



Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Bioactivity of Biodentine and Mineral Trioxide Aggregate: An In Vitro Assessment

Sawsan T. Abu Zeid^{1,2,*}, Ensanya A. Abou Neel^{3,4,5}, Loai Al Sofi¹, Hadeel Edress¹, Osama Alothmani¹, Amna Siddiqui¹, Abeer A. Mokeem-Saleh¹

¹Department of Endodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.

²Endodontic Department, Faculty of Dentistry, Cairo University, Cairo, Egypt.

³Department of Restorative Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.

⁴Biomaterials Division, Faculty of Oral and Dental Medicine, Tanta University, Cairo, Egypt.

⁵Biomaterials and Tissue Engineering Division, UCL Eastman Dental Institute, 256 Gray's Inn Road, London, WC1X 8LD

E-Mail : sawsanabuzeid55@hotmail.com

ABSTRACT

Aim: to evaluate the bioactivity of Biodentine compared with ProRoot-MTA. **Methods:** Standardized cavities were prepared in 20 dentin blocks and filled with either Biodentine or ProRoot-MTA (10 each). Each group was then subdivided into two subgroups; one was kept in deionized water, and the other in phosphate buffer solution (PBS) for 28 days. Bioactivity was determined at the material surface and material-dentin interface using Scanning Electron Microscope with Energy Dispersive X-ray. **Results:** Samples of Biodentine and ProRoot-MTA stored in deionized water showed microporous granular structure interrupted by few large hexagonal-shaped grains. Their surface elemental analysis showed similar main constituents including carbon, oxygen, and silicon with no statistically significant difference between both materials. Biodentine contained significantly more calcium than ProRoot-MTA. Unlike ProRoot-MTA, calcium and strontium were significantly decreased at the interface of Biodentine than that at the material's surface. After 28 days incubation in PBS, an apatite layer was formed at both the material surfaces, and the material-dentin interface exhibited as globular structure with entangled rod-like appearance for Biodentine or circular-shaped with flower-like deposits for ProRoot-MTA. There was no significant difference in calcium/phosphate ratio between Biodentine (3.52 ± 1.34 wt. %) and ProRoot-MTA (3.89 ± 1.27 wt. %) filled samples. **Conclusion:** The immersion of Biodentine and ProRoot-MTA in PBS resulted in the formation of comparable apatite layer that wasn't formed when these materials were immersed in deionized water indicating their bioactive property.

Keywords: Bioactivity, Biodentine, MTA Calcium silicate, elemental analysis.

INTRODUCTION

Radicular perforations negatively impact the outcome of root canal treatment (1, 2). In a two-year follow up study, around 40% of perforations sealed with Super EBA or amalgam failed (3). However, the use of Mineral Trioxide Aggregate (MTA) improved the success rate of perforation repair to 80% (4). This enhanced outcome has been attributed to MTA's biocompatibility and bioactivity (5-7).

Bioactivity reflects the ability of the material to release calcium ions, produce calcium hydroxide and form an interfacial layer between the material and dentinal wall leading to the deposition of apatite crystals over the surface of the material when it is placed in a synthetic tissue fluid environment such as phosphate buffer saline (PBS) (7). The ability of MTA to react chemically with the simulated body fluids in a manner compatible with the repair processes of the tissue leading to the deposition of apatite crystals at the material surface has been demonstrated confirming its bioactivity (8, 9). Nevertheless, the long setting reaction and difficult manipulation have been two main drawbacks of MTA (10).

Biodentine has been a recently introduced dentin replacement material that is claimed to be bioactive. It is composed of tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide and zirconium oxide while the liquid component includes calcium chloride, a hydrosoluble polymer and water. It sets within 6.5-45 minutes (11, 12). Exposing immortalized murine cells to Biodentine led to their transformation to odontoblast-like cells confirming the bioactive capacity of the material (13). The formation of "Mineral Infiltration Zone" has been described at Biodentine-dentin interface (14). Few studies have reported that Biodentine led to more calcium release at its interface compared to MTA (15, 16). Further insight on this aspect has been needed in order to verify whether Biodentine possesses higher bioactivity compared to MTA.

