Comparison of calcium ion release from MTA-Angelus® and Biodentine®

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Abstract. Bioactive materials undergo hydration by releasing a number of ions during the setting stage. The reaction on the surface of these materials can release and alter the concentration of dissolved ions, which triggers both intracellular and extracellular responses. This process also leads to remineralization. The released Ca\(^{2+}\) ions increase alkalinizing activity, have a bactericidal effect, suppress osteoclast activity, and stimulate fibroblast formation. The present study aimed to analyze Ca\(^{2+}\) ion release from the bioactive materials MTA-Angelus® and Biodentine®. As many as 46 samples (23 MTA-Angelus® and 23 Biodentine®) were prepared (2 mm in diameter and 2 mm in height). Both the materials were immersed in deionized water for 1 h and 48 h followed by measurement of the released Ca\(^{2+}\) ions. An atom absorption spectrophotometer was used to measure Ca\(^{2+}\) ion release. The results were statistically tested using the Kruskal–Wallis test. The Mann–Whitney post-hoc statistic test showed a significant difference between all the groups (p ≤ 0.05). Biodentine® released more Ca\(^{2+}\) ions compared to MTA-Angelus® at the 1h and 48 h measurements. MTA-Angelus® released Ca\(^{2+}\) ions faster than Biodentine®.

1. Introduction

The term bioactive material refers to materials that can induce a biological response in body tissues. Bioactive glass is a bioactive material that can induce bone formation. It comprises silica (glass) and other materials containing calcium ions and is available in powder form or mould form [1].

Hydration of bioactive materials leads to the release of a number of ions during the setting stage. The reaction on the surface of these materials can alter the concentration of the dissolved ions which in turn triggers an intracellular and extracellular response that results in remineralization. The released Ca\(^{2+}\) ions increase alkalinizing activity, have a bactericidal effect, suppress osteoclast activity, and trigger fibroblast formation [2]. Ca\(^{2+}\) can activate Ca\(^{2+}\)-dependent ATPase and react with carbon in the tissues; the subsequent formation of calcium carbonate represents the initiation of remineralization. Ca\(^{2+}\) ions are also required for cell migration and differentiation [3]. These properties of bioactive materials are used in endodontic cases requiring hard tissue formation such as during vital pulp treatment (pulp capping, pulpotomy), apexogenesis, apexification and for closure of perforation. Mineral trioxide aggregate (MTA) and Biodentine® are some of the bioactive materials.
Both MTA-Angelus® and Biodentine® undergo similar hydration processes and release a number of ions. However, the similarity, particularly in terms of the number of Ca\(^{2+}\) ions released by MTA-Angelus® and Biodentine® that could induce hard tissue formation, remains unknown. The present study aimed to analyze and compare the number of Ca\(^{2+}\) ions released by MTA-Angelus® and Biodentine®.

2. Methods

This was a laboratory experiment (in vitro) conducted in chemistry analysis laboratory, ITB, Bandung, Indonesia, during September 6–17, 2014. The samples used in this research were prepared using Teflon moulds (2 mm in diameter, 2 mm in height) for MTA-Angelus® and Biodentine® until the final setting. The sample size (n = 23) was determined using Federer Formula: \((t - 1)(n - 1) > 15\), where \(t\) is the number of groups (MTA-Angelus® and Biodentine®), and \(n\) is the sample size.

The tools and materials used in this study were white MTA (MTA-Angelus®, Angelus, Londrina, PR, Brazil), Biodentine® (Septodont, Saint-Maur-des-Fosses, France), deionized water (Amidis®), a Teflon mould (2 mm in diameter and 2 mm in height), hand instrument plugger, incubator set at 37°C, cement spatula, plaster instrument, amalgamator, mixing slab, plastic test tube, Atom Absorption Spectrophotometer, and stopwatch.

MTA-Angelus® and Biodentine® were mixed until a paste was obtained according to the manufacturer’s instructions. The paste was immediately placed in the Teflon mould. Specimens were then removed from the mould after final setting (±15 min for MTA-Angelus® and ±12 min for Biodentine®). MTA-Angelus® and Biodentine® disks were placed in a tube containing 10 mL deionized water and incubated at 37°C.

Twenty-three samples were used for each material. All the samples were placed in plastic tubes containing 10 mL deionized water and the released calcium ions were measured at 1 h and 49 h in duplicate post setting using an atomic absorption spectrophotometer.

Data pertaining to calcium ion release was collected according to the standard criteria for the following groups: MTA-Angelus® 1 h, MTA-Angelus® 49 h, Biodentine® 1 h, and Biodentine® 49 h. Data of this research was analytic descriptively evaluated. The Ca\(^{2+}\) ion release data and numeric and non-parametric data were analyzed using the Kruskal–Wallis and post-hoc Mann–Whitney tests.

