

The Ability of Biodentine™ of Guided Tissue Remineralization (GTR): Analysis Using SEM, EDX and TEM

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Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 20 September 2018 / Accepted: 05 January 2019 / Published: 18 January 2019

Abstract

Objective: To analyze the Biodentine™ capability in guided tissue remineralization. **Material and Methods:** Four premolar with two cavities per tooth of 3 mm depth were demineralized with EDTA 17% in shaking incubator at 37°C temperature. After 7 days, the sample were washed with aquabidest then were soaked in 20 ml NaCl 1 M (pH 7.0) at 25°C temperature for 8 hours. The samples were divided into two groups: G1: The control group (cavity directly restored with composite resin); G2: Biodentine™ group (cavity with Biodentine™ as a base then restored with composite resin). All samples were stored in shaking incubator under PBS solution at 37°C temperature. SEM, EDX and TEM analysis were performed on the 7th and 14th day. **Results:** The 14th day Biodentine group had the best SEM remineralization feature with irregular dentine tubular features covered by density of mass. In the EDX analysis, the concentration of calcium ion of the Biodentine group was higher than the control group on the 7th day analysis (Biodentin™ 10.2167 and control 1.9667) and on the 14th day analysis (Biodentine™ 29.833 and Control 22.080). The Biodentine™ group and control group of the 7th and 14th day experienced significant increases in calcium ion concentration while the concentration of phosphate ion in the Biodentine™ and control group had a much lower value of calcium either on the 7th or 14th day. The TEM analysis of Biodentine™ group showed more intrafibrillar remineralization than the control group. The feature of intrafibrillar dentin remineralization is analyzed by looking at the density of black dots in collagen. **Conclusion:** Biodentine™ is able to trigger the process of remineralization by guided tissue remineralization.

Keywords: Tooth Remineralization; Dentin; Calcium Phosphates.

Introduction

Dentin remineralization can occur in two ways: extrafibrillar and intrafibrillar. Extrafibrillar remineralization occurs over the remaining apatite crystals in epitaxial demineralization dentin [1-3]. In conditions of little or no mineral content, remineralization occurs in the intrafibrillar part that still contains minerals, the process is called Guided Tissue Remineralization (GTR). Guided Tissue Remineralization (GTR) can occur if there is still collagen as a scaffold for the apatite crystal deposit [4].

The dentin collagen consists of a triple helix tropocollagen arranged parallel to the ladder and fibril-shaped [5]. Among tropocollagen, there are gap zone and overlap zone. The gap zone contains intrafibrillar minerals while extrafibrillar minerals are present in the cavities between collagen fibrils. The intrafibrillar mineral contributes to the mechanical properties of dentin [5,6].

Intrafibrillar remineralization process requires non-collagen protein, Dentin Matrix Protein 1 (DMP 1). DMP 1 binds to collagen and stabilizes Amorphous Calcium Phosphate (ACP) in order not to aggregate and remain nano-sized. GTR is a concept of "biomineralization of collagen" nano technology with biomimetic principles. In GTR, nanoprecursor Amorphous Calcium Phosphate (ACP) is formed by ions bonding of bioactive materials with DMP 1 collagen. Biomimetic remineralization through a bottom-up process is the formation of nano crystals that can enter into gap zones derived from larger apatite structures [7].

Biodentine™ is a bioactive material based on pure silica cement, most of which is composed of tricalcium silicate ($3\text{CaO}\cdot\text{SiO}_2$) with addition of calcium carbonate (CaCO_3) and zirconium dioxide (ZrO_2) [8]. It is liquid contains calcium chloride (CaCl_2) with a hardening time of about 12 minutes [9]. Biodentine™ is made by Active Biosilicate Technology™, which removes heavy metals such as aluminate and calcium sulfate and releases calcium ions with a beneficial pH for remineralization [10]. Biodentine™ has small particle size and dentine-like compression strength with calcium silica content that can trigger non-classical remineralization process. Biodentine™ layer interfaces with dentin form a mineral-rich micromechanical tag. In addition, Biodentine™ has been shown to increase TGF- β 1 pulp cell secretion in triggering angiogenesis, cell differentiation, mineralization process [11], can promote cell proliferation and migration [9,12]. Clinically, Biodentine™ can be used for apexification treatment, perforation cover, root resorption treatment, filling the root tip after apical resection [13].

The role of Biodentine™ as an agent of GTR dentin has not been analyzed. This study aims to analyze the ability of Biodentine™ in remineralizing dentin by Guided Tissue Remineralization (GTR).

