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The Sealing Ability of Biodentine and MTA as a Root Sealer in the Management of Open Apices of Permanent Teeth

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Abstract

Objectives: Introduction: The root seal should provide an impermeable seal in different environments to prevent the egress of bacteria from the canal into the peri-radicular tissues and the ingress of peri-radicular fluid into the canal.

Aim: The aim of this pilot study is to assess, by means of an in-vitro investigation using micro-CT and an optical microscope, the quality of the root apical seal achieved with either MTA® or Biodentine™ when placed in a moist environment that simulates the various clinical peri-apical wet environments.

Materials and methods: A total of thirty-six freshly extracted human teeth were randomly allocated to 2 groups: MTA® and Biodentine™. Each group was subdivided into 3 subgroups containing 6 teeth each. Materials insertion and packing occurred while the teeth were immersed in the environmental fluids (Dry, SBF and Acid), following the standard apical divergence and instrumentation. Then 3 mm of the materials were scanned and analysed using the micro-CT scan (MCT) and an optical microscope was used to investigate the integrity of the root-apex at the surface interface seal.

Results: The mean porosity percentage of MTA® and Biodentine™ in the 3 different environments; Dry: 24.08% and 45.42%, SBF: 38.28% and 56.03%, Acid: 46.78% and 50.43% subsequently. There was not any statistically significant difference between the three environments at a P-value=0.16.

Conclusion: Moisture and acidic environment do not have a statistically significant effect on the sealing ability of both materials MTA® and Biodentine™. But they generate morphological changes in both materials.

Keywords: Bioceramics • Micro-computed tomographic • Apical plug • Apexification • Immature teeth • Microleakage • Root resorption

Introduction

The aim of root canal treatment is to disinfect the root canal complex shape and achieve an impermeable seal within the canal and the peri-apical foramina to prevent a peri-radicular infection. An adequate endodontic treatment with a fluid tight barrier and apical seal is difficult to achieve in non-vital immature open-apex teeth, due to apex widening and the lack of an apical stop. Open apices exist in the developing root of immature teeth, when the pulp of the teeth experience necrosis. Apexification and the apical barrier technique is the method used to create an apical barrier in non-vital open apex teeth [1,2].

Bioceramics are the materials of choice for the treatment of teeth with an open apex. There are two main bioceramics materials available for creating an artificial apical barrier: Mineral Trioxide Aggregate (ProRoot MTA®) (Dentsply, Cauk, USA) and Biodentine™ (St Maur des Fosses, Septodont, France) [3].

MTA® had many properties [4,5]. Several clinical studies have reported a clinical outcome success rate ranging from 94% to 100% and survival rate of 96% over 15 years. MTA® provided scaffolding for hard tissue formation and a better biological seal, with a reduction in the number of fractures in thin roots [6-11]. Biodentine™ was recently used and could be considered as a substitute for MTA®, because of its properties and the fact that it can overcome the drawbacks of MTA®. Biodentine™ showed highly successful outcomes and a similar success rate to MTA® when used for apical closure [12-14].

In clinical situations, the presence of blood, tissue fluids, inflammation and infection from the surrounding peri-radicular area might affect the material's properties and its adaptation to root dentin, which may in turn influence the material's sealing ability. In-vitro studies showed that presence of blood, acid and moisture affect the material's structure and sealing ability. Presence of moisture could affect ProRoot-MTA® and Biodentine™ properties and their apical seal [15-17]. The acidic environment (PH 5) affects the setting of MTA® with presence of erosive surfaces and the use of Biodentine™ was preferred in the presence of infection [18,19]. The effect of the blood on the MTA®'s sealing ability by formation of gabs in presence of blood during the hydration process [20]. There is lack of in-vitro studies investigating the sealing ability of MTA® and Biodentine™ while placing them in immature teeth in different environments.

Various methodologies have been introduced to assess the quality of root canal fillings, such as scanning electron microscopy (SEM), fluid filtration, bacterial microleakage, toxin infiltration, the micro-computed tomographic method (micro-CT) and optical microscope [21,22]. The most commonly used method was dye penetration in-vitro for examining microleakage, which is no longer considered a valid method [23-25].

