TIME-DEPENDENT EFFECT OF DIFFERENT INTRACANAL MEDICAMENTS ON DENTIN MICROHARDNESS AND DISLOCATION RESISTANCE OF MTA USED DURING REGENERATIVE ENDODONTIC TREATMENT

Hamdy M.R*», Elddamony E.M** and Abdelgawad R.A*”

Abstract

Objectives: This study was designed to measure and compare dentin microhardness and MTA (ProRoot, Dentsply Tulsa Dental, Tulsa, OK) dislocation resistance (DR) used in regenerative endodontic treatment (RET) after application of three different intracanal medication (ICM) for 2, 4 and 12 weeks’ time intervals.

Material and methods: One-hundred sixty eight human maxillary central incisors were selected for the study. Teeth were cut apically 12 mm below and coronally 2 mm above the cemento-enamel junction. Canals were instrumented up to instrument F5 (ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland), then Peeso drills (Mani, Tochigi, Japan) were used from No. 1 up to No. 6, passing 1 mm beyond the apical foramen to obtain larger root canals. Between every 2 consecutive instruments 2 mL of 2.5% sodium hypochlorite (NaOCl) used for canals irrigation, final flush using 5 mL of 2.5% NaOCl and 5 mL of 17% ethylene-diamine-tetra-acetic acid (EDTA, Sigma) followed by 10 mL of distilled water. Samples were randomly divided according to the type of the ICM used inside the canal into 4 equal groups (n=42); Group 1: Double antibiotic paste (DAP) paste: 1:1 mixture of ciprofloxacin (Cipro 500 mg, Schering Plough, Kenilworth, NJ, USA) and metronidazole (Flagyl 500 mg, Sanofi-Aventis, Tours, France), Group 2: Bioactive glass powders (BAG S53P4) of 60 mol% SiO2, 12 mol% P2O5 and 28 mol% CaO composition were prepared through sol gel processing route, all reagents were purchased from Sigma-Aldrich (Dorset, UK). Group 3: Non-setting Calcium hydroxide (CH) (Merk, Germany) was used, and finally, Group 4 (Control): where no ICM was applied. Samples were kept in saline solution for either 2, 4, and 12 weeks, randomly selected samples from each group n=14 at each interval where intracanal medication removed, half of them n=7 were subjected to dentin Vickers microhardness test, in remaining half n=7 MTA (ProRoot, Dentsply Tulsa Dental, Tulsa, OK) placed 4 mm deep into the coronal third of the roots having a 4 mm-long chamber, samples were stored for a week at 37 °C at 100 % humidity to allow the complete setting of MTA, then push-out test was used to measure the dislocation resistance DR of MTA. Collected data were analyzed using a two-way ANOVA followed by Bonferroni’s post-hoc test was used for pair-wise comparisons (P ≤ 0.05).

* Lecturer in Endodontic Department, Faculty of Dentistry, Suez Canal University
** Lecturer in Dental Materials Department, Faculty of Dentistry, Suez Canal University
INTRODUCTION

Achieving the goal of endodontic treatment while dealing with immature teeth is a great challenge, considering capability to disinfect weak, thin, infected dentinal walls and poor crown/root ratio together with establishing apical seal are jeopardizing their entity. Apexification using long term calcium hydroxide application or mineral trioxide aggregate artificial apical plug application has been traditionally used for management of such cases. However such treatment modalities take long time and proved inability to reinforce weak incomplete roots, moreover long term use of calcium hydroxide reduces the roots fracture resistance significantly. The concept of regenerative endodontic treatment (RET) was introduced by Nygaard-Ostby in 1961, but unfortunately there were drawbacks including; apical cytotoxicity, increased risk for tooth fracture, and discoloration. In 2004 the concept was reintroduced to modernistic endodontics by Banchs and Trope through canal disinfection, bleeding establishment and final sealing with MTA. As long as; a sterile environment is critical for pulp tissue regeneration, so the treatment protocol involves frequent procedures for disinfection of the pulp space using irrigation solutions and effective intracanal medication between sessions. Triple antibiotic paste (TAP) containing metronidazole, ciprofloxacin, and minocycline is proved to be a successful regimen for RET in controlling the root canal pathogen.

