

The comparison of calcium ion release and pH changes from modified MTA and bioceramics in regeneration

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Abstract. The surface reactions of bioactive materials release and change dissolutions triggering intracellular and extracellular responses. Calcium ion release can promote alkalinizing activity, which is needed in tissue regeneration. To analyze calcium ion release and pH changes in modified MTA and bioceramics as bioactive materials. Thirty samples, measuring 3 mm in diameter and 3 mm in height, were prepared, with 15 consisting of modified MTA and 15 consisting of bioceramics. Both materials were immersed in deionized water for an hour, then measured and transferred into fresh solutions and soaked for 48 hours or 168 hours. The measurements were conducted using an atom absorption spectrophotometer and pHmeter. Mann Whitney's post hoc statistic test showed a significant difference among all the 1-hour, 48-hour, and 168-hour measurement groups, with a value of $p \leq 0.05$. Bioceramics released more calcium ions and raised pH levels higher than modified MTA for each of the three soak-time groups. Bioceramics released more calcium ion and had higher pH level compared to modified MTA which contributed to the tissue regeneration.

1. Introduction

Mineral Trioxide Aggregate (MTA) is widely used for its sealing properties, as well as its biocompatibility, antimicrobial effect, radiopacity, dimensional stability, and higher humidity tolerance compared to materials used before MTA was available [1]. MTA is an aggregate powder that contains mineral oxides with excellent biological action. MTA chemical reactions produce calcium hydroxide, which has antibacterial effects and induces bone formation, making it useful as a scaffold in alveolar bone regeneration processes [2]. Calcium silicate has a major role in the bone healing processes due to its biocompatibility and ability to induce calcium phosphate formation on periodontium tissue, as demonstrated in previous studies by Tay *et al.* [3], Reyes-Carmona *et al.* [4], and Torabinejad-Parirokh [5]. Monalisa showed MTA's ability to induce regeneration and hard tissue formation. This ability may be due to its high pH levels (10.2–12.5) and substance release, which may activate cement to blasts to produce matrices in cementum formation [6]. Current MTA products have some disadvantages, such as slow setting times and difficult handling. Anew material, known as modified MTA, overcomes these disadvantages. This new material is based on calcium silicate cement consisting of a powder and gel component, with a powder particle size $<10 \mu\text{m}$ —a smaller particle size than in standard MTA. This modification enables a shorter setting times and mixes into a smooth, non-sandy consistency, thus, facilitating its application on the tooth apex.

Along with this technology, bioceramics have similar properties. Bioceramics are nanostructured biomaterials with a calcium silicate-base, are insoluble, radiopaque, and aluminum-free. Bioceramic



producers claim this material is incredibly biocompatible, hydrophilic, does not shrink during setting, and is easier to use due to its ready-to-use packaging.

The release of calcium ions and the increase of pH levels are connected to bioactive material hydration. Bioactive material hydrates by releasing some of its ions, such as calcium ions. Reactions on the surface of the material may release and change the concentration of dissolved ions, inducing both intracellular and extracellular responses, and resulting in hard tissue formation. Calcium ions are the dominant ions in this surface reaction. As bioactive materials release calcium ions, there is a bactericidal effect, suppressing osteoclast activity and stimulating fibroblast formation. Calcium ions also activate Ca-dependent Adenosine Triphosphatase (Ca-dependent ATPase) and react with carbon in the tissue to form calcium carbonates, initiating remineralization. Calcium ions are also needed for cell migration and differentiation processes. Since only a few studies have compared modified MTA and bioceramics, this study aimed to test the effectiveness of regeneration by measuring the rates of calcium ion release and pH level indices.