There are limited studies investigating the sealing ability of both materials, but they are not clinically relevant as they don't simulate the clinical placement procedure. Such as, placement of the materials in a dry environment [26], compaction of both materials in dry environment and then subsequently, stored them in different environmental solutions [27,28]. In addition, these studies didn't mention the application of their condensation pressure, which could have affected the outcomes as it was variable and uncontrolled

[29]. No study investigated the effect of condensation pressure on the sealing ability of both materials. And finally, Diversity between studies evaluated the sealing ability of MTA® using different unstandardized methods that might affect the outcomes [30-32].

Since there is a lack of studies investigating the sealing ability of Biodentine™ and MTA® in immature teeth while placing them in moist environments, especially acidic ones, this pilot study was conducted with reference to the clinical situation to replicate true clinical placement procedures with the root end submerged in the solution of interest and compaction of both materials against sponge at the root-tip; so that the setting of the material may be affected by the solution during placement, setting and maturation. Additionally, using highly sensitive and standard method (MCT) to measure the sealing ability [33].

The hypothesis is that the quality and integrity of the root-apex seal achieved with either MTA® or Biodentine™ in an open-apex configuration are affected by the nature of the periapical environment into which it is placed. Moreover, there is no difference in the sealing ability of either Biodentine™ or MTA® in the presence of simulated apical bio-fluids. Also, there is no difference in the quality and integrity of the root-apex at the surface interface seal achieved as a function of the different periapical environments tested. Based on this, the aim of this study is to assess, by means of an in-vitro investigation using micro-CT and an optical microscope, the quality of the root apical seal achieved with either MTA® or Biodentine™ when placed in a moist environment that simulates various clinical peri-apical wet environments.

Materials and Methods

A total of thirty-six freshly extracted human teeth were selected and stored in buffered saline. These teeth were obtained in accordance with STHNHS Ethics approval REC reference 16/WM/0236, amendment SA01 (Protocol STH18841). The selected tooth samples had straight single roots with completely formed apices. Only teeth with no previously filled root canal, caries or cracks were included. Thirty-six teeth were randomly allocated to 2 groups (MTA® and Biodentine™ materials) and then 3 subgroups contain 6 teeth each. 2 simulated environmental sub-groups: Simulated Body Fluid (SBF) simulating the moisture environment, butyric acid at 5 PH simulating the acidic environment and a dry environment as a control using a foam sponge with PTFE tape not soaked in any fluid. Teeth were prepared by de-coronation at the cement-enamel junction with a diamond disc at high speed with a water spray coolant for the single rooted tooth.

Apical divergence

Apical divergence was performed by removing 2 mm of the apical tip with a diamond disc for removing any apical delta and standardisation of the canal exits to the centre of the tooth. The apical divergence was performed following the Hachmeister et al. in-vitro experimental model method for open apex [34,35]. The divergent open apex was prepared using retrograde apical preparation with a Sx gold Protaper file and F5 with 0.5 taper Protaper Gold® files. A standardised root width of 1.2 mm was used (Figure 1).

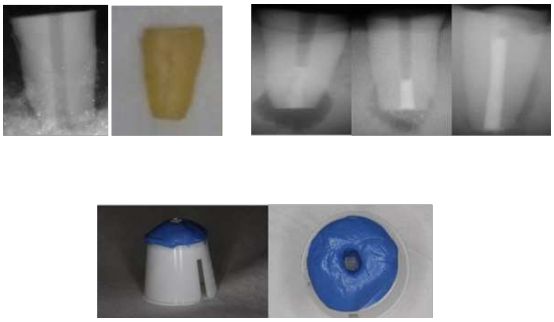


Figure 1. (A) the teeth after de-coronation, apical divergence and canal instrumentation. (B) Radiographs following a Biodentine™ and MTA® apical-plug and final post-operative obturation, to determine the length and voids within the obturation. Presence of the radiolucency at the root-tip due to presence of the sponge that simulate periapical granuloma tissues (C) The tooth's vertical position and the foam sponge to simulate the clinical situation and procedure.