Unfortunately; minocycline present in this mixture proved the cause of discoloration of treated teeth. Double-antibiotic paste (DAP) containing metronidazole only and ciprofloxacin is an alternative to avoid discoloration been proposed. However, these antibiotic formulas are developing bacterial resistance and allergic reactions. Considering these disadvantages, calcium hydroxide (CH) may be another promising alternative because of its antimicrobial properties. However, discouraging information about its effectiveness against E.faecalis because of the buffering action of dentin recorded.

Bioactive glass (BAG S53P4) is basically a component system of oxides containing SiO$_2$, Na$_2$O, CaO, and P$_2$O$_5$, was invented by Dr. Hench in 1969 and since then it has been used to treat a variety of medical conditions. It possess antibacterial and acid neutralizing properties through pH increase following alkali ions release in an aqueous environment, reported able to stimulate bone regeneration. Moreover, it has remineralizing effect on the demineralized tooth structure through calcium and phosphate ions releasing. The addition of powdered enamel and dentin definitely enhanced its antimicrobial efficacy. Regarding all these promising criteria, BAGs have been studied in variety of uses in dental field including; alveolar ridges augmentation, periodontal pocket treatment, a filler in dental materials, such as restorative materials, cements, pit and fissure sealants, root canal sealer.

Results: showed that both DAP, CH groups of intracanal medication and time interval had a significant decreasing effect on dentine microhardness and MTA dislocation resistance the DR of MTA (P-value <0.001). While, BAG group’s results revealed significant increase in dentine microhardness and MTA dislocation resistance (P-value <0.001). The time factor displayed a significant effect on dentin microhardness and the DR of MTA (P-value <0.001).

Conclusion: Regarding situations in this study, intracanal medications type and duration of application used in root canals disinfection through RET must be carefully chosen to avoid negative effect on dentin microhardness or DR of MTA jeopardizing the success of the treatment. BAG (S53P4) showed promising results, further studies needed to complete investigations about it as intracanal medication in RET.

KEYWORDS: Regenerative endodontic treatment, MTA, Bioactive glass, Dentin microhardness, Dislocation resistance.
and for hypersensitive teeth treatment. But, although these amazing properties, its efficacy as intracanal medication for RET hasn’t been studied yet. As long as; obtaining the therapeutic effects of intracanal medication requires treatment periods varying from 1 week to several months. As a final step during regenerative endodontic treatment; MTA is placed on the coronal part of the root canal which is a biocompatible, conductive, and inductive calcium silicate-based material that is able to bond to dentin chemically. Bond strength of MTA is an important factor since teeth are exposed to occlusal and procedural forces that might dislodge the MTA after its placement. Alterations in dentin surface properties and reduction of its mechanical properties; flexure strength, microhardness and root resistance to fracture during RET steps may influence the bond strength of MTA that placed on the coronal part of the root canal. Therefore, the aim of the present study was to evaluate the time-dependent effect of different intracanal medicaments on the dentin microhardness and dislocation resistance (DR) of MTA.