Combining Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) provides qualitative and semi-quantitative assessment of the superficial calcium to phosphate ratio (Ca/P). Such tests have been used to assess the bioactivity of materials (17). Utilizing this approach, this study aimed at comparing the bioactivity potential of MTA and Biodentine when placed in PBS for four weeks. The null hypothesis was that there was no difference between both materials in their bioactivity.

MATERIALS AND METHODS

Twenty standardized blocks of radicular dentin (8x8x3 mm³) were prepared from recently extracted human single-rooted teeth. In each block, a 4-mm diameter hole was prepared using carbide round bur # 2 under copious amount of water. Blocks were equally and randomly divided into two main groups; one group was filled with Biodentine (Septodont, Saint-Maur-des-Fossés, France), while the second one was filled with white ProRoot-MTA (Dentsply Tulsa Dental, OK, USA). Both materials were mixed according to manufactures' instructions. The specimens were kept in an incubator at 100 % humidity and 37°C for 48 hours until complete setting. Each group was then equally subdivided into two subgroups; specimens were either immersed in deionized water or phosphate buffer solution (PBS) for 28 days. Each solution was refreshed every three days. After 28 days, the specimens were analyzed using SEM/EDX to evaluate the surface morphology and elemental composition of the materials' surface as well as materials' dentin interface. Ca/P was calculated for samples incubated in PBS, and compared between groups as an indication of bioactivity.

The data were statistically analyzed by ANOVA and Tukey's Post Hoc test for the elemental composition and student's *t* test for Ca/P, respectively at significance level of 5%.

RESULTS

SEM results

Figure 1 (A & B) shows the scanning electron microphotographs of Biodentine and ProRoot-MTA filled dentin samples after the incubation in deionized water for 28 days. Both groups exhibited multiple clusters of small grains producing a micro-porous granular morphology. Few large hexagonal-shaped grains, marked with vertical white arrows, were seen between these clusters. The surface of ProRoot- MTA also showed the presence of multiple scattered white dots, marked with a horizontal white small arrow- figure 1B.

Figure 2 (A & B) shows the scanning electron microphotographs of Biodentine and ProRoot-MTA filled dentin samples after the incubation in PBS for 28 days. As seen from the figure, an apatite layer was formed on the entire surface as well as at the material-dentin interface for both materials. The granular surface of Biodentine was covered by an entangled rod-like apatite layer, indicated by a horizontal white arrow (figure 2A). ProRoot-MTA filled dentin samples however showed the presence of flower-like deposits, indicated by a vertical white arrow (figure 2B) on their surfaces.

EDX results

Biodentine samples showed significantly lower oxygen and calcium, but significantly higher carbon, sodium, phosphate, magnesium, manganese, strontium, zirconium and potassium when incubated in PBS than when immersed in deionized water. Meanwhile, the amount of silicon, chloride, arsenic, niobium and bismuth was not affected by the storage medium (Figure 2 C & E).

ProRoot-MTA samples had significantly lower oxygen and bismuth amounts, but higher silicon, phosphate, sodium, aluminum, magnesium, potassium when immersed in PBS than when immersed in deionized water. Meanwhile, the amount of carbon, calcium, manganese, chloride, arsenic and strontium was not affected by the storage medium (Figure 2 D & F).

Comparing the elemental composition of the surface of Biodentine and ProRoot-MTA [Figure 1 (C-F)] revealed that there was no significant difference between both materials regarding potassium, manganese, arsenic, strontium, carbon, oxygen and silicon contents ($P>0.05$). Calcium content was significantly higher in Biodentine ($32.3\pm 5.24\text{wt.}\%$) compared to ProRoot-MTA ($25.89\pm 2.47\text{wt.}\%$). The opposite was true for bismuth. Regarding trace elements, Biodentine contained significantly greater magnesium than ProRoot-MTA. Sodium, zirconium and niobium were only detected in Biodentine samples while aluminum was only present in ProRoot-MTA.

The analysis of the material-dentin interface in samples immersed in PBS for both materials showed that the amount of carbon, oxygen and silicon were significantly higher than those detected at the materials' surfaces, whereas calcium and phosphate amounts were significantly lower ($P<0.05$).

