Biocompatibility Assessment of A New MTA Versus Ceramic Based Endodontic Root Canal Sealers

Noha Abdel-Mawla El-Wassef1, Dina Sami Farahat2, Mahitabe Elgamily1, Mohamed Ibrahim Salman1,2, Hussein Abdel Hameed El shikha2

1Associate professor of Dental Biomaterials Faculty of Dentistry Mansoura University, postal code 35516
2Lecturer of Dental Biomaterials Faculty of Dentistry Mansoura University, postal code 35516
3Lecturer of Oral Biology Faculty of Dentistry Mansoura University postal code 35516
4Associate professor of Endodontic Faculty of Dentistry Mansoura University, postal code 35516
5Research intern, Faculty of Dentistry, Mansoura University, postal code 35516
*Corresponding author: mahitabe.fathy@yahoo.com

Received December 27, 2019; Revised February 06, 2020; Accepted February 23, 2020

Abstract Connective tissue response and push-out bond strengths of a new (mineral trioxide aggregate) MTA based endodontic sealer were evaluated. Polyethylene tubes containing ProRoot ES and Endosequence BC sealers were subcutaneously implanted in 45 rats. After 3, 7, 30 days the animals were euthanized, and the specimens were evaluated histologically. Bond strengths of the sealers to root dentine were measured using a push-out bond strength test. Scores of the inflammatory tissue reaction for ProRoot® ES were significantly lower than BC sealer group at 3 days with a thinner fibrous capsule at 30 days. Also, they were not significantly different from the control group. The push-out bond strength of ProRoot® ES could not be evaluated due to handling problems, nevertheless the measured Endosequence BC bond strengths were within an acceptable range. ProRoot® ES had better biocompatibility, seen as more favorable tissue response, than the BC Sealer, although the latter had improved handling characteristics.

Keywords: endodontic sealers, biocompatibility, push out bond strength, MTA, Endosequence


1. Introduction

A significant objective of the obturation stage in endodontic therapy is to provide a hermetic seal of the prepared root canal and its accessory spaces [1]. Endodontic sealers, along with core filling materials like gutta-percha, have an important role for a successful root canal treatment. According to Grossman, an ideal endodontic sealer should provide a bacteria resistant seal with strong bonding to canal walls, possess a reasonable working time, exhibit low solubility in oral and tissue fluids and show a strong antimicrobial influence [2]. Since these materials may contact periapical tissues directly through their extrusion from the apex or indirectly due to leach out of their degradation products, their cytotoxicity may affect wound healing or result in cellular degeneration [3]. Therefore, the root canal sealer’s biocompatibility remains a fundamental concern for choosing a suitable sealer.

ProRoot ES Endo Root Canal Sealer (Dentsply, Tulsa Dental, Tulsa, OK, USA) is a recently introduced mineral trioxide aggregate (MTA) based sealer manufactured from an enhanced formula of ProRoot MTA root repair material. MTAs are bioactive calcium-silicate hydraulic materials whose structure is mainly based on Portland cement constituents [4]. They have been used in endodontic applications such as apexogenesis, pulp capping and as root repair materials due to their biocompatibility, bioactivity and sealing ability. These materials can stimulate the precipitation of apatite via a reaction between the calcium they release and the phosphate present in physiologic fluids [5]. ProRoot MTA root repair materials are mainly composed of tri-calcium silicate, di-calcium silicate, and bismuth-oxide as a radiopacifier [6]. ProRoot ES sealers are supplied as a powder and liquid system in which the powder is described to have a finer particle size that may contribute to a higher calcium ion release and an increased pH level [7]. The liquid is a water based gel, formed of a viscid solution of water-soluble polymers. Mixing the powder and liquid yields a colloidal gel that hardens to form an impermeable barrier against microleakage.