**MATERIAL & METHODS**

**Samples preparation and grouping**

The protocol of this study was approved by the Ethics Committee of Suez Canal University (Protocol No. 229/2019). One hundred sixty eight human maxillary incisors that had been recently extracted were used and immature, carious, cracked, resorbed, or calcified teeth were discarded. The buccolingual and mesiodistal dimensions of the teeth were obtained radiographically and their mean is calculated and teeth with a maximum of 10% deviation from the mean in each dimension were included and stored in distilled water for 4 months till the time of use. The teeth were shortened to 12 mm apical and 2 mm above the cemento-enamel junction with a low-speed rotary saw (Isomet, Buhler, Lake Bluff, IL, USA) to standardize the teeth length and mimic immature roots. A single operator instrumented all the canals using a rotary nickel-titanium system (ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland). The canals were instrumented up to instrument F5, and then Peeso drills (Mani, Tochigi, Japan) were used from No. 1 up to No. 6 (1.7 mm in diameter), passing 1 mm beyond the apical foramen to obtain larger root canals. The canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) between every 2 consecutive instruments. A final flush was then done using 5 mL of 2.5% NaOCl and 5 mL of 17% ethylene-diamine-tetra-acetic acid (EDTA, Sigma) followed by 10 mL of distilled water. The canals were then dried with paper points (Dentsply Maillefer). Samples were randomly divided according to the type of the ICM used inside the canal into 4 equal groups (n = 42): **Group 1**: DAP paste: 1:1 mixture of ciprofloxacin (Cipro 500 mg, ScheringPlough, Kenilworth, NJ, USA) and metronidazole (Flagyl 500 mg, Sanofi-Aventis, Tours, France) prepared with sterile distilled water (w/v 2.5:1) was delivered into the canals as described by . **Group 2**: Bioactive glass powders (BAG S53P4) of 60 mol% SiO$_2$, 12 mol% P$_2$O$_5$ and 28 mol% CaO composition were prepared through sol gel processing route as previously described by . All reagents were purchased from Sigma-Aldrich (Dorset, UK). The particle size of 70-710 µm which is not ideal to be used as a medicament was transferred into a sterile glass mortar and pestle, crushed into smaller size and then passed through a sieve having mesh size of 45 µm suitable to be used as a medicament, powder was mixed with sterile saline in the ratio of 1.5:1 (wt/vol) to obtain a paste-like consistency. **Group 3**: Non setting CH (Merk, Germany) was used. CH powder was mixed with sterile saline in the ratio of 1.5:1 wt/v to obtain a paste delivered into the canals as described previously. **Group 4(C) Control**: where no ICM was applied.
Intracanal medicaments application

All canals including control group samples were apically sealed using modeling wax (Dentsply DeTrey, Bois Colombes, France) to ensure containment of the ICMs inside the root canal throughout the required duration, and the coronal orifices were sealed with glass ionomer cement (Fuji, GC, Tokyo, Japan). All samples were kept in saline solution, which was replenished every 7 days to avoid dehydration throughout the evaluation period, which lasted up to 12 weeks. After 2, 4, and 12 weeks, randomly selected samples from each group n=14 (representing the samples of each group at each interval). The temporary filling material was removed with a size 3 round bur (Dentsply Maillefer) under water cooling, followed by the removal of DAP, BAG, and CH using 2 mL of 2.5 % NaOCl and 17 % EDTA, then root canals were dried using absorbent paper points.

Microhardness evaluation:

After ICM removal at each time interval, half of randomly selected specimens from each group (n=7) were longitudinally sectioned in a buccolingual direction by using a double faced diamond disk at low speed, without passing through the canal space. This was followed by using a chisel & mallet to split the root. The root segments were then horizontally embedded in auto-polymerizing acrylic resin (Acrotone, Dent Product. Egypt) leaving their dentin surface exposed. The dentin surface was ground flat and smooth with a series of ascending grades of carbide abrasive papers 500, 800, 1,000, and 1,200 grit (Bigo, Dent Product. Germany), under distilled water to remove any surface scratches and finally polished with 0.1-Mm alumina suspension on a rotary felt disc (Microdont LDA. Brazil) to obtain a smooth glossy mirror-like surface. Microhardness measurements were performed using a microhardness tester Tukon 1102 (Wilson Instrument, Norwood, MA) with a Vickers diamond indenter. For each specimen, three indentations were made along a line approximately 0.5 mm from the root canal space at three different dentin levels (the inner, middle, and outer dentin) using a load of 50g/10sec. After the load was removed, the diamond-shaped indentations were carefully observed in an optical microscope with a digital camera and image analysis software, allowing the accurate digital measurement of their diagonals. The average length of the two diagonals (usually to the nearest 0.1-μm) was used to calculate the microhardness value. The representative hardness value was obtained as the average of the results for the three indentations.