2. Materials and Methods

A total of 30 samples were prepared and divided into two groups of 15, one a modified MTA sample group and the other a bioceramic sample group. Representing modified MTA, the MTA Flow® (Ultradent Products, USA) material was mixed until it reached a putty consistency. The MTA Flow® mixture and ready-to-use bioceramic material, iRoot® BP Plus, (Innovative BioCeramix Inc, Canada) were placed into Teflon molds. The samples set (15 minutes for MTA Flow® and 120 minutes for iRoot® BP Plus), and were removed from the molds. Both material groups were immersed in deionization water for 1 hour, 48 hours, and 168 hours. The levels of calcium ion release were measured using an atom absorption spectrophotometer from the immersion water and the pH level was measured using a pH meter. The results of the data were analyzed using Shapiro-Wilk test for normality, Kruskal-Wallis test for variance and Mann Whitney's post hoc analysis on SPSS software version 24.0.

3. Results and Discussion

3.1 Results

An atom absorption spectrophotometer was used to analyze calcium ion release and a pHmeter was used to analyze pH levels of both samples. The release of calcium ions and pH change were measured three times for each material. The first measurement was conducted after one hour of immersion in the deionization water, the second measurement was conducted after 48 hours of immersion in the deionization water, and the last measurement was conducted after 168 hours of immersion in the deionization water. The release of calcium ions occurred in both material samples for each of the soak-time variables. Based on the observations of all samples, the mean values of the amount of calcium ions released by modified MTA and bioceramic samples are displayed in Table 1.

Table 1. Mean of the release of calcium ions in modified mta and bioceramics for 1 hour, 48 hours, and 168 hours

| Material | Release of Calcium Ions (ppm) | | |
|--------------|-------------------------------|--------------|---------------|
| | 1 hour | 48 hours | 168 hours |
| Modified MTA | 4.69(±1.67) | 23.82(±6.51) | 25.77(±14.98) |
| Bioceramics | 8.49(±0.93) | 47.96(±4.48) | 66.43(±19.13) |

Table 1 shows the release of calcium ions in both material groups for 1-hour, 48-hour, and 168-hour immersion times. Based on the Shapiro-Wilk data distribution, an abnormal data distribution was found

for group that was immersed for 1 hour, while a normal data distribution was found for the samples that were immersed for 48 and 168 hours.

Table 2. p-values of the release of calcium ions between test groups

| Test Group | Immersion Time | p-values |
|------------------------------|----------------|----------|
| Modified MTA vs. Bioceramics | 1 hours | 0.000 |
| Modified MTA vs. Bioceramics | 48 hours | 0.000 |
| Modified MTA vs. Bioceramics | 168 hours | 0.000 |

p-values < 0.05

Post hoc test was used to determine the data presented in Table 2, comparing the release of calcium ions between the modified MTA and bioceramic material. There was a significant difference found between MTA Flow® and iRoot® BP Plus for all three soak-time variables ($p = 0.000$). The mean pH levels of the modified MTA and bioceramic samples are displayed in Table 3.

Table 3. Mean pH level of modified mta and bioceramics for 1 hour, 48 hours, and 168 hours

| Material | pH Level | | |
|--------------|-------------|--------------|--------------|
| | 1 hour | 48 hours | 168 hours |
| Modified MTA | 7.48(±0.31) | 10.22(±0.60) | 11.39(±0.77) |
| Bioceramics | 7.72(±0.14) | 11.41(±0.39) | 12.78(±0.20) |

A normality test was conducted using Shapiro-Wilk's method, and abnormal data distribution was found in the samples that were immersed for 48 hours, while normal data was found for the 1-hour and 168-hour immersion groups.

Table 4. p-values of pH between test groups

| Test Group | Immersion Time | p-values |
|------------------------------|----------------|----------|
| Modified MTA vs. Bioceramics | 1 hour | 0.013 |
| Modified MTA vs. Bioceramics | 48 hours | 0.000 |
| Modified MTA vs. Bioceramics | 168 hours | 0.000 |

p-values < 0.05

Post hoc test analysis showed in Table 4, comparing the pH level changes between the modified MTA and bioceramic shows a significant difference between MTA Flow® and iRoot® BP Plus after 1 hour, 48 hours, and 168 hours immersion.