EndoSequence BC Sealer (Brasseler, Savannah, GA, USA) is also a calcium-silicate based bioceramic material that is supplied as a readily mixed injectable paste. It consists of tricalcium silicate, dicalcium silicate, calcium phosphate, zirconium oxide, colloidal silica, and calcium
hydroxide [8]. The presence of water is essential for the hardening of this material, hence it utilizes dentin water content for its setting reaction [9]. It shows powerful antimicrobial effect, biocompatibility, and osteoconductivity. Moreover, this sealer can produce hydroxyapatite during its setting resulting in a chemical bond with the dentin [10].

One of the most important causes of failure of root canal treatment is the apical and coronal escape of bacteria and their byproducts in a leaking endodontically treated root canal [11]. An ideal endodontic sealer should strongly cling to the root dentinal walls to fill in the spaces between dentin and filling materials through which percolation of fluids and bacteria may occur. A strong bond also ensures the stabilization of the apical seal during subsequent manipulation [12]. To determine the efficiency of bond between endodontic materials and dentin, bond strength methods such as the push out test, which utilizes dentin cylinders filled with sealers, have been used [13].

The aim of this study was to assess the biocompatibility and dentin bond strength of ProRoot ES sealer compared to Endosequence BC sealer. The following null hypotheses were tested:

1. There is no difference in the connective tissue reactions caused by subcutaneous implants of the tested materials in rats.
2. There is no difference in dentin bond strength between the tested materials.

2. Materials and Methods

The study was conducted in accordance with the guidelines of Mansoura University Research Ethics Committee (no. 111311118) according to NIH guide for the care and use of laboratory animals, Eighth edition 2011.

2.1. Biocompatibility of Tested Sealers in Subcutaneous Tissue of Rats

Forty-five adult male Sprague Dawley rats weighing between 200g - 250g were divided into three groups, fifteen animals each.

Control group: received empty polyethylene tubes (10 mm length x 3mm diameter).

Group 1: received polyethylene tube filled with ProRoot ES.

Group 2: received polyethylene tube filled with Endosequence BC Sealer.

The animals were intramuscularly injected with xylazine hydrochloride 0.33 ml/100 g (Bayer, Leverkusen, Germany) and zolazepam (Virbac SA, Carros, France). The dorsal fur was shaved, and the skin was disinfected then incised and divulged to insert the testing materials.

Each tube had one end closed with heat to prevent the sealer from extruding. The sealers were injected into the sterile tubes, then inserted into the rats’ dorsal subcutaneous tissue. The incisions were stitched by a 5-0 Vicryl suture material (Johnson & Johnson, Belgium).

Five animals from each group were sacrificed by overdose anesthesia at 3,7 and 30 days. Tissue biopsies containing the implanted tubes (with one cm safety margins) were excised and fixed in 4 % formaldehyde for 24 h. After removal of the tubes, the tissues were fixed in paraffin blocks and processed for histological examination using hematoxylin-eosin stains.

Quantitative assessments of inflammatory cells were done at ×400 magnifications, and the inflammatory reaction was scored as previously described [14];

0 - none or few inflammatory cells and no reaction;
1- less than 25 cells and mild reaction;
2- between 25 - 125 cells and moderate reaction;
3- equal or more than 125 cells and severe reaction.

Statistical methods

Data presented in mean and standard deviation and evaluated using (SPSS, Inc., Chicago, IL, USA) version 23. For comparison of different groups, one way Analysis of variance (ANOVA) and tukey were used. Two-way ANOVA was used to detect the effect of groups & time on the number of inflammatory cells. P value less than 0.05 was considered statistically significant.