MTA application

The remaining half of the specimens at different intervals 2, 4 and 12 weeks from each group (n=7) were used for evaluation of displacement resistance (DR) of MTA at every time interval. After removal of ICM as mentioned before, MTA (ProRoot, Dentsply Tulsa Dental, Tulsa, OK) was prepared in accordance with the manufacturer’s instructions and placed 4 mm deep into the coronal third of the roots with an amalgam carrier having a 4 mm-long chamber. A saline-moistened cotton pellet was placed on top of the MTA to accelerate its homogenous setting. The samples were stored for a week at 37 °C at 100 % humidity to allow the setting of the MTA completely.

Push-out test

For each specimen, a transverse section perpendicular to the long axis of the root to obtain a single slice from each root using an Isomet saw (Buehler, Lake Bluff, IL) under water cooling. Slice thickness was adjusted to 2 mm±0.1 mm utilizing a digital caliper (Mitoyo, Tokyo, Japan), while the root canal space was filled with cement material (MTA). A universal testing machine (Model 3345, Instron, Buckinghamshire, UK) was used to dislodge the plug materials utilizing a 1.3-mm-diameter cylindrical plunger in an apical-coronal direction at
a load cell of 500 N and a crosshead speed of 0.5 mm/min until failure. The maximal force applied to the material before displacement was recorded in newtons (N). The following equation was used to calculate the push-out bond strength (MPa), as an indicator of retention: Push-out bond strength (MPa) = Force to dislodgement (N)/Adhesive surface area (mm²). For each section, the adhesion surface area (A) and MTA retention with intracanal medications was calculated as follows: (πr1 + πr2) × L, and the value of L was calculated as the square root of (r1 − r2)² + h², where π is a constant equal to 3.14, r1 is the smaller radius, r2 is the larger radius, and h is the thickness of the section in millimeters as measured using a digital caliper.

Statistical analysis

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). All data showed normal (parametric) distribution. Data were presented as mean and standard deviation (SD) values. Two-way Analysis of Variance (ANOVA) was used to study the effect of material, time and their interaction on mean microhardness and push-out bond strength. Bonferroni’s post-hoc test was used for pair-wise comparisons when ANOVA test is significant. Pearson’s correlation coefficient was used to study the correlation between cement thickness and microhardness. The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

Dentin Microhardness

The results showed that material regardless of time had a statistically significant effect on mean microhardness table 1. BAG showed the statistically significantly highest mean microhardness. Control showed statistically significantly lower mean value followed by DAP. CH showed the statistically significantly lowest mean microhardness (P-value <0.001, Effect size = 0.991).

Time regardless of material also had a statistically significant effect on mean microhardness (P-value <0.001, Effect size = 0.728), 2 weeks interval showed the statistically significantly highest mean microhardness followed by 4 weeks showed statistically significantly lower mean value, and 12 interval showed the statistically significantly lowest mean microhardness table(2).

Comparison between materials, time intervals:

Comparisons between the materials revealed significant difference between mean microhardness of different materials (P-value <0.001, Effect size = 0.541) table (3) and figure (1). At 2 weeks; DAP group showed the statistically significantly highest mean microhardness followed by BAG and CH groups with no statistically significant difference between them and Control group showed the statistically significantly lowest mean microhardness.

<table>
<thead>
<tr>
<th>TABLE (1)</th>
<th>The mean, standard deviation (SD) values and results of two-way ANOVA test for comparison between microhardness values of the four materials regardless of time</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAP</td>
<td>BAG</td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>88.4 C</td>
<td>11.6</td>
</tr>
</tbody>
</table>

P-value <0.001* Effect size (Partial eta squared) 0.991

*: Significant at P ≤ 0.05, Different superscripts are statistically significantly different
while after 4 as well as 12 weeks BAG group showed the statistically significantly highest mean microhardness followed by Control group then DAP and CH groups showed the statistically significantly lowest mean microhardness.

**Time intervals Comparison revealed that;** DAP as well as CH groups showed the statistically significantly highest mean microhardness at 2 weeks, statistically significant lower mean value at 4 weeks, and statistically significant lowest mean microhardness at 12 weeks. **While, BAG group** showed statistically significant highest mean microhardness at 12 weeks, statistically significant lower mean value at 4 weeks, and statistically significant lowest mean microhardness at 2 weeks. **Control group;** showed no statistically significant difference between mean microhardness at different time intervals.