Material and Methods

Samples using four single root teeth after post-extraction were immediately immersed in a phosphate buffered saline (PBS) solution and stored in a refrigerator at 4°C which should not exceed two weeks of storage. Each tooth was made two cavities on mesial and distal using diamond

cylindrical bur no.16 to a depth of 3 mm. The entire surface of the tooth was coated with nail polish except the walls and cavity base. The cavities were demineralized by applying ethylene diamine tetraacetic acid (EDTA) 17% for 1 week and were stored in shaking incubator at 37°C. The root apexes were soaked in PBS solution during the incubation process. After 1 week, the teeth were rinsed with aquabidest for 30 minutes and soaked in 20 ml of 1 M (Sodium Chloride) solution (pH 7.0) at 25°C for 8 hours to eliminate the soluble part and to keep the non-collagen protein remains on dentin.

The Biodentine™ group using a mesial cavity prior to cavity with Biodentine™ as a base then restored with composite resin. The control group used a distal cavity that was directly restored with composite resin.

Sample analysis was performed on the 7th and 14th day using Scanning Electron Microscope (SEM), Energy Dispersive X-ray Analysis (EDX) and Transmission Electron Microscopy (TEM). Before starting the SEM and EDX analysis, the samples were cut to dentin base and cleaned with aquadest. The steam was performed by sterilization of dehydration method, which was soaked with ethanol concentration of 50%, 70%, 80%, 90% for 20 minutes, and 96% for 2 hours. Samples were analyzed using SEM to see dentin surface morphology while using EDX to measure the calcium and phosphate content on dentine surfaces in the control group and Biodentine™ group.

For TEM analysis, the samples were scraped on the cavity basis to remove the particles that had previously soaked in 96% alcohol. The alcohol is allowed to evaporate leaving the particles and placed on top of a 3 mm diameter TEM grid made of carbon-coated copper. Analysis with TEM was used to see whether intrafibrillar remineralization occurs in the gap zone.

Results

SEM Analysis

SEM overview of the demineralized dentin (control) surface morphology, visible open dentin tubules and collagen around the tubules were seen regularly (Figure 1A). This indicated the loss of apatite minerals in the demineralized dentin group. SEM image of the control group showed the process of remineralization with a picture of the edge of dentinal tubules that slightly experienced irregularity (Figure 1B and 1C). SEM image of demineralized dentin surface post application after the 7th day was seen more white irregular dentin edge of the Biodentine™ that indicate a remineralization (Figure 1D and 1E). After the 14th day, Biodentine™ (Figure 1D) in addition to irregular dentin tubules are also covered by density of mass.

EDX Analysis

In Table 1, calcium and phosphate levels in the control and Biodentine™ group increased. Calcium level increased from 1.9667 at the 7th day to 22.080 at the 14th day in the control group. Calcium level also increased from 10.2167 at the 7th day to 29.833 at the 14th day in the Biodentine™ group. Phosphate levels in both group also increased from the 7th day and the 14th day but its value is

not as high as the value of calcium. In conclusion: remineralization occurs both the control group and the Biodentine™ group.