Apical plug

Endodontic instrumentation using hand K-flex files #10, 15 and rotary gold pro-taper files #S1, S2. Irrigations used are 5.25% NaOCl and 17% EDTA as a pre-final flush. The teeth were randomised and kept in moist gauze at a temperature of 37°C. The teeth were stabilised by positioning them on a vertical axis that faced the floor using a silicon impression. Teeth were embedded in a pot with a silicon impression base containing a hole in the middle for a PTFE tape and a sponge soaked in the simulating periapical fluid where the root-end will be submerged (Figure 1). Both the MTA® and Biodentine™ were mixed according to the manufacturer's instructions followed by Orthograde condensation up to the apical end at 3 mm thickness while the root-end were submerged in the dry and environmental fluids. Packing and condensation of the material (MTA®/ Biodentine™) was carried out in a manner analogous to a clinical situation. The root apex was resting on a sponge that offers a similar resistance to compaction to that of periapical granulation tissue, then immediately after, placed in humidity chamber. And then one by one was retrieved for gutta-percha back-filling. All canals were back-filled with gutta-percha vertical condensation using Elements™ and sealer (Tubli-seal™) followed by 2 mm of composite filling (3M™). Radiographs were taken after the apical divergence, after the apical plugs and after the gutta-percha back-filling (Figure 1). The samples were stored in the humidity chamber for 7 days of 37°C before the Micro-CT scanning.

MCT-CT scan

The samples were scanned after adjusting appropriate parameters for scanning according to the most accurate, based on a previous study [36]. Each tooth was scanned from the apex to 3 mm above the divergence root tip; using a resolution of mm at 75 kV and 118 µA with a (2000 × 1048) pixel size of 4.8 mm. Scanning was performed with a rotational angle of 180° around the root longitudinal axis, using a 0.7° rotation step and a 3 sec. exposure time. The scan estimated 17 mins. An AL 1.0 mm filter was used to reduce the image noise. Then, images were created for each tooth and transferred to Skyscan CT-analyzer (CTan) software and reconstructed with NRecon (SkyScan) software. A set Volume of Interest (VOI) was defined as a full cross-sectional area including the material and the dentine walls and the material apical plug along the long axis of the 3 mm of the root. The CTan and CTVol (Skyscan) software was used for the 3-Dimensional (3D) volumetric visualization, analysis, and measurement of the porosity within the structure of the material (voids) and at the interface between the dentin walls and the materials' apical plugs (gabs) were calculated combined together. The percentage of voids and gaps was calculated as the Mean Percentage of the Porosity (MPP).

Light optical microscope

The images in this study were obtained using a light optical microscope with a magnification (2X, 4X and 8X), then analysed and captured using an axio-cam ICC camera. To analyse qualitatively the surface topography of the root end sealer and the interface with the dentine following placement and maturation of the material.

Statistical Analysis

A Two-way Anova and Bonferroni post hoc test were used to determine any statistically significant differences between the two groups by using IBM SPSS software version 16. The level of significance was set at P<0.05. The power of the calculation was set at 1-ß=0.8, and a group sample size of 75 (power set at 80%) was needed to show the significance differences between the three environments.

Results

The mean porosity percentage (MPP) of MTA® and Biodentine™ in the 3 different environments; Dry: 24.08% and 45.42%, SBF: 38.28% and 56.03%, Acid: 46.78% and 50.43% subsequently. MPP increased when both materials were inserted in SBF and acid, but this was not statistically significant different, at a P-value=0.16. Although the control subgroups of both materials in dry environment was statistically significant different, at a P-value=0.00. Presence of small standard deviation in the control subgroups while large standard deviation in both materials in the tested subgroups. Which illustrated the changes and variability in structure occurred to both material while submerging in different environments.

