.