Regarding the calcium/phosphate ratio, calculated for the samples incubated in PBS, there was no significant difference ($P=0.861$) observed between Biodentine (3.52 ± 1.34) and ProRoot-MTA (3.89 ± 1.27) samples.

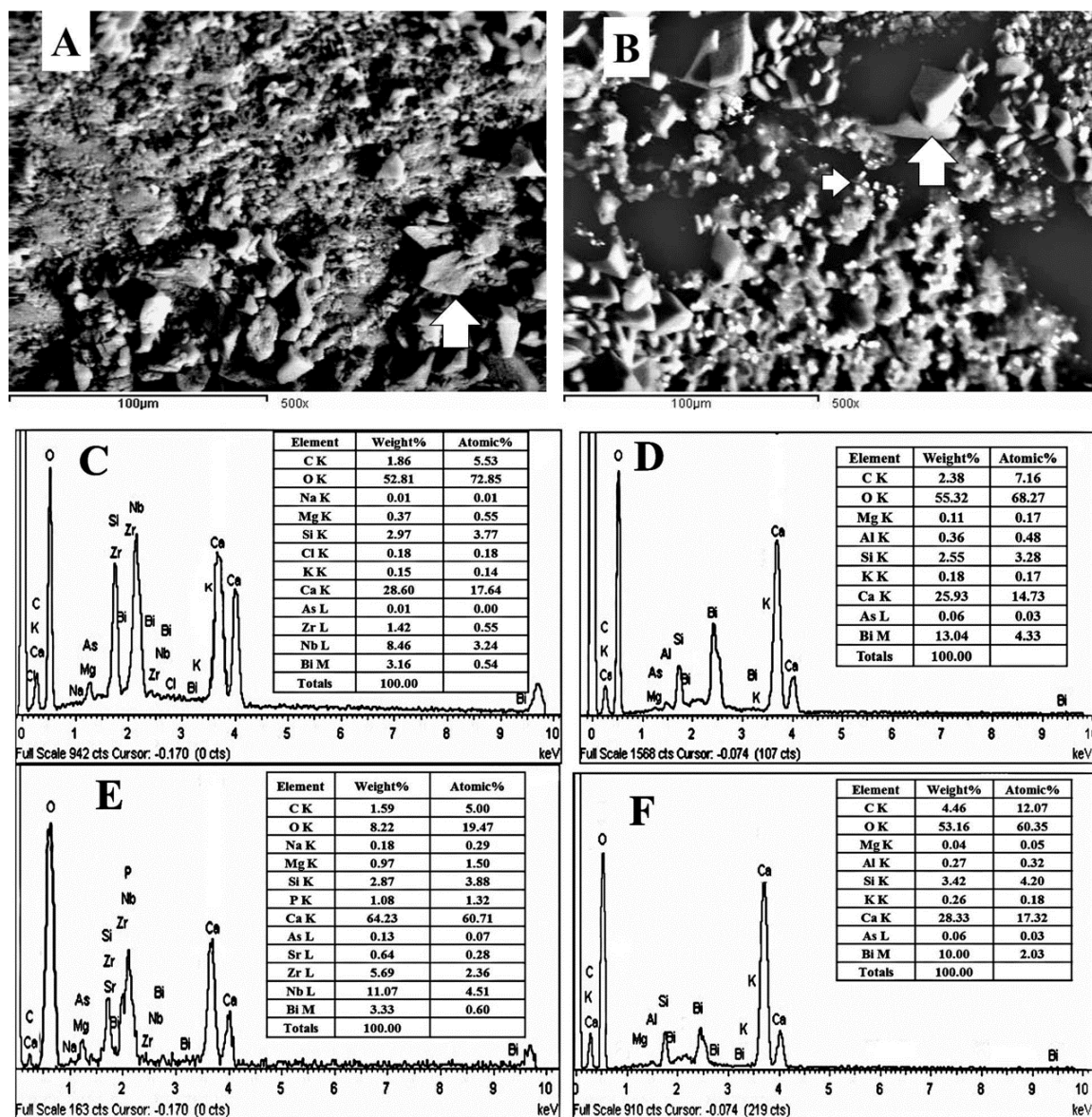


Figure 1: Scanning electron microphotographs of Biodentine (A) and ProRoot-MTA (B) filled dentin samples incubated in deionized water for 28 days. Both samples showed micro-porous granular structures with clusters of minute grains interrupted with large hexagonal-shaped grains (vertical white arrows). There are scattered white dots (a horizontal white small arrow) of radiopaque constituents of ProRoot-MTA. Energy dispersed X-ray analysis of the elemental composition at the surface and material-dentin interface of Biodentine (C & E respectively) and ProRoot-MTA (D & F respectively) showed nearly similar composition but with different proportions of their ingredients.