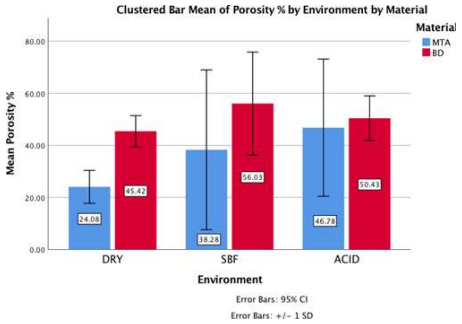
Discussion

This study measures quantitatively the Mean Percentage of Porosity (MPP) within the material in each sample, and qualitatively the 3D and 2D reconstruction images of the filling material in the root as well as the images from the optical microscope to show the integrity of the materials at the interface with the dentine wall.

There is no standard method that can be used to measure the apical sealing ability of any root filling. The available methodologies for measuring micro leakage are not standardised. Since the study results depend on the test method used, it is very important to choose highly sensitive, reproducible and standardised method. That's why the MCT method was preferred to measure the sealing ability of both materials. As, it can be standardised, provide both qualitative and quantitative analysis of the material, it is rapid, with high accuracy and sensitive results, non-destructive, and comparable with histologic studies [37-40].

SBF is considered an acellular simulated body fluid that has inorganic ion concentrations similar to those of human extracellular fluid and body plasma fluid. It was prepared at 7.5 pH according to Kokubo's protocol [41,42]. Butyric acid was used, as it is a by-product produced by the metabolism of anaerobic bacteria. Acidity at a 5 PH value was chosen because the pulpal infection and periapical inflammation lowers the peri-radicular tissue pH to around 5.5 near the involved tooth. Freshly-mixed Biodentine™ and MTA® materials were packed inside the canal and then inserted into a different environment to expose their surfaces to various environments, as that might affect their hydration reaction. The rationale for using a 3 mm apical plug is because a plug of thickness 3-5 mm is considered optimal and produces a reasonable seal for MTA® application. Also, 3 and 4 mm thick apical plugs reveal a good sealing ability with less microleakage of fluid filtration [43]. A PTFE was used to prevent attachment of the materials to the sponge.

The efficacy of a material is affected by its sealing ability, which in turn is affected by the percentage of voids and gaps (porosity), it decreases as the percentage of porosity increases. In the acidic environment, MTA® had the highest MPP of 46.78% compared to the other two environments that might be as MTA® crystals dissolve in pH 5 acidic environments. Regarding Biodentine™, the MPP was 50.43% higher than the dry environment 45.42% but lowers than SBF. It was claimed that the sealing ability of Biodentine™ in acid was enhanced by the acidic environment over time, as it showed morphological changes different from MTA®, but MPP increased in both materials in this study (Graph 1, Figure 2). Also, two of the samples showed Biodentine™ separation and extrusion out of the dentinal wall and the presence of large porosities in the presence of acid (Figure 3).



Graph 1: MPP of MTA® and Biodentine™ in the three different environments with their standard deviation.

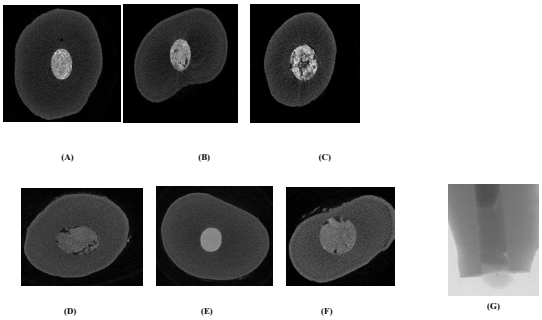


Figure 2: (A, B, C) The 2D reconstruction of cross-sectional view for MTA® subgroups, voids are in radiolucent appearance. (A) MTA® in a dry environment shows homogenous filling with a small percentage of voids. (B) MTA® in SBF fluid shows an increase in the percentage of voids. (C) MTA® in butyric acid shows the presence of the largest percentage of voids in the subgroups. (D, E, F) The 2D reconstruction images of cross-sectional view for Biodentine™ subgroups. (D) Biodentine™ in a dry environment showing the presence of voids. (E) Biodentine™ in SBF fluid showing no presence of voids. (F) Biodentine™ in butyric acid shows the presence of voids. (G) Shows the separation of Biodentine™ apical plug from the dentinal wall in the apical part after 1 week.