**While after 4 as well as 12 weeks** BAG group showed the statistically significantly highest mean microhardness followed by Control group then DAP and CH groups showed the statistically significantly lowest mean microhardness.

**Time intervals Comparison revealed that;** DAP as well as CH groups showed the statistically significantly highest mean microhardness at 2 weeks, statistically significant lower mean value at 4 weeks, and statistically significant lowest mean microhardness at 12 weeks. **While, BAG group** showed statistically significant highest mean microhardness at 12 weeks, statistically significant lower mean value at 4 weeks, and statistically significant lowest mean microhardness at 2 weeks. **Control group;** showed no statistically significant difference between mean microhardness at different time intervals.

**While after 4 as well as 12 weeks** BAG group showed the statistically significantly highest mean microhardness followed by Control group then DAP and CH groups showed the statistically significantly lowest mean microhardness.

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**Time intervals Comparison revealed that;** DAP as well as CH groups showed the statistically significantly highest mean microhardness at 2 weeks, statistically significant lower mean value at 4 weeks, and statistically significant lowest mean microhardness at 12 weeks. **While, BAG group** showed statistically significant highest mean microhardness at 12 weeks, statistically significant lower mean value at 4 weeks, and statistically significant lowest mean microhardness at 2 weeks. **Control group;** showed no statistically significant difference between mean microhardness at different time intervals.
**Push-out bond strength**

The results showed that material regardless of time had a statistically significant effect on mean push-out bond strength (\(P\)-value <0.001, Effect size = 0.853) table (4). BAG group showed the statistically significant highest mean push-out bond strength followed by CH group showed statistically significant lower mean value. There was no statistically significant difference between DAP and control groups; both showed the statistically significantly lowest mean push-out bond strength values.

Time regardless of material showed statistically significant difference between mean push-out bond strength (\(P\)-value <0.001, Effect size = 0.955) table (5). 12 weeks interval showed the statistically significantly highest mean push-out bond strength, 4 weeks showed statistically significantly lower mean value, and 2 weeks period showed the statistically significantly lowest mean push-out bond strength.

**Comparison between materials, time intervals:**

There were statistically significant differences between mean push-out bond strength of different materials at all intervals table (6), figure (2). **At 2 weeks;** BAG showed the highest statistically significant mean push-out bond strength values, control groups showed lower statistically significant values. DAP and CH groups; both showed the statistically significantly lowest mean push-out bond strength values with no statistically significant difference between. **After 4 weeks;** BAG group showed the statistically significant highest statistically significant mean push-out bond strength values, followed by CH, Control with no statistically significant difference between them, DAP showed the statistically significantly lowest mean push-out bond strength. **At 12 weeks;** BAG group showed the statistically significantly highest mean push-out bond strength, followed by Control group, then both DAP and CH groups showed the statistically significantly lowest mean push-out bond strength values, no significant difference between both.

**Table (4) The mean, standard deviation (SD) values and results of two-way ANOVA test for comparison between push-out bond strength values of the four materials regardless of time**

<table>
<thead>
<tr>
<th></th>
<th>DAP</th>
<th>BAG</th>
<th>CH</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.95(^c)</td>
<td>6.19(^a)</td>
<td>3.97(^b)</td>
<td>4.64(^c)</td>
</tr>
<tr>
<td>SD</td>
<td>1.98</td>
<td>2.14</td>
<td>1.49</td>
<td>0.98</td>
</tr>
</tbody>
</table>

\(P\)-value <0.001*  Effect size (Partial eta squared) 0.853

*: Significant at \(P \leq 0.05\), Different superscripts are statistically significantly different

**Table (5). The mean, standard deviation (SD) values and results of two-way ANOVA test for comparison between push-out bond strength at different times regardless of material**

<table>
<thead>
<tr>
<th></th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.02(^c)</td>
<td>4.99(^b)</td>
<td>6.81(^a)</td>
</tr>
<tr>
<td>SD</td>
<td>0.62</td>
<td>1.05</td>
<td>1.27</td>
</tr>
</tbody>
</table>