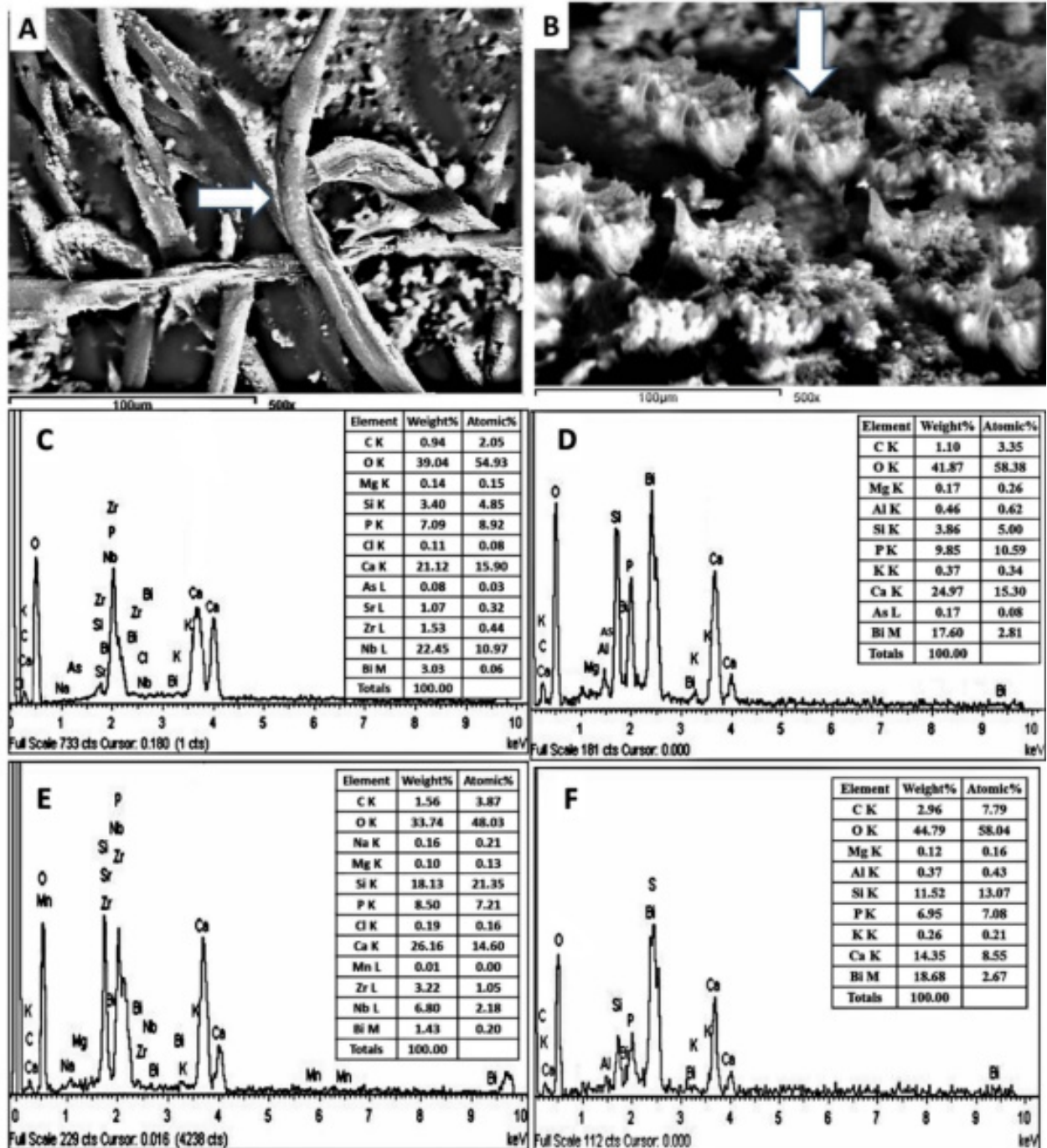


Figure 2: Scanning Electron Micrographs of Biodentine (A) and ProRoot-MTA (B) filled dentin samples incubated in phosphate buffer solution for 28 days. Both samples showed the formation of an apatite layer on their surface. The surface of Biodentine-filled samples had a globular structure overlapped by an apatite layer of fiber or rod-like (horizontal white arrow) structure as tangled branches of a tree. ProRoot-MTA-filled samples however had circular-shaped or flower-like deposits (vertical white arrow). Energy Dispersed X-ray analysis at the surface of both investigated materials (C and D) and their dentin interface (E, and F) revealed calcium phosphate deposits with similar composition but with different proportions of their ingredients.

DISCUSSION

Since the bioactivity of repair material is crucial for achieving an optimal outcome for the perforation repair process, this study compared the bioactivity of Biodentine and ProRoot-MTA when they were placed in PBS for an extended period of four weeks. Determining the Ca/P at the interface and surface of each material was used as an indicative of bioactivity.

The results indicated that Biodentine and ProRoot-MTA showed no significant difference in their carbon, oxygen and silicon ingredients. The calcium content of Biodentine was significantly higher than ProRoot-MTA. Aluminum was only detected in ProRoot-MTA; it was responsible for ettringite formation during the hydration reaction and final setting of MTA (18-20). The absence of aluminum from Biodentine, however, could be related to the active biosilicate technology employed to remove the impurities, and hence improve the biological characteristic of Biodentine (21).

As shown from SEM images, the white dots seen on the surface of ProRoot-MTA could be due to the presence of bismuth that normally was added as a radiopacifier (18). The high content of radiopacifier in ProRoot-MTA could be accounted to its long setting time (22). Biodentine had zirconium and niobium has been indicated from elemental analysis. Recently, niobium was used as a filler in dental restorations; it increases