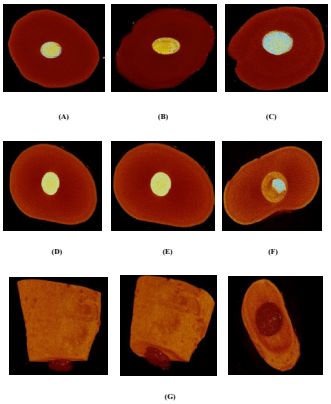


Figure 3. (A, B, C) The 3D reconstruction images for MTA® subgroups and the presence of voids are indicated in blue. (A) MTA® in a dry environment (B) MTA® in SBF fluid (C) MTA® in butyric acid. (D, E, F) illustrate the 3D reconstruction images for Biodentine™. (D) Biodentine™ in a dry environment. (E) Biodentine™ in SBF. (F) Biodentine™ in butyric acid. (G) Showing the apical third of the root including the Biodentine™ material separation and extrusion and a cross-section of Biodentine™ that was in interface with the acid.

SBF could simulate both environments' moisture and blood contamination. The presence of MTA® in the SBF had a higher MPP of 38.28% than the dry environment 24.08%, but it was lower than MTA® in acid, while the presence of Biodentine™ in SBF had the highest MPP of 56.03% (Graph 1). It was claimed that excessive moisture might delay the setting reaction of Biodentine™, which results in its separation from dentin, thus affecting its sealing ability. But in this study, that occurred in the presence of acid (Figures 2, Figure 3) [44]. It was observed that, MTA® was significantly less porous than Biodentine™, with MPP of 36% and 51% for Biodentine™ at P value=0.03(P<0.05) in total. And it was significantly less porous with MPP of 24.08% and 45.42% for Biodentine™ at a P-value=0.00 in the control subgroups as well, which conflicts with Bani et al.

There was not any statistical significance difference between the 3 subgroups, possibly due to the large variability in both materials when they were immersed in the tested environmental solutions. This was illustrated by the large standard deviation, as a result of changes in the materials' morphology in the different solutions. And not due to technique fault as the control subgroups showed small standard deviation. When both materials are used as apical plug barriers in immature teeth, different pressures are applicable. Higher condensation pressures were associated with less porosity. MTA® images have been used, as they offer a higher radio-opacity and distinguish between the material and the porosity. The porosity tends to increase with apex proximity, possibly due to operative technique combined with material dissolution; as the porosity in the first layer of MTA® faces the acidic environment increases more than in the dry environment and more than 3 mm away from the apex in both dry and acid environments (Figure 4). While the operative technique could result in porosity closer to the apex, as the condensation pressure may be reduced in the first layers and subsequently increased, to prevent material extrusion into the sponge (as periapical tissues), similar to a clinical situation. The operator who undertook the experiment is an experienced operator. Also, training was undertaken prior to the experiment to avoid any technique-related errors arising. In addition, a periapical radiograph confirmed correct material packing and void absence. A sponge placed below each root resembled periapical tissue apical plug resistance, has not been mentioned before in any of the previous studies. That is a valid point to discuss, as varying amounts of pressure were exerted when placing the material's first and other layers. A 4.8 mm pixel size was used to identify the smallest sized voids/porosity compared to bacteria size (0.5-5 mm), which porosities might allow bacteria transportation. That suggests that the well-condensed apical plug's 2D radiograph might contain voids and that presence of infection might result in material dissolution. For these reasons, apical plug thickness should not be less than 3 mm.

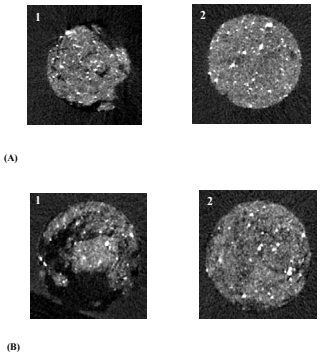


Figure 4. (A) These cross-sectional images from the same sample of MTA® material in dry environment, to show the effect of the applied condensation pressure on the material apical plug layers. Image 1: shows MTA® layer facing the dry sponge with presence of porosity. Image 2: shows MTA® plug at 3mm from the apex with less porosity. (B) These cross-sectional images from the same sample of MTA® in acid environment, to show the effect of the acidic environment on the material apical plug layers. Image 1: shows MTA® layer facing the sponge soaked in butyric acid with presence of large number of porosities. Image 2: shows MTA® plug at 3mm from the apex with less porosity.