\(P\)-value <0.001*  Effect size (Partial eta squared) 0.955

*: Significant at \(P \leq 0.05\), Different superscripts are statistically significantly different

**DISCUSSION**

Regenerative Endodontic Treatment (RET) was proposed to achieve a significant increase in the thickness and length of immature roots compared to teeth treated with Ca(OH)2 apexification or MTA apical plug.\(^{38,39}\) In RET procedure, a gentle mechanical instrumentation is performed to preserve weak roots,\(^{40}\) as long as disinfection of root canals is mandatory for pulp tissue regeneration.\(^{9}\) American Association of Endodontists (AAE) recommends root canal irrigation using 1.5% sodium hypochlorite (NaOCl), then intracanal medicament application during the first visit for 1-4 weeks.\(^{41}\) Upon absence of clinical signs and symptoms confirmation in the following visit, 17% ethylenediaminetetraacetic acid (EDTA) irrigation in order to release dentin endogenous proteins then bleeding induction to deliver stem cells into the canal and produce natural scaffold.\(^{42,43}\) Root canals dentin is composed of approximately 70: 20:10% ratio of inorganic, organic material, and water respectively, 90% of the organic matter is collagen, which has a major role in mechanical properties of dentin.\(^{44}\) Root canal irrigation using NaOCl causes organic matrix oxidation and

**TABLE (6)** The mean, standard deviation (SD) values and results of two-way ANOVA test for comparison between push-out bond strength values with different interactions of variables

<table>
<thead>
<tr>
<th>Time</th>
<th>DAP</th>
<th>BAG</th>
<th>CH</th>
<th>Control</th>
<th>P-value</th>
<th>Effect size (Partial eta squared)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>2 weeks</td>
<td>5.62&lt;sup&gt;CE&lt;/sup&gt;</td>
<td>0.4</td>
<td>8.5&lt;sup&gt;AE&lt;/sup&gt;</td>
<td>0.54</td>
<td>5.81&lt;sup&gt;CE&lt;/sup&gt;</td>
<td>0.16</td>
</tr>
<tr>
<td>4 weeks</td>
<td>3.74&lt;sup&gt;CF&lt;/sup&gt;</td>
<td>0.21</td>
<td>10.51&lt;sup&gt;AF&lt;/sup&gt;</td>
<td>0.5</td>
<td>4.85&lt;sup&gt;BF&lt;/sup&gt;</td>
<td>0.27</td>
</tr>
<tr>
<td>12 weeks</td>
<td>2.35&lt;sup&gt;CG&lt;/sup&gt;</td>
<td>0.35</td>
<td>12.37&lt;sup&gt;AG&lt;/sup&gt;</td>
<td>0.62</td>
<td>2.7&lt;sup&gt;CG&lt;/sup&gt;</td>
<td>0.3</td>
</tr>
</tbody>
</table>

<sup>*</sup>: Significant at P ≤ 0.05. A,B,C,D superscripts in the same row indicate statistically significant difference between materials, E,F,G superscripts in the same column indicate statistically significant difference between times.