3. Results

The mean values of Ca\(^{2+}\) ions released from the MTA-Angelus® and Biodentine® specimens (2 mm in diameter and 2 mm in height) are presented in Table 1.

|                  | 1 h     | 49 h    | Difference | Speed of Ion Release Percentage |
|------------------|---------|---------|------------|---------------------------------|
| **MTA Angelus®** | 2.24 (±0.62) | 12.97 (±0.37) | 10.73       | 479%                            |
| **Biodentine®**  | 5.38 (±0.42) | 15.67 (±0.66) | 10.29       | 191%                            |
| **Difference**   | 3.14    | 2.7     |            |                                 |

A 10.73 ppm difference in the amount of Ca\(^{2+}\) ions released by MTA-Angelus® at 1 h and 49 h and a 10.29 ppm difference in the amount of Ca\(^{2+}\) ions released by Biodentine® at 1 h and 49 h as shown in Table 1. There was a 3.14 ppm difference in the amounts of calcium ion released by MTA-Angelus® at 1 h and Biodentine® at 1 h, while there was 2.7 ppm difference in the amount of calcium ions released by MTA-
Angelus® at 49 h and Biodentine® at 49 h. Table 1 shows that the release of calcium ions from Biodentine® was higher than that from MTA-Angelus® at both 1 h and 49 h.

The speed of ion release from 1 h to 49 h of immersion in the MTA-Angelus® group increased 4.7 times (479%), while that in the Biodentine® group increased 1.9 times (191%). The results showed that MTA-Angelus® releases Ca²⁺ ions at a faster rate than does Biodentine®.

Data obtained from the two groups exhibited non-normal distribution; therefore, the Kruskal–Wallis test was used for data analysis. The results showed a significant difference in the amounts of calcium ions between all the groups (p≤0.05). Therefore, it can be concluded that the amount of calcium ions released was significantly different between the two groups. Post-hoc analysis using the Mann–Whitney test was performed between each treatment group to identify the groups that showed a significant difference.

Table 2. p values of Ca²⁺ release between each group

| Treatment                        | p value |
|----------------------------------|---------|
| MTA 1 h versus MTA 49 h hours    | 0.000^b |
| MTA 1 h versus Biodentine 1 h hour | 0.000^b |
| Biodentine 1 h versus Biodentine 49 h hours | 0.000^b |
| Biodentine 49 h versus MTA 49 h  | 0.000^b |
| p<0.05; n = 23 for each group    |         |

Statistically significant differences (p<0.05) with regard to the amount of Ca²⁺ ions released were observed on comparing MTA Angeleus® 1 h versus MTA Angeleus® 49 h, MTA Angeleus® 1 h versus Biodentine® 1 h, MTA Angeleus® 49 h versus Biodentine® 1 h, and Biodentine® 1 h versus Biodentine® 49 h (Table 2).

4. Discussion
Both MTA Angeleus® and Biodentine® are class A bioactive materials with osteoproductive properties. According to Wilson (1994), osteoproductive properties enable the colonization of a bioactive surface by free osteogenic stem cells within the contact area. These bioactive materials trigger both intra and extracellular response on the surface. Class A bioactive materials can bond well with both hard and soft tissues.

Regarding the mechanism of action, MTA Angeleus® and Biodentine® are similar to calcium hydroxide. Both these materials are used as substitutes for calcium hydroxide owing to their better biocompatibility and good closure. Several studies have shown that the end product of hydration of these materials is calcium hydroxide. The predominant ions released during the setting process are calcium ions. The process of ion release from both materials enables crystal deposition on the surface, which initiates hydroxyapatite (HA) precipitation. HA is a material that contains calcium ions, which results in good biocompatibility, reduces toxicity to tissues and foreign body reaction, facilitates osteoid induction, and produces a good osteogenic effect.

Specimens were prepared by adaptation and modification of methods used in previous research regarding ion release. The dimensions of specimens used in this research were not identical to those in clinical settings where the diameter of apical foramen is smaller by 1 mm and the width of perforation that could be sealed is < 2 mm; however, 2 × 2 mm² specimens were used in the present study to simplify the process and avoid the washing-out of the test material during immersion in deionized water. Deionized water at neutral pH was chosen for specimen immersion to obtain accurate measurements of ion release without ion contamination from the immersion liquid.
The physical difference between MTA Angeleus® and Biodentine® after setting was affected by the alumina content. Alumina content in MTA Angeleus® renders the product more brittle. Furthermore, water significantly influences the rigidity of materials. For MTA Angeleus®, distilled water is used as the solvent. Excess water content increases porosity and reduces mechanical resistance, at the microscopic scale. In contrast, water deficiency reduces the homogeneity of the powder and liquid mixture. Solvent used for Biodentine® contains a hydro soluble polymer as reducing agent that helps to maintain the balance between the low water content of Biodentine® and consistency of the mixture. The higher the porosity of a material, the lower is its mechanical strength. Owing to this phenomenon, a number of MTA Angeleus® specimens were rendered defective during their removal from the mould and were thus excluded from the study.