2.2. Push-Out Bond Strength to Root Dentin

Twenty human premolar teeth extracted for orthodontic purposes with mature single root canals were selected from Oral Surgery clinic, Faculty of Dentistry, Mansoura University. Only sound teeth with straight roots and a completely formed apex were chosen, while, defected roots were excluded. A radiograph was taken to exclude the presence of other canals and any other anatomical complexities. The roots were prepared using step-back technique up to master apical file #40. Root canals were irrigated after each file using 2 mL 5.25% sodium hypochlorite in a syringe with a 30-gage side vented needle (ProRinse, DENTSPLY, Tulsa Dental Specialties, TN, USA). To remove the smear layer, irrigation of each canal consisted of 2 mL EDTA (17%) (Inter Med-Vista Dental. Racine, WI, USA), which remained in the canal for 1 min, followed by 5 mL NaOCl (2.5%). All canals received a final rinse with 2 mL normal saline and then dried using paper points before receiving the sealers.

For the first group, all teeth were filled with "EndoSequence BC sealer using the BC tip and condensed incrementally a plunger and paper points under magnification. After obturation, teeth were radiographed to make sure their canals are fully-filled. For the second group, however, it was noticed that the ProRoot ES sealer was remarkably difficult in manipulation after mixing, although a syringe was used as a tribunal to facilitate injection into canals. After the material was injected and during condensation, it got pulled out during outward movement because of its high viscosity and stickiness, this prevented the canals from being fully obturated. All teeth were stored for one week at 37°C with 100% humidity to allow sealers to fully set.

Each tooth was poured in acrylic blocks and horizontally sectioned into 1 mm thick slices using a low speed diamond disk under water-cooling. Push-out bond strength test was performed using a universal testing machine [15]. The load was applied in an apico-coronal direction at a crosshead speed of 0.5 mm/min. The test could not be done for the second group (the ProRoot ES) due to the incomplete filling of the canals.
The push-out bond strength was calculated using the formula:

Bond strength in MPa = maximum load in N/adhesion area in mm²

The adhesion area was gauged by using the formula (1):

\[ \pi \left( \eta_1 + r_2 \right) \sqrt{\left( \eta_1 - r_2 \right)^2 + h^2} \]  

Where \( \pi = 3.14 \), \( r_1 \), \( r_2 \) are the coronal and apical radii respectively, and \( h \) is the slice’ thickness

3. Results

3.1. Biocompatibility of Tested Sealers in Subcutaneous Tissue of Rats

3.1.1. At 3 Days

A moderate inflammatory reaction was observed in all of the three groups (Figure 1). The tissues were less organized and infiltrated with remnants of neutrophils, lymphocytes and macrophages, however no areas of necrosis were detected. The score of the inflammatory reaction in ProRoot ES sealer group was significantly lower than that of EndoSequence BC group but not significantly different from the control group (Table 1).

3.1.2. At 7 Days

The tissue began to be more organized with the beginning of the appearance of collagen fibers and fibroblast cells in between (Figure 2). The inflammatory cells were mostly lymphocytes with remnants of neutrophils and macrophages. Multinucleated giant cells were also spotted in specimens of the EndoSequence BC. The EndoSequence BC group showed significantly higher amounts of inflammatory cells relative to the control group, and was also higher than the ProRoot ES group, but not significant. The intensity of inflammation significantly decreased compared to that at 3 days in both the control and EndoSequence BC groups, however, the decrease in the ProRoot ES group was not significant. The number of inflammatory cells in specimens of ProRoot ES group was not significantly different from the control or the EndoSequence BC groups (Table 2).

3.1.3. At 30 Days

The tissue was more organized showing a milder inflammatory reaction with fewer lymphocytes (Figure 3). The number of inflammatory cells significantly decreased in all of the groups compared to their numbers at 3 and 7 days (Table 2). Fibrous capsules were formed with collagen fibers and fibroblast cells in between in all of the specimens. The capsule observed in the ProRoot ES group was significantly thinner than that observed in EndoSequence BC group which had more densely packed collagen fibers (Table 3). There was no significant difference between the scores of the inflammatory reactions for the three groups after 30 days. Additionally, there was no significant difference between the fibrous capsule thickness in control group and ProRoot ES group.