**Fig. (2)** Bar chart representing mean and standard deviation values for push-out bond strength of different variables

**Time intervals Comparison revealed that,** all study groups was a statistically significant difference between mean push-out bond strength at different times (P-value <0.001, Effect size = 0.887), (P-value <0.001, Effect size = 0.901), (P-value <0.001, Effect size = 0.818) and (P-value <0.001, Effect size = 0.644), respectively.
collagen denaturation which changes the mechanical properties, including microhardness due to depletion of the organic phase. \textcite{45,46} Oliveira et al. \textcite{47} reported that 1% NaOCl decreased root dentin microhardness to depths up to 1,000 µm from the canal lumen. Subsequent demineralization of dentin inorganic components by calcium ions chelation effect of EDTA reduces the microhardness \textcite{36}. Cruz-Filho et al. \textcite{48} reported dentin microhardness reduction within the first minute after EDTA application. After then; to complete root canals disinfection, obtain the antimicrobial effect of intracanal medication, it should be placed with time interval 1: 4 weeks’ and treatment periods varying from 1 week to several months which may reach up to 11 weeks \textcite{49}. But, drawbacks of chemical composition and long duration application including; teeth discoloration, cytotoxicity and decreased fracture resistance were recorded \textcite{26,50}. Calcium hydroxide (CH) was one of selected intracanal medications in this study. It is the golden intracanal medication, it has been approved by AAE as an alternative intracanal medication RET \textcite{5}. It doesn’t cause tooth structure staining and enhance the growth factors and biomolecules release from dentin \textcite{51}, it has high pH of 12 couldn’t be maintained due to dentine buffering effect that leads to E. faecalis survival, replication and bacterial biofilm formation which risk the success of RET \textcite{37}. Double antibiotic paste (DAP) was also used in this study owing to its proved efficacy in RET as alternative to TAP to avoid drawbacks of minocycline including; staining of teeth and cytotoxicity against SCAPs \textcite{52,53}. However, bacterial resistance and allergic reactions developed by these antibiotic formulas must be considered \textcite{54}. Bioactive glass (BAG S53P4) was invented by Dr. Hench in 1969 and was prepared using the melting technique and ground to fine particles \textcite{55}. FDA approved bioactive glass (BAG S53P4) for clinical use \textcite{56}. In this study, searching for a better alternative intracanal medication, bioactive glass (BAG S53P4) has been introduced as it proved antimicrobial effect as intracanal medication which inferred to its high pH \textcite{57,58}. In this study; root canal dentin microhardness measurement was performed using Knoop indenter microhardness test, as it can provide evidence about mechanical changes of root canal walls treated with used chemical agents at different time intervals \textcite{13}. In this study; both Calcium hydroxide and double antibiotic paste showed significant decrease in dentin microhardness after 2, 4 and 12 weeks’ time interval. These results are related to their effect on dentin chemical structure which directly related to its microhardness. Previous studies \textcite{59,60} studied the effect of calcium hydroxide intracanal medication for 5 weeks or more, suggested that the alkaline pH of calcium hydroxide denature the organic matrix of the dentine. On the other hand; double antibiotic paste is an acidic material (pH = 3.4), shows a demineralizing effect on the dental hard tissues leading to exposure of collagen-rich matrix of the dentin \textcite{61}. In this study; Bioactive glass used as intracanal medication in RET and its effect on root canal dentine is not previously studied. BAG showed significantly highest mean microhardness which significantly increased after 4 and 12 weeks’ time intervals. These results can be inferred to its composition contains calcium oxide, sodium phosphor and silicon oxides in specific proportions that are responsible for the ability of the material to strongly bond to calcified tissues producing hydroxyapatite layer on its surface that bonded to hard tissues and used in calcified tissues regeneration (e.g., bone, enamel) \textcite{63,64}. Studies described its remineralizing effect as; BAG dissolved and released their ions forming hydroxy-carbonate apatite crystals that obliterate the dentinal tubules more efficiently and protect dentine, inhibit demineralization and facilitate remineralization \textcite{65-67}. Its rule in remineralization of enamel and increase its microhardness also has been reported \textcite{58}. The results showed a negative effect of application period of both CH and DAP where twelve weeks period showed significantly lowest mean microhardness that revealed that the relatively long-term exposure of radicular dentine to intracanal medication might be the reason for the significant reduction in root
dentin microhardness except for BAG. These interesting results shed a light on Bioactive glass use in RET, further in-vitro and in-vivo studies are required to show its bioactive ability enhance regeneration of pulp and root, examine the topography of dentin wall after its application.

Again and according to AAE recommendation, final RET steps are root canal coronal seal through MTA application and tooth coronal final restoration completion. MTA is inductive, conductive, and bio-compatible calcium silicate-based material (tri and di calcium silicate) has ability to chemically bond with dentin. MTA displacement resistance (DR) might be influenced by its thickness, dentin humidity, pH the environment, and dentin pretreatment using either irrigants or medicaments. Complete removal of intracanal medication from root canals is mandatory to allow and maintain bonding efficacy of MTA or other permanent biomaterial filling. As complete intracanal medicaments removal is more challenging in RET because of thin dentinal walls that abolish dentin wall debridement protocols.