radiopacity, microhardness and adhesive property. Niobium may also reduce solubility and setting time, but improve the dimensional stability and bioactivity of root canal materials (23, 24). Both Biodentine and ProRoot-MTA contained traces of manganese, and strontium. It was believed that these elements may help improve their biocompatibility (18).

After incubation in phosphate buffer solution, the formation of an apatite layer was detected on the surface of both Biodentine and Pro-Root MTA as well as at the material-dentin interface for both materials. The apatite layer has a rod-like structure as tangled branches of a tree in the case of Biodentine. The morphology of the apatite layer seen on Pro-Root MTA however, is different, because of its circular-shaped or flower-like deposits. The difference in morphology of the apatite layer could be attributed to the difference existing in their calcium and silicon constituents particularly at the dentin interface. Biodentine showed higher calcium and silicon than ProRoot-MTA (15). The calcium-silicon constituents of calcium silicate-based material could interact with dentin in the presence of phosphate buffer solution to form intrafibrillar apatite deposition, and tag-like structure that improve the material's sealing ability (15). Regardless of this variation in the morphology of the apatite layer, its calcium/phosphate ratio was 3.52 ± 1.34 for Biodentine and 3.89 ± 1.27 for ProRoot-MTA. This ratio could indicate that this apatite layer has been a combination of hydroxyapatite and calcium carbonate as previously observed in other studies (25, 26). Based on the current results of bioactivity, there was no reason to reject the null hypothesis.

CONCLUSION

After the incubation for 28 days, both materials showed the formation of an apatite layer in their surfaces as well as at material-dentin interface. Ca/P ratio showed no significant difference between both materials in these layers. The similarity in Ca/P ratio between the two materials indicated similar behavior under similar conditions. Due to comparable bioactivity, Biodentine could be used as an alternative to ProRoot-MTA as a root repair material.