MTA Angeleus® and Biodentine® undergo dissolution on the surface in physiological environments to form a Hydroxy Carbonate Apatite (HCA) layer. The greater the degree of solubility of the bioactive glass material, the more obvious is its effect on tissue growth [4]. At the end of the first hour, an HCA layer was formed as a result of crystallization of the CaO-P₂O₅ amorphous layer because of the presence of HO⁻ and CO₃²⁻ in the solution [4]. Hence, for each specimen, the initial Ca²⁺ ion release was measured after 1 h of immersion.

In addition to HCA layer formation and dissolved ion release from bioactive materials, calcium ion concentration plays an important role in bone regeneration. The main mechanism to increase new bone growth involves controlling the release of dissolved ions in the bioactive materials, particularly the critical calcium ion concentration [5]. Several studies have shown that in addition to forming HCA, the constant and slow release of calcium ions from the bioactive material may increase osteogenesis by regulating osteoblast proliferation, differentiation, and gene expression.

Osteoprogenitor cells should be able to undergo mitosis and receive proper chemical stimuli from the environment for bone regeneration to occur. Bioactive materials can induce fibroblast proliferation by accelerating cell growth cycle; cells do not go through the G1 and S phases and directly enter the G2 phase. The critical calcium ion concentration causes osteoblasts to differentiate into mature osteoblasts and proliferate and regenerate new bone within 49 h. Osteoblasts that do not enter the cell cycle and fail to differentiate undergo apoptosis caused by the dissolved ion product [5]. Calcium ion release 49 h after final setting was measured to determine whether the presence or absence of calcium ions was sufficient and constant to support the differentiation of osteoblasts into mature osteoblasts and proliferate and regenerate bone tissue.

Table 1 shows that calcium ions released in the Biodentine® group at 1 h and 49 h after setting were higher than that in the MTA Angeleus® group. Ion release depends on several factors, including the structure and constituent mineral particles. Both of these factors are responsible for water resorption and solubility which induces porosity. Biodentine® forms calcium phosphate particles that measure < 1 micron, which results in a more compact surface layer. MTA Angeleus® has a larger particle size (diameter: 1–5 micron). This is one of the factors responsible for greater Ca²⁺ ion release from Biodentine® compared to that from MTA Angeleus® [6].

The amount of calcium ion release from Biodentine® is also related to the calcium carbonate content as filler. Natural or synthetic calcium carbonate is the most important biomaterial for mineralization. Calcium carbonate is deposited by osteoblasts that have been exposed to calcium phosphate naturally during the mineralization process.

MTA Angeleus® released calcium ions faster than Biodentine® (Table 1). This was because of the low solubility of Biodentine® compared to MTA Angeleus®. The low solubility of Biodentine® is caused by the hydro soluble polymer comprising the Biodentine® liquid, which acts as the water reducing agent.

Abnormal distribution of data pertaining to MTA Angeleus® was noted in the present study. Based on the preparation of the specimens according to the manufacturer’s instructions, the only variation that was
possible was during the mixing of MTA Angelus®. Lack of accuracy of the powder quantity during its removal from the package (Biodentine® was not mixed outside the factory package, while MTA Angelus® was mixed after its removal from the factory package) may have caused uncontrolled variation in mixture composition. Furthermore, manual mixing could have resulted in unidentical mixing of the samples. Biodentine® package is in the form of capsule; the mixing was done using an amalgamator for identical time periods (30 s according to factory manual), and a relatively more homogenous mixture could be the reason for similar and normal distribution in the Biodentine® group.

In clinical settings, both silicate-based bioactive materials are used for direct pulp capping, indirect pulp capping, apexogenesis, apexification, perforation closure and in cases that require hard tissue formation. Tricalcium silicate on both materials induces reparative dentin synthesis by modulating pulp cells to secrete TGF-ß, BMP-2, BMP-4, BMP-7 (osteogenik-1 protein), dentin matrix protein (DMP-1), matrix extracellular phosphoglycoprotein (MEPE), bone sialoprotein (BSP), email matrix derivate, stem cell, and stimulate pulp remineralization by forming tertiary dentin.

Tertiary dentin formation by both bioactive materials was achieved 3 months after application, along with the establishment of a thick dentin barrier. The presence of calcified barrier causes the environment to be strongly alkaline, which prevents ion exchange on the surface of the bioactive material. Use of silicate bioactive cement as a pulp capping agent and in pulpotomy cases showed that the material is well tolerated even despite direct contact with the pulp. The quality of dentinal bridge produced by silicate bioactive cement is better and harder compared to that produced by calcium hydroxide [7].

5. Conclusion
Biodentine® released more Ca²⁺ ions than MTA-Angelus®. MTA-Angelus® released CA²⁺ ion Faster than Biodentine®

6. References
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