Table 5. Correlation between the release of calcium ions and the change in pH levels

| | Correlation Values | p-values |
|--------------------|--------------------|----------|
| 1-Hour Immersion | 0.298 | 0.110 |
| 48-Hour Immersion | 0.752 | 0.000 |
| 168-Hour Immersion | 0.662 | 0.000 |

*Pearson correlation test

Table 5 shows the results of correlation test, and a positive correlation was found between the release of calcium ions and pH level changes for the 1-hour immersion group. This implies that as a

more calcium ions were released, the pH level also increased. The correlation for this group ($r = 0.298$), however, was insignificant ($p > 0.05$). The correlation test for the 48-hour immersion group showed a positive correlation between the release of calcium ions and change in pH level ($r = 0.752$). This correlation was statistically significant ($p < 0.01$). The correlation test for the 168-hour immersion group also showed a positive correlation (parallel) between the release of calcium ions and pH level changes ($r = 0.662$). This correlation was also statistically significant ($p < 0.01$).

3.2 Discussion

The preparation of the samples for this research was adapted and modified based on previous research methods regarding ion release and pH level changes. There were some advantages to using different dimensions for the samples in this study than the clinical conditions (where the diameter of the one third apical post preparation was less than 3 mm). The 3 x 3 mm dimension of the samples facilitated not only making the specimens, but avoiding material wash out during the immersion process. Deionized water, with a neutral pH, was chosen as the immersion liquid to enable an accurate measurement of released ions and pH level changes as the material was not previously contaminated with ions from the immersion liquid.

Calcium silicate-based materials were chosen as the primary material for retrograde obturation due to the physio-chemical properties and ability to stimulate tissue repair by regenerating mineralized tissue. Hench *et al.*, stated that on the first hour after the bioactive material set, an Hydroxy Carbanon Apatite HCA layer will form as the effect of the crystallization of CaO-P₂O₅ amorphous layer due to OH⁻ and CO₃²⁻ in the solution [7]. Therefore, a measurement of the release of calcium ions was conducted after the first hour of immersion in this study. Other studies showed that calcium ions are constantly and slowly released from bioactive material, and thus, improve osteogenesis by regulating osteoblast proliferation, differentiation and gene expression. In the process of bone regeneration, osteoprogenitor cells undergo mitosis and precise environmental chemicals stimulate these cells to go through complete cell cycles. The primary mechanism to increase new bone growth relies on the release of soluble ion products from bioactive material, particularly on a critical concentration of calcium ions. In 48 hours, due to the critical concentration of calcium ions, the osteoblasts differentiated into mature osteoblast phenotypes and started to proliferate and regenerate new bone.

Osteoblasts that do not go through cell cycles, and do not differentiate, undergo apoptosis. Measuring the release of calcium ions after 48 hours determined whether the conditions to support the differentiation of osteoblasts into mature osteoblast phenotypes, proliferate, and regenerate new bone, were met. The measurements of the release of calcium ions and pH levels after 168 hours were taken because bone matrix crystallizations occur on the surface of bioceramic material seven days after injury. Osteoblasts secrete longitudinal organic matrices made of fibril collagen matrices (mainly type 1 collagen). The increase in pH level was likely caused by alkaline phosphatase enzymes secreted by osteoblasts along with other cells that have an important role in the mineralization process. The interaction between alkaline phosphatase and phosphoprotein inside the bone and dentin are crucial in mineralization. Table 1 shows that the mean value of the release of calcium ions from the bioceramic sample was greater than from the modified MTA sample for 1-hour, 48-hour, and 168-hour immersion groups. P values from each group were measured, as shown in Table 5. Sarkar *et al.*, stated that calcium ions released from MTA will react with phosphates in synthetic tissue liquid and will form hydroxy apatite. The result of this research revealed that the amount of calcium ions released by bioceramics was greater than in the modified MTA [8]. This result corresponds with the previous research conducted by Zhu *et al.*, that reveals that bioceramics (iRoot® BP Plus) form apatite better than modified MTA when immersed in a body liquid simulation [9].

