Evaluation of Calcium Ion Release from Two Calcium Silicate-Based Endodontic Materials

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Abstract
Objectives: Determine the concentration of free calcium ion in extracts delivered from MTA and Biodentine materials.

Materials and methods: Test materials were mixed according manufacturer instructions and shaped into discs using sterile polyethylene tubes. Material extracts were delivered as materials discs were immersed in culture medium for either 1, 3 or 7 days. Amount of free calcium ion was analyzed using Quantichrom calcium colorimetric assay kit. All data obtained were analyzed using student T-test and one way anova.

Results: T-test revealed that Biodentine significantly released more calcium ions than MTA at all-time points. One way ANOVA revealed that calcium concentration is significantly different among different extract preparation time within the Biodentine group while no significant difference was found between different MTA extracts. 7th day Biodentine extract significantly contained less ionized calcium than that 1st and 3rd day extracts.

Conclusions: In spite of chemical similarity, Biodentine released more ionized calcium than MTA in culture media.

Introduction
Periapical surgery may represent valuable option for saving the teeth when root canal treatment or retreatment proved to be unsuccessful. During endodontic surgery, apical part of the root is resected and retrograde filling material is usually used to seal a prepared root-end cavity. In such situation, the material comes in direct contact with periapical tissues; thus; it must not only provide an adequate physical seal at apical area, but also must possess properties which promote healing and regeneration of the relevant tissues [1].

Recently, bioactivity has been introduced as pre-requisite for retrograde material, the property which allow the material to interact with the host to stimulate normal anabolic function of the tissues by ionic exchange between material and surrounding tissues [2]. Bioactive materials are mainly characterized with its ability to release free calcium ion which is very important for differentiation of undifferentiated mesenchymal cells into specialized cells [2]. In spite of its regularity action, the level of the free calcium ions is very crucial during repair process as high concentration of ionized calcium can cause cytotoxicity and trigger cell death [3,4].

Calcium silicate based materials have gained popularity in recent years due to their good sealing ability, adaptation and excellent bioactivity [5,6].

Mineral trioxide aggregate (MTA) was developed as a retrograde filling material at Loma Linda University, California, USA. Unlike a number of dental materials that are not moisture-tolerant, MTA actually requires moisture to set [7]. In a study by Von Arx, et al. [8] they reported 92.4% success after 5 years follow up [8]. Bioden-
tine (Septodont, Saint Maur des Faussés, France) is a calcium silicate based product which became commercially available in 2009 and that was specifically designed as a “dentine replacement” material. Manufacturer present Biodentine as a material that has a wide range of applications including endodontic root repair material (root perforations, apexification, resorptive lesions, and retrograde filling material in endodontic surgery) and can be used as a dentine replacement material in restorative dentistry [9]. Caron, et al. [10] reported that two cases treated with biodentine as retrograde filling material were considered to be completely healed at the 1-year follow-up and this was confirmed at the 2-year follow-up evaluation.

The present study was aiming to laboratory determine the concentration of free calcium ion in extracts delivered from MTA and Biodentine materials.

Materials and Methods

Gray mineral trioxide aggregate (MTA; Angelus, Londrina, Brazil) and Biodentine (Septodont, Saint Maur des Faussés, France) were mixed following the manufacturers’ instructions under aseptic conditions with the aid of ultraviolet sterilization chamber (Aura 2000 MAC4, Euroclone S.p.A., v. Lombardia, Italy). Materials were prepared and shaped into material discs (Twelve samples of each material) by using a sterile cylindrical polyethylene tube (8-mm diameter and 3-mm height). Increments of materials were placed inside the polyethylene tubes and packed with light pressure against sterile glass slab using sterile metal spatula, then samples were covered with cover slab to remove excess material.