In the microscopic images; over all, both Biodentine™ and MTA® groups showed high quality and integrity of the root-apex at the surface interface. Only one sample showed presence of a gab in MTA® group (5) while, in the Biodentine™ group, four samples showed the presence of gaps and a lack of integrity of the root-apex at the surface interface. Between the subgroups, the SBF subgroup showed the high quality and integrity of the root-apex at the surface interface with no presence of gaps in either material, possibly due to the effect of moisture on the material, resulting in its expansion. The acid subgroup images showed a difference in the appearance and morphology of both materials, with presence of smooth and rough surface at the surface interface. That was reported in previous studies, suggesting that formation of spheroidal crystals when Biodentine™ is exposed to an acidic environment. While, for MTA® due to the absence of the needle like crystals and erosion of the cubic crystal surfaces in pH5 environment (Figures 5 and 6) [45]. The hypotheses of this study were accepted, although it is limited to small sample size and being in-vitro pilot study. In order to quantitate the effects of the environment on the sealing ability of the materials, a very large group size is needed for definite conclusions. This pilot study could be used as an experimental model for open apex-teeth simulating the clinical situation and procedure, to measure the sealing ability of the apical plug fillings.

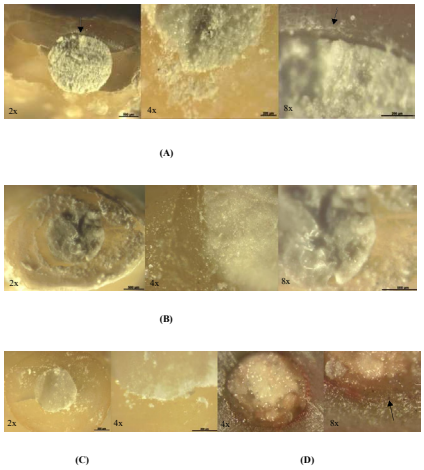


Figure 5. The microscopic images of MTA® apical plug at 2x and 4x magnification, to show the quality and integrity of the root-apex at the surface interface. With the presence of gaps, an 8x magnification was applied to illustrate the lack of integrity of the root-apex at the surface interface. (A) MTA® in the dry environment with no presence of a gap at the interface. In the 8x magnification image, the presence of a gap at the interface was observed in one of the dentine walls. (B) MTA® in the SBF with no presence of gaps. (C) MTA® in the acidic environment with no presence of gaps. (D) Shows changes in the morphology and microstructure of MTA® at the interface in some of the samples.

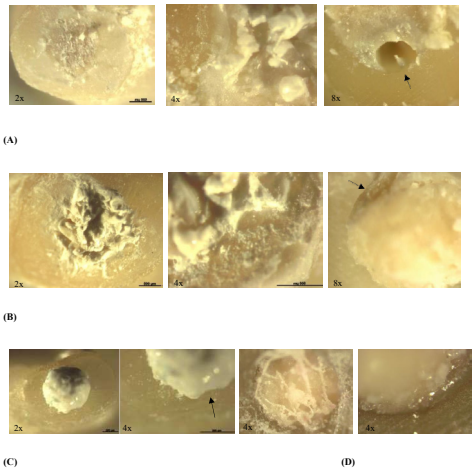


Figure 6. The microscopic images of Biodentine™ apical plug at 2x and 4x magnification, to show the quality and integrity of the root-apex at the surface interface. With the presence of gaps, an 8x magnification was applied to illustrate the lack of integrity of the root-apex at the surface interface. (A) Biodentine™ in the dry environment with no presence of gaps. (B) Biodentine™ in the SBF environment with no presence of gaps. (C) Biodentine™ in the acidic environment, it shows the sample that has separated and Biodentine™ extrusion (D) Shows different samples without extrusion with no presence of any gaps between the dentine wall and the Biodentine™, but changes in the morphology and microstructure of the Biodentine™ in some of the samples.