The release of calcium ions depends on several factors, such as structure form and material mineral particles. These factors are responsible for water resorption, solubility, and porosity. Reducing the particle size of the cement increases surface area and facilitates faster cement particle hydration. In this study, the particle size of modified MTA was 10 µm while the particle size of the bioceramic sample was 2 µm. This smaller particle size increased the surface area of the material, thus, increasing

the release of calcium ions. The physiologic process that supports regeneration is caused by extracellular matrices, which regulate tissue regeneration. Extracellular matrices strengthen the defect area in regeneration, and act as a barrier to prevent the intrusion of the surrounding tissues, thus serving as a frame for cell migration and proliferation. According to Holand *et al.* [10], in calcium silicate-based materials, the calcium oxide compound reacts with bodily fluids to produce calcium hydroxide, which then divides into calcium ions and hydroxide ions. Hydroxide ions are responsible for the alkalization of their environment and activate alkaline phosphatase enzymes. Calcium ions also react with carbonate ions in periapical tissue which precipitates of calcite granules that trigger the deposition mineralized tissue process [2].

Calcium silicate particles in modified MTA and bioceramics react with water and produce solutions with high pH levels that contain calcium, hydroxide, and silica ions. Calcium silicate hydrate gel undergoes precipitation in cement particles, whereas calcium hydroxide undergoes nucleation. Calcium silicate hydrate gel undergoes polymerization to form compact tissue while releasing calcium hydroxide, thus, increasing its environmental pH level [2]. Calcium ion release analysis for the experiment groups indicated that bioceramic samples showed a significant increase of calcium concentration in their environment compared to modified MTA. The smaller particle size of bioceramic increased the surface area for surface reactions, and produced better particle hydration and more calcium hydroxides. The greater amount of hydroxyl clusters formed from calcium hydroxide led to the increase of pH level. For each of the soak-time groups, the mean pH level for the bioceramic samples were higher than for modified MTA, as shown in Table 3. P values from each group were measured, as shown in Table 4. The second hypothesis, that there is difference of pH level between modified MTA and bioceramic, was also confirmed.

The data distribution in this study implies an abnormal distribution for the modified MTA group. The only variation that might have occurred was in the inaccuracy of powder and liquid quantities for mixing the modified MTA. Meanwhile for the bioceramic sample, no mixing process was needed because it came in ready-to-use packaging. Therefore, homogenic dough was more common and probably why the bioceramics group data was more consistently and normally distributed. The manual mixing technique for modified MTA resulted in non-identical mixing for each sample. The correlation test between the release of calcium ions and pH level changes for both material types is shown in Table 5. The positive correlation between the release of calcium ions and pH level changes after 1 hour indicates that an increase in calcium ions also increased pH level. However, the correlation was not significant for the 1-hour group. The result for groups immersed for 48 hours and 168 hours showed significant positive correlations between the release of calcium ions and pH level changes. This result is consistent with research conducted by Saghiri *et al.* [11], that indicated that for material with smaller particle sizes, more calcium ions were released, and increased pH levels. Both modified MTA and bioceramics are near-ideal root-end filling materials because both materials are easy to manipulate, have good radiopacity, good long term stability, attach well with dentinal wall, and have excellent biocompatibility. Nevertheless, bioceramics had better bacteriostatic and bactericidal effects than modified MTA, as well as a greater ability to regenerate tissue because it released more calcium ions for a longer period.

4. Conclusion

Bioceramics release more calcium ions and increase pH levels more than modified MTA, and can thus increase tissue regeneration. Further studies about the physical properties of modified MTA and bioceramics need to be conducted in regards to the release of calcium ions by modified MTA and bioceramics.

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