To obtain the initial setting, discs contained within polyethylene tubes were kept for 3 hours in incubator (Shellab incubator, Sheldon Manufacturing, Inc., Oregon, USA) at 37 °C and 95% relative humidity, after initial setting, material discs were exposed to ultraviolet light for 20 minute on each surface using ultraviolet cabinet (Aura 2000 MAC4, Euroclone S.p.A., v. Lombardia, Italy) to ensure sterility of material samples.

Four samples of each material were transferred separately into 24-well culture plates and incubated in 1.5 mL serum free Dulbecco’s Modified Eagle Medium (DMEM) (Lonza, Verviers, Belgium) supplemented with 1% Pen-Strep antibiotic (Pen-Strep; 1000 U/mL penicillin, 1000 mg/mL streptomycin) (Lonza, Verviers, Belgium) at 37 °C in a humidified atmosphere containing 5% CO₂ for 24 hours to allow the leachable materials to leach from the samples into the medium. The surface area of tested material to volume ratio used for extract preparation was about 125 mm²/mL in accordance with ISO standard 10993-5 [11]. The supernatant was then collected and referred to as “material day-1 extract”.

Extracts of longer periods (3 and 7 days) were prepared with the same protocol used for preparing day-1 extract. After each time point of sample incubation, the conditioned medium (material extract) was then filtered using 0.20 µm sterile syringe filter (Corning Incorporated, Corning, Germany).

Ionized calcium concentration in each material extract was measured using Quantichrom calcium colorimetric assay kit (Bioassay Systems, Hayward, CA, USA).

To apply this method an internal standard was prepared and 3 Separate wells were used for each sample. For the internal standard, 250 µL 10 mg/dL Ca²⁺ Standard were prepared by mixing 125 µL 20 mg/dL Standard and 125 µL distilled water. 5 µL of the sample extract were transferred to the three separate wells. 5 µL of 10 mg/dL Ca²⁺ were added to the first well (Sample plus Standard well), while 5 µL distilled water were added to the second well (Sample alone well). For the third well, 5 µL 20 mM EDTA were added (sample Blank well). 200 µL Working Reagent were added to the three wells and tapped lightly to mix. In case of if any particulates or turbidity were seen, the pipette was moved up and down to dissolve this turbidity. The mix was incubated for 3 min at room temperature and then the optical density at 610 nm was read for each well using a VERSA max microplate reader (Molecular Devices, Sunnyvale, CA, USA).
The free calcium ion concentration was calculated using the following formula:

$$[Ca^{2+}] = \frac{(OD_{SAMPLE} - OD_{BLANK})}{(OD_{STANDARD} - OD_{SAMPLE})} \times 10 \times n \text{ (mg/dL)}$$

Where:
- $OD_{SAMPLE}$: Optical density of the sample alone well.
- $OD_{BLANK}$: Optical density of the sample blank well.
- $OD_{STANDARD}$: Optical density of the Sample plus Standard well.
- $10$: Is the concentration of the standard in mg/dL.
- $n$: is the sample dilution factor.

Data obtained from quantification of calcium ions were subjected to student T-test to verify the presence of significance between MTA and Biodentine extracts. Within the same group, data were subjected to one way analysis of variance (ANOVA) to compare calcium content between different days extracts.

### Results

Detailed values of free calcium ions concentrations in different extracts derived from MTA and Biodentine are summarized in Table 1. T-test revealed that Biodentine significantly released more calcium ions than MTA at all-time points (1, 3 and 7 days extracts). Regarding time of extract, ANOVA revealed that calcium concentration is significantly different among different extract preparation time within the Biodentine group while no significant difference was found between different MTA extracts. Post HOC test revealed that the 7th day Biodentine extract significantly contained less ionized calcium than that 1st and 3rd day extracts while there was no significant difference between 1st and 3rd day extracts.

As an Observational result, a white dispersed precipitate was noticed covering the samples of tested materials as well as the periphery of wells used for extract preparation.

### Discussion

An annual estimation of endodontic procedures suggests that approximately 5.5% of all treatments performed involve apical root-end, that’s need to be restored with root repair material that come in direct contact with living tissues. That’s why an ideal root repair material should maintain a sufficient seal, and be biocompatible with human tissues [11,12].