Conclusion

Within the limitations of this study, moisture and acidic environment did not have a statistically significant effect on the sealing ability of both materials MTA® and Biodentine™. But they generated morphological changes in both materials. This pilot study was designed to provide an indication about MTA® and Biodentine's™ potential behaviour when they are placed in different environments. Further research should be undertaken to establish a correlation between the exposure to different environments especially in the cases of infection and trauma cases and the clinical performance of these materials.

References

1. Flanagan, T.A. "What can cause the pulps of immature, permanent teeth with open apices to become necrotic and what treatment options are available for these teeth". Aust Endod J 40.3(2014):95-100.
2. Rafter, M. "Apexification: a review". Dent. Traumatol 21.1(2005):1-8.
3. Wang, Z. "Bioceramic materials in endodontics" Endodontic topics 32.1(2015):3-36.
4. Torabinejad, M., et al. "Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part II: Leakage and Biocompatibility Investigations". J Endod 36(2010):190- 202.
5. Parirokh, M., et al. "Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part III: Clinical Applications, Drawbacks, and Mechanism of Action". J Endod 36(2010a):400-413.
6. Nicoloso, G.F., et al. "A comparative evaluation of endodontic treatments for immature necrotic permanent teeth based on clinical and radiographic outcomes: a systematic review and meta- analysis". Int J Paediatr Dent (2017).
7. Lu, J.X., et al. "Comparison of mineral trioxide aggregate and calcium hydroxide for apexification of immature permanent teeth: A systematic review and meta-analysis". J Formos Med Assoc 115(2016):523-530.
8. Parirokh, M., et al. "Mineral trioxide aggregate: A comprehensive literature review—part i: Clinical applications, drawbacks, and mechanism of action". J Endod 36(2010):16-27.
9. Torabinejad, M., et al. "Regenerative Endodontic Treatment or Mineral Trioxide Aggregate Apical Plug in Teeth with Necrotic Pulps and Open Apices: A Systematic Review and Meta-analysis". J Endod 43(2017):1806-1820.
10. Torabinejad, M., et al. "Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview – part II: other clinical applications and complications". Int Endod J 51.3(2018):284-317.
11. Ree, M.H., et al. "Long-term Success of Nonvital, Immature Permanent Incisors Treated With a Mineral Trioxide Aggregate Plug and Adhesive Restorations: A Case Series from a Private Endodontic Practice". J Endod 43(2017):1370-1377.
12. Rajasekharan, S., et al. "Biodentine™ material characteristics and clinical applications: A 3 year literature review and update". Eur Arch Paediatr Dent 19(2018):1-22.
13. Vidal, K., et al. "Apical Closure In Apexification: A Review And Case Report Of Apexification Treatment Of An Immature Permanent Tooth With Biodentine". J Endod 42(2016):730-734.
14. About, I., "Biodentine: from biochemical and bioactive properties to clinical applications". G Ital Endod 30(2016):81-88.
15. Uzunoglu, E., et al. "Effects of mixing techniques and dentin moisture conditions on push-out bond strength of ProRoot MTA and Biodentine". J Adh Sci Tech 30(2016):1891-1898.
16. Kim, J., et al. "Interfacial characteristics of Biodentine and MTA with dentine in simulated body fluid". J Dent 43(2015):241-247.
17. Mathew, L., et al. "A comparative evaluation of the microleakage of blood-contaminated mineral trioxide aggregate and Biodentine as root-end filling materials: An in vitro study". J Interdisc Dent 6(2016):19-24.
18. Lee, Y., et al. "Effects of physiological environments on the hydration behaviour of mineral trioxide aggregate". Biomaterials 25(2004):787-793.
19. Elnaghy, A.M. "Influence of Acidic Environment on Properties of Biodentine and White Mineral Trioxide Aggregate: A Comparative Study". J Endod 40(2014):953-957.
20. Zeid, A., et al. "The Effect of Blood Contamination on the Chemical Characterization of Hydrated Mineral Trioxide Aggregates and Their Dentin-Interface: A Comparative Study". Spectroscopy Letters 48(2015):631-637.
21. Wu, M., et al. "Endodontic leakage studies reconsidered. 1. methodology, application and relevance". Int Endod J 26(1993):37-43.
22. Jafari, F., et al. "Importance and methodologies of endodontic microleakage studies: A systematic review". J Clin Exp Dentistry 9(2017):e812.
23. Ver, A., et al. "Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review". J Oral Sci 48(2006):93-98.
24. Camps, J., et al. "Reliability of the Dye Penetration Studies". J Endod 29(2003):592-594.
25. Oliver, C.M., et al. "Correlation between clinical success and apical dye penetration". Int Endod J, 34(2001):637-644.
26. Bani, M., et al. "Efficacy of Biodentine as an Apical Plug in Nonvital Permanent Teeth with Open Apices: An In Vitro Study". Biomed Res Int (2015).
27. Agrafioti, A., et al. "Comparative evaluation of sealing ability and microstructure of MTA and Biodentine after exposure to