![Figure 1](image1.png)

**Figure 1.** Rat subcutaneous tissue reaction at 3 days (A) control group (B) EndoSequence BC group (C) ProRoot® ES group. The tissue was not organized, moderate inflammatory reaction was observed in the 3 groups but with greater intensity in EndoSequence BC, chronic inflammatory cells were observed, lymphocytes (yellow arrows), macrophages (white arrows) H & E, X 400.

![Figure 2](image2.png)

![Figure 3](image3.png)
Figure 2. Rat subcutaneous tissue reaction at 7 days. (A) control group (B) Endosequence BC group (C) ProRoot® ES group. Inflammatory cells were observed including Remnant of neutrophils (black arrows), lymphocytes (yellow arrows), multi nucleated giant cells (red arrows), macrophage (white arrows), collagen fibers (blue arrow) H & E, X 400.

Figure 3. Rat subcutaneous tissue reaction at 30 days. (A) control group (B) Endosequence BC group (C) ProRoot® ES group. Fibrous capsule observed with collagen fibers (blue arrow), fibroblasts (green arrows) and remnant of lymphocytes (yellow arrows), the collagen fibers were densely packed and thicker in Endosequence BC group H & E, X 400.

Table 1. Means and Standard Deviations of The number of Inflammatory Cells Between Different Groups of the Study at Different Time Points.

<table>
<thead>
<tr>
<th>Number of inflammatory cells</th>
<th>Control</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td>28.25 ±1.58</td>
<td>41.00*</td>
<td>1.69</td>
<td>30.00± 3.46</td>
</tr>
<tr>
<td>7 days</td>
<td>25.00 ±2.27</td>
<td>31.50*</td>
<td>6.07</td>
<td>27.00 ±3.63</td>
</tr>
<tr>
<td>30 days</td>
<td>11.75 ±5.47</td>
<td>13.25</td>
<td>3.24</td>
<td>15.00± 3.85</td>
</tr>
</tbody>
</table>

Compared by using One-way ANOVA followed by post-hoc Tukey.
SD: standard deviation
*: significance relative to Control group
X*: significance relative to Group 1
*: significance <0.05

Table 2. Comparison Between Means and Standard Deviations of The number of Inflammatory Cells at Different Time Points Between Different Groups.

<table>
<thead>
<tr>
<th>Number of inflammatory cells</th>
<th>3 days</th>
<th>7 days</th>
<th>30 days</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>28.25 ±1.58</td>
<td>25.00*</td>
<td>11.75*</td>
<td>5.47</td>
</tr>
<tr>
<td>Group 1</td>
<td>41.00 ±1.69</td>
<td>31.50*</td>
<td>13.25*</td>
<td>3.24</td>
</tr>
<tr>
<td>Group 2</td>
<td>30.00 ±3.46</td>
<td>27.00</td>
<td>15.00*</td>
<td>3.85</td>
</tr>
</tbody>
</table>

Compared by repeated measures ANOVA followed by post-hoc Bonferroni.
*: significance relative to 3 days group
*: significance relative to 7 days group
*: significance <0.05

Table 3. Thickness of Fibrous Capsule (µm) after 30 Days of Surgical Implantation.

<table>
<thead>
<tr>
<th>Fibrous capsule thickness</th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48.44±3.796</td>
<td>87.87±7.834*</td>
<td>59.00±12.37*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Means and standard deviations compared by using One-way ANOVA followed by post-hoc Tukey.
*: significance <0.05
a: significance relative to Control group
b: significance relative to Group 1

Table 4. Means and Standard Deviations of Push-out bond Strength Values for Different Root Sections of EndoSequence BC Sealer group in MPa

<table>
<thead>
<tr>
<th>Root sections</th>
<th>Min-Max EndoSequence BC sealer in MPa</th>
<th>Mean, Median (SD) EndoSequence BC sealer in MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical</td>
<td>3.00- 9.21</td>
<td>6.33, 6.69 (2.46)</td>
</tr>
</tbody>
</table>
3.2. Push Out Bond Strength to Root Dentin

Means of push-out bond strength of Endosequence BC sealer group in was 6.33, 6.5 and 5.17MPa for apical, middle and coronal sections respectively (Table 4).