REFERENCES

1. Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: the Toronto study. Phases I and II: Orthograde retreatment. *J Endod* 2004;30(9):627-633.
2. de Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, et al. Treatment outcome in endodontics: the Toronto study--phases 3 and 4: orthograde retreatment. *J Endod* 2008;34(2):131-137.
3. Gorni FG, Gagliani MM. The outcome of endodontic retreatment: a 2-yr follow-up. *J Endod* 2004;30(1):1-4.
4. Siew K, Lee AH, Cheung GS. Treatment Outcome of Repaired Root Perforation: A Systematic Review and Meta-analysis. *J Endod* 2015;41(11):1795-1804.
5. Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995;79(6):756-763.
6. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review-part III: clinical applications, drawbacks, and mechanism of action. *Journal of Endodontics* 2010;36(3):400-413.
7. Torabinejad M, Parirokh M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part II: other clinical applications and complications. *Int Endod J* 2018;51(3):284-317.

8. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. *Journal of Endodontics* 2005;31(2):97-100.
9. Gandolfi MG, Taddei P, Tinti A, Prati C. Apatite-forming ability (bioactivity) of ProRoot MTA. *International Endodontic Journal* 2010;43(10):917-929.
10. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--Part I: chemical, physical, and antibacterial properties. *J Endod* 2010;36(1):16-27.
11. WANG Z. Bioceramic materials in endodontics. *ENDODONTIC TOPICS* 2015;32(1601-1538):27.
12. Parirokh M, Torabinejad M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part I: vital pulp therapy. *Int Endod J* 2018;51(2):177-205.
13. Zanini M, Sautier JM, Berdal A, Simon S. Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization. *J Endod* 2012;38(9):1220-1226.
14. Atmeh AR, Chong EZ, Richard G, Festy F, Watson TF. Dentin-cement interfacial interaction: calcium silicates and polyalkenoates. *J Dent Res* 2012;91(5):454-459.
15. Han L, Okiji T. Bioactivity evaluation of three calcium silicate-based endodontic materials. *International Endodontic Journal* 2013;46(9):808-814.
16. Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentine. *Int Endod J* 2011;44(12):1081-1087.
17. Ya shen bp, yan yang, jingzhi ma & markus haapasalo. What do different tests tell about the mechanical and biological properties of bioceramic materials. *Endodontic Topics* 2015;32:38.
18. Dammaschke T, Gerth HUV, Züchner H, Schäfer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. *Dental Materials* 2005;21(8):731-738.
19. Camilleri J. Characterization of hydration products of mineral trioxide aggregate. *International Endodontic Journal* 2008;41(5):408-417.
20. Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR. The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. *International Endodontic Journal* 2005;38(11):834-842.
21. Arora V, Nikhil V, Sharma N, Arora P. Bioactive dentin replacement. *Journal of Dental and Medical Sciences* 2013;12(4):51-57.
22. Formosa LM, Mallia B, Bull T, Camilleri J. The microstructure and surface morphology of radiopaque tricalcium silicate cement exposed to different curing conditions. *Dental Materials* 2012;28(5):584-595.
23. Viapiana R, Guerreiro-Tanomaru JM, Hungaro-Duarte MA, Tanomaru-Filho M, Camilleri J. Chemical characterization and bioactivity of epoxy resin and Portland cement-based sealers with niobium and zirconium oxide radiopacifiers. *Dental Materials* 2014;30(9):1005-1020.
24. Leitune VCB, Collares FM, Takimi A, Lima GBd, Petzhold CsL, Bergmann CPr, et al. Niobium pentoxide as a novel filler for dental adhesive resin. *Journal of dentistry* 2013;41(2):106-113.
25. Abu Zeid ST, Alamoudi NM, Khafagi MG, Abou Neel EA. Chemistry and Bioactivity of NeoMTA Plus™ versus MTA Angelus® Root Repair Materials. *Journal of Spectroscopy* 2017;2017(Article ID 8736428):1-9.
26. Alqedairi A, Muñoz-Viveros CA, Pantera EA, Campillo-Funollet M, Alfawaz H, Neel A, et al. Superfast Set, Strong and Less Degradable Mineral Trioxide Aggregate Cement. *International journal of dentistry* 2017;2017(Article ID 3019136):1-9.