The ability to release calcium is an essential for successful endodontic root end procedure because of the action of calcium on osteoblasts and cementoblast cells differentiation and hard tissue mineralization [13]. Hunter, et al. [14] reported that calcium ions released from silicate based material may play an important role in the repair process because they can pass through the cell membranes by depolarization or activation of membrane-bound calcium channels [14], however, a high concentration of intracellular calcium ions can cause cytotoxicity and trigger cell death [2-4].

This study demonstrated that all the materials tested released calcium ions and had the ability to form surface apatite crystals (observational), both of which indicate their bioactivity. Results obtained from current study indicated that the two

### Table 1: Mean and standard deviation of free calcium ions concentration in the day 1, day 3 and day 7 extracts of tested materials (mg/dL).

<table>
<thead>
<tr>
<th></th>
<th>Free calcium ion concentration (mg/dL)</th>
<th>Day 1 extract</th>
<th>Day 3 extract</th>
<th>Day 7 extract</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA</td>
<td></td>
<td>10.6 ± 1.86(\text{A})</td>
<td>10.17 ± 1.9(\text{A})</td>
<td>8.2 ± 1.1(\text{A})</td>
<td>0.148</td>
</tr>
<tr>
<td>Biodentine</td>
<td></td>
<td>23.4 ± 2.4(\text{B, a})</td>
<td>20.7 ± 1.4(\text{B, a})</td>
<td>15.4 ± 2.1(\text{B, b})</td>
<td>0.001</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Values with different superscripts (A or B) at the same column are significantly different at p value < 0.05; Values with different superscripts (a or b) at the same row are significantly different at p value < 0.05.
materials differ regarding the ability to produce apatite crystals and the amount of calcium released, as Biodentine released significantly more calcium ions and produced more surface crystals.

Results obtained from current research are in agreement with previous researches [6,15-17]. Bozeman, et al. [17] reported that both gray and white MTA materials released more Ca initially, followed by a decline and then rise in elution, also SIR Khan [16] reported that MTA and Biodentine released significantly less calcium ions in 7th day than the initial 5 hours of contact of those materials with distilled water. Han and Okiji [6] reported that Biodentine significantly released more calcium ions than MTA when materials were in contact with simulated body fluid while Gandolfi, et al. [15] reported significantly increased calcium release by Biodentine compared with MTA when materials were immersed in water.

Setting reaction of calcium silicate includes the hydration of calcium silicates particles that results in the production of calcium hydroxide as byproduct of the reaction, the major source of calcium ions released from these materials [18-20]. In this regard, the higher calcium ion release of Biodentine material may be attributable to its higher calcium silicate content and thus, the amount of calcium hydroxide produced after the hydration reaction may be higher compared with the MTA. Moreover, the increased calcium release in Biodentine may be related to increased content of calcium carbonate and calcium chloride. Contrary to our results, SIR Khan, et al. [16] reported more calcium released from MTA in comparison with Biodentine. A possible explanation of that may be related to different actions occurred with materials when subjected to different culturing fluids, the current study utilized phosphate containing culturing media while the former authors utilized distilled water.

At the present study calcium ion release was greater in 1st day extract then subsid- ed in 7th day extract. This maybe as a result of complete hydration of materials as the materials samples were totally immersed inside the culture media, thus rapid release of calcium ions occurred at the first day. Regression of calcium ions concentration inside day 7 extracts could be explained as a result of increased formation of hydroxyapatite on materials surfaces; which is confirmed in present study. It can be supposed that the consumption of calcium hydroxide for the formation of hydroxyapatite like crystals may also be a mechanism leading to a decreased level of calcium ions.

Conclusion

Within the limitations of this study and in spite of chemical similarity, Biodentine released more ionized calcium than MTA in culture media.

References

8. Von Arx T, Hanni S, Jensen SS. 5-year results comparing mineral trioxide aggregate and