4. Discussion

Materials used for root canal filling are critical to successful endodontic treatment. An important characteristic is biocompatibility which is the capability of the material to act with an appropriate host tissue response [16]. In vivo tests are founded on clinical and histologic evaluation especially for the inflammatory response [16]. Subcutaneous and intraosseous implantation have been widely accepted as one of the most dependable techniques that can be implemented in animal model studies [17]. In this study, polyethylene tubes were utilized as they maintain the tested materials in contact with tissues in a controlled manner [18].

Both the Endosequence BC and the ProRoot ES sealers groups showed moderate inflammatory reactions with predominance of chronic inflammatory cells at days 3 and 7 that significantly decreased to a milder reaction at day 30 during the study. This was in accordance with the results of a study by Khalil et al. [19] who found that MTA and Endosequence materials provoked initial moderate to severe inflammatory reactions at 7 days that were significantly reduced by 30 days. The initial inflammatory reaction observed on the third day in all of the tested groups may have been due to the surgical procedures and the tissue reaction to receiving a subcutaneous implant [20]. However, the inflammatory response was significantly higher with the Endosequence BC sealer than with ProRoot ES sealer at 3 days. The inflammation also extended deep in the subcutaneous tissue and was not limited to the end of the tube. Multinucleated giant cells were noted in the Endosequence specimens at 7 days and absent in those of ProRoot ES which means that the Endosequence induced a more irritative effect, which led us to reject the first null hypothesis. This was in agreement with Taha NA et al. who noted the presence of more inflammation and multinucleated giant cells in Endosequence specimens compared with specimens of MTA material [21]. On the other hand, these results were in contrast with the study by Khalil et al. which reported a stronger inflammatory reaction to MTA repair material [19]. Other studies showed longer tissue reactions up to 60 days after MTA materials implantation [22,23].

Throughout the study, the intensity of the inflammation in ProRoot ES group was not significantly different from the control group. When MTA comes into contact with soft tissue, it dissolves into calcium hydroxide, which has a high pH. This alkaline pH can promote apical obliteration with calcified tissue formation. It also interferes with osteoclastic activity and encourages alkalization of the adjacent tissue, which helps healing and explains the more favorable effect of the sealer [24].

The fibrous capsule formation started early at 7 days in the Endosequence group till reaching a well formed mature one at 30 days. In the ProRoot ES group, the collagen fibers of the capsule were significantly thinner and not so packed at 30 days. The increase of the fibrous tissue capsule thickness may be accredited to a stronger inflammatory reaction and to the effect of mast cells on fibroblast proliferation and the fibrosis activity as a part of the inflammatory process. Mussel et al [25] and Shabiet al [26], suggested that the quality and thickness of the fibrous capsule around a material is inversely related to the biocompatibility of that material and is an indication of inflammation. Whereas, others suggested that the fibrous capsule deposition around an implanted material is a sign of tissue tolerance [27]. Undeniably, all dental materials produce some irritation once they interact with the living tissues [28].

The second null hypothesis could not be verified as the ProRoot ES Sealer showed great limitation during canal filling, that prevented bond-strength measurements. This raises questions about its clinical feasibility and further sustainability. On the other hand, Endosequence BC Sealer was much easier in handling and its mean bond-strength value was within the accepted range [29,30].

5. Conclusions

ProRoot ES Sealer has better biocompatibility by having favorable tissue cellular responses and thinner fibrous capsule, when surgically implanted into the subcutaneous tissue of rats, than the Endo sequence BC Sealer, although the later has much improved handling and manipulation characteristics.

Conflict of Interest

The authors deny any conflicts of interest in connection with this article.

References