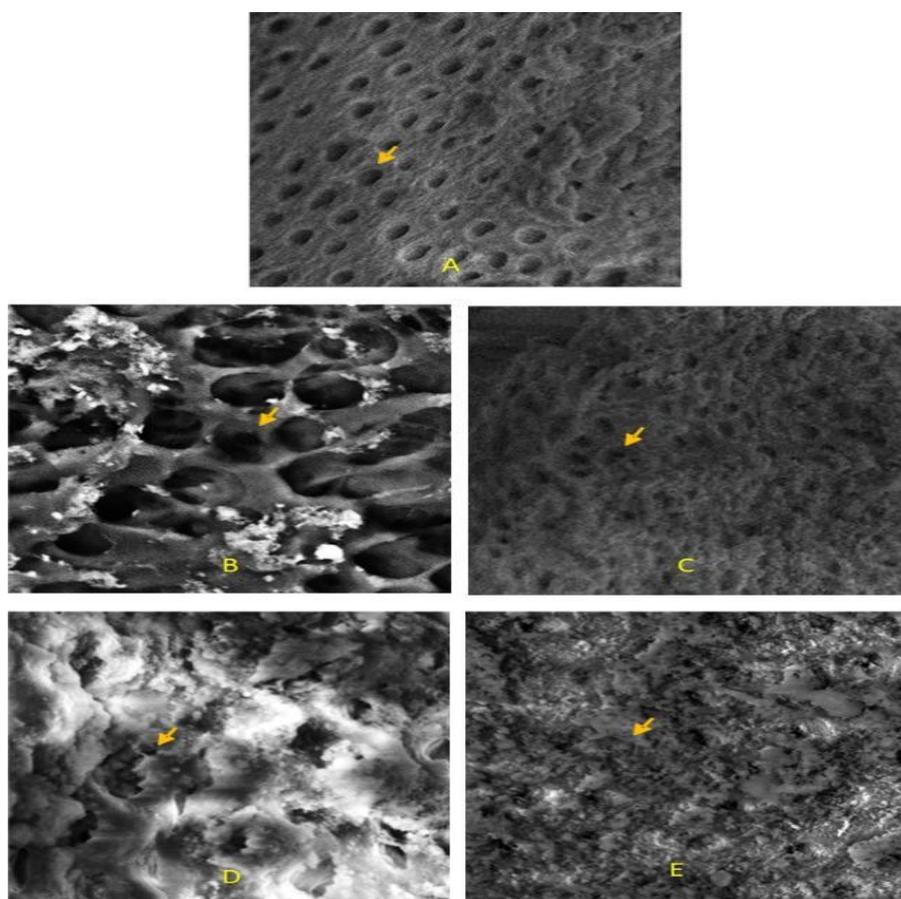


Figure 1. SEM description: A. dentin demineralization; B. The 7th day dentin control group; C. The 14th day dentin control group; D. The 7th day dentin Biodentine™ group; E. The 14th day dentin Biodentine™ group.

Table 1. Mean value and standard deviations of calcium and phosphate in the control and Biodentine™ group.

| Group | 7 days | | 14 days | |
|-------------|------------------|------------------|-----------------|-----------------|
| | Calcium | Phosphate | Calcium | Phosphate |
| Control | 1.9667 ± 1.799 | 0.9297 ± 0.69463 | 22.080 ± 0.1136 | 13.17 ± 1.50841 |
| Biodentine™ | 10.2167 ± 1.4609 | 4.90 ± 4.80204 | 29.833 ± 10.661 | 6.3200 ± 1.8355 |

In Table 2, Calcium significance values between control group the 7th and 14th day was 0,015 whereas in the Biodentine™ group between the 7th and 14th day were not significantly different (0,407). If the control and Biodentine™ group were compared, significant differences occurred between the 7th day of control and the 7th day of Biodentine™ (0.024) and between the 7th day of Biodentine™ and the 14th day of control (0.029). It could be concluded that Biodentine™ release of calcium on the 7th and 14th day with no significance difference whereas in the control group of calcium concentration on the 7th to 14th day increased significantly.

In Table 3, the significance values of phosphate levels were only between the control group of the 7th and 14th day (0.008) and between the 14th day control and Biodentine™ group (0.049). In

conclusion, phosphate levels in the control group experienced a very high increase while the Biodentine™ group consistently incremented.

Table 2. Significancy value of calcium levels between the control and Biodentine™ groups.

| Group | | Control | | Biodentine™ | |
|-------------|---------|---------|---------|-------------|---------|
| | | 7 days | 14 days | 7 days | 14 days |
| Control | 7 days | - | 0.015* | 0.024* | 0.228 |
| | 14 days | 0.015* | - | 0.029* | 0.913 |
| Biodentine™ | 7 days | 0.024* | 0.029* | - | 0.407 |
| | 14 days | 0.228 | 0.913 | 0.407 | - |

*Statistically Significant.

Table 3. Significancy value of phosphate levels between control and Biodentine™ groups.

| Group | | Control | | Biodentine™ | |
|-------------|---------|---------|---------|-------------|---------|
| | | 7 days | 14 days | 7 days | 14 days |
| Control | 7 days | - | 0.008* | 0.869 | 0.141 |
| | 14 days | 0.008* | - | 0.414 | 0.049* |
| Biodentine™ | 7 days | 0.869 | 0.414 | - | 0.999 |
| | 14 days | 0.141 | 0.049* | 0.999 | - |

*Statistically Significant.

TEM Analysis

In Figure 2, intrafibrillar remineralization on the 7th day of the control and Biodentine™ groups has begun to be seen with a slight black dotage (Figure 2A) and in the 14th day analysis the control and Biodentine™ group became clearer and denser picture of the black dots contained in collagen. In the Biodentine™ group have a denser dot (Figure 2D). The control and Biodentine™ groups can induce GTR process, but Biodentine™ group produce more GTR.