Berkhoff et al. 2014. In this study; gentle manual agitation with a ProTaper F5 file accompanied with 5.25% NaOCl and 17% EDTA irrigation. Dentin wall abrasion should be avoided to prevent formation of intracanal medication, smear layer mix that decrease its removal potentials. The integrity of the root filling (MTA)–dentine interface should be preserved as it subjected to displacement forces through function or operative procedures. In this study, the MTA dislocation resistance investigated in vitro it terms of bond strength using push-out test that is widely accepted way for evaluation. Chemical and mechanical dentin properties change after intracanal medicaments application previously proved to significantly decrease MTA dislocation resistance after 2 weeks application of both TAP & DAP, and 4 weeks for CH. In this study; CH & DAP showed significant decrease in MTA dislocation resistance starting from 2 weeks, continued decreasing over 4 and 12 weeks intervals both was lower than control group where no intracanal medication used, in accordance with Patil et al 2019. Physiochemical bond between MTA & dentine wall passes through two stages; initial (mechanical) negatively affected by surface roughness and erosions and final (chemical) through formation of an interfacial layer (hydroxyapatite or carbonated apatite) when MTA comes into close contact with dentin, a chemical bond is formed. Recently, the use of DAP and CH intracanal medication proved to cause Ca and P alteration and increases dentine surface roughness. Moreover, Yassen et al. explained time-dependent deteriorating dislocation resistance of MTA following CH & DAP intracanal medication use as follow; collagen degradation of dentin occurs after CH use, acidic nature of DAP cause dentin demineralization. On the other hand; MTA showed higher DR when used following CH than DAP intracanal medications in accordance with Shokounejad et al., who explained the role of alkaline pH of CH, in addition to reaction of residual Ca ions on remains of intracanal medication with MTA forming calcium carbonate are the cause of higher DR in CH treated group than DAP group. Others studies attributed this to; less quantity and more superficial CH intracanal medication remnants than deeply extended DAP resulted in pH decrease, dentine demineralization, moreover calcium ions chelation deteriorates adhesion of MTA on dentin. In this study; BAG showed a surprising results regarding MTA dislocation resistance which was the highest among used intracanal medications and significantly increased over the study period, as mentioned up to our knowledge no study yet has investigated its effect as intracanal medication on MTA dislocation resistance. The initial (mechanical) high dislocation resistance of MTA could be inferred to large particles size 45 µm of BAG intracanal medication residues that improve the initial (mechanical) bond between MTA and dentin wall, as proved through other studies explained that larger size residues of intracanal medication may improve MTA marginal adaptation due to its physical bond. As well, high pH value 11.65 of BAG-S53P4 gives rise to higher disloca-
tion resistance of MTA as its bond strength proved to become stronger at pH values higher than 8.4. On the other hand; BAG composed calcium oxide, sodium, phosphorus and silica of calcium and phosphate in a proportion mimics bone, it is surface-active material where minerals can bind to hydroxyapatite. When BAG used in dentin mineralization or remineralization it showed the ability to release calcium and phosphate ions and form a bioactive layer hydroxyapatite (HCA) at the interface which is equivalent to the mineral phase of hard human tissues. The (chemical) bond of MTA with dentin walls described by Sarkar et al. as follow; when MTA is hydrated calcium hydroxide and calcium silicate hydrate (CSH) are formed, then calcium hydroxide interaction with phosphate ions in the body forming amorphous calcium phosphate (ACP), that transforms into interfacial layer (hydroxyapatite or carbonated apatite) that improves bond of MTA. As well, it might chemically bind with BAG residues on the dentin wall giving stronger bond to dentin wall, reasonable explanation for high, increasing DR of MTA all over the treatment intervals.

CONCLUSION

Regarding situations in this study, intracanal medicaments type and duration of application used in root canals disinfection through RET must be carefully chosen to avoid negative effect on dentin microhardness or DR of MTA jeopardizing the success of the treatment. BAG (S53P4) showed promising results, further studies needed to complete investigations about it as an alternative intracanal medication in RET.

REFERENCES


41. AAE: Clinical Considerations for a Regenerative Procedure (2016).