different environments". Clin Oral Investig 20(2016):1535-1540.

28. Yazdi, K., et al. "Microstructure and chemical analysis of four calcium silicate-based cements in different environmental conditions". Clin Oral Investig (2018):1-10.
29. Nekoofar, M., et al. "The effect of condensation pressure on selected physical properties of mineral trioxide aggregate". Int. Endod. J. 40(2007):453-461.
30. Yazdizadeh, M., et al. "Evaluation of Apical Microleakage in Open Apex Teeth Using MTA Apical Plug in Different Sessions". ISRN Dentistry 2013.
31. Torabinejad, M., et al. "Dye leakage of four root end filling materials: Effects of blood contamination". J Endod 20(1994):159-163.
32. Torabinejad, M., et al. "Bacterial leakage of mineral trioxide aggregate as a root-end filling material". J Endod 21(1995):109-112.
33. Jung, M., et al. "The imaging of root canal obturation using micro- CT". Int Endod J 38(2005):617-626.
34. Hachmeister, D.R., et al. "The Sealing Ability and Retention Characteristics of Mineral Trioxide Aggregate in a Model of Apexification". J Endod 28(2002):386-390.
35. Martin, R.L., et al. "Sealing Properties of Mineral Trioxide Aggregate Orthograde Apical Plugs and Root Fillings in an In Vitro Apexification Model". J Endod 33(2007):272-275.
36. Sisli, S.N., et al. "Comparative Micro– computed Tomographic Evaluation of the Sealing Quality of ProRoot MTA and MTA Angelus Apical Plugs Placed with Various Techniques". J Endod 43(2017):147-151.
37. Zaslansky, P., et al. "Identification of root filling interfaces by microscopy and tomography methods". Int Endod J 44(2011):395.
38. Rhodes, J., et al. "Micro- computed tomography: a new tool for experimental endodontology". Int. Endod J 32(1999):165-170.
39. Nielsen, R.B., et al. "Microcomputed tomography: An advanced system for detailed endodontic research". J Endod 21(1995):561-568.
40. Von Stechow, D., et al. "Three- Dimensional Quantitation of Periradicular Bone Destruction by Micro- Computed Tomography". J Endod 29(2003):252-256.
41. Kokubo, T., et al. "Apatite Formation on Ceramics, Metals and Polymers Induced by a CaO SiO2 Based Glass in a Simulated Body Fluid". Bioceramics 113-120.
42. Oyane, A., et al. "Preparation and assessment of revised simulated body fluids". J Biomed Mater Res 65(2003):188-195.
43. Valois, C.R.A., et al. "Influence of the thickness of mineral trioxide aggregate on sealing ability of root-end fillings in vitro". Oral Surg Oral Med Oral Pathol Oral Radiol Endod 97(2004):108-111.
44. Caronna, V., et al. "Comparison of the Surface Hardness among 3 Materials Used in an Experimental Apexification Model under Moist and Dry Environments". J Endod 40(2014):986-989.
45. Ballal, V., et al. "Effects of chelating agent and acids on Biodentine". Aust Dent J 63(2018):170-176.