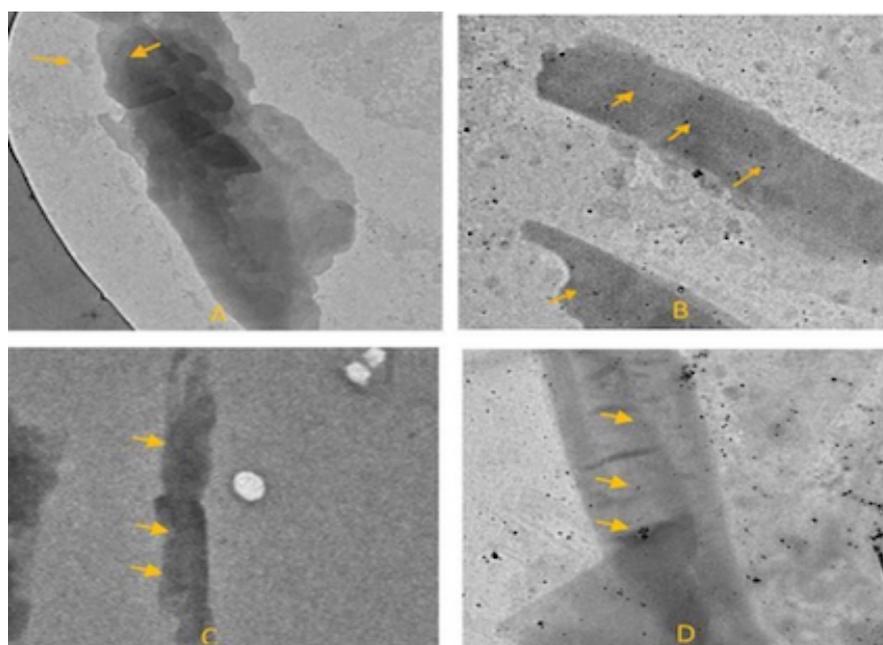


Figure 2. Overview of TEM analysis of GTR occurrence in dentine collagen: A. The 7th day of control group; B. The 14th day of control group; C. The 7th day of Biodentine™ group; D. The 14th day of Biodentine™ group.

Discussion

In this study, the samples were immediately stored at 4°C and soaked in a PBS solution in order to keep the type I collagen structure vital [14]. The use of PBS as a sample immersion medium because PBS has ionic concentrations comprising sodium chloride, sodium bicarbonate, potassium chloride and potassium phosphate, so it can be a source of calcium and phosphate for remineralization [15]. The teeth were demineralized by immersion in a 17% EDTA solution for day 7th at 37°C to obtain condensed affected dentin. The use of EDTA 17% resulted in dentin demineralization with collagen crosslinks that remain intact [2]. According to some authors, 17% EDTA has a good sealing ability against calcium ions so that only minerals of calcium are released while still leaving intact collagen cross-linking with conditions resembling affected dentine [16].

For the GTR process in addition to the presence of collagen is also required the presence of non-collagen protein is Dentin Matrix Protein 1 (DMP1) which acts as a regulator and stabilizer to prevent the formation of amorphous calcium phosphate nucleation before it can reach intrafibrillar region [2,17]. Non-collagen protein in addition to functioning nano-particles also prevent the transformation of nano-particles into apatite crystals before entering the intrafibrillar gap zone. Non-collagen proteins will bind to collagen in the gap zone, then the complex nanoscale precursors will turn into apatite nano crystals to form electrostatic intrafibrillar mineralization. This biomimetic remineralization is a bottom-up approach that is, remineralization through the formation of nano crystals that can enter the gap zone between collagen [1].

In GTR, non-collagen proteins act as stabilizer agents whereas mineral agent precursors can be obtained from bioactive materials and minerals remaining in dentin. In the bioactive materials of the dominantly released ions are calcium and phosphate. In the process of remineralizing calcium and phosphate ions that control this process, without the existence of these ions the process of remineralization will not occur.

Evaluation of remineralization in this study was conducted using SEM, EDX and TEM on the 7th and 14th day. This is based on the 7th day already formed octa-calcium phosphate while the new hydroxyapatite mineral was seen on the 14th day. Calcium and phosphate are ions that play a role in remineralization. The major sources of calcium and phosphate in this remineralization process can be obtained from bioactive materials such as Biodentine™. In addition, the source of calcium and phosphate ions can also be obtained from the PBS solution used as a soaking medium.

In SEM analysis, in the control group and Biodentine™ after the 7th and 14th day, it appears that in both groups the remineralization occurred which showed the irregularity of the dentin tubule wall. Although the form of irregularity of the dentinal tubules between the control group and Biodentine™ is different (Figure 1). In the Biodentine™ group the irregularity is much more real, but it shows that demineralization dentine in the physiological environment is still capable of independently performing remineralization process. This condition illustrates that remineralization can occur physiologically without any involvement of bioactive materials. While the addition of Biodentine™ in this study can improve remineralization which results better than physiologically.

In the EDX analysis is in Table 1, calcium and phosphate levels in the control and Biodentine™ group increased. In the control group, calcium content increased from 1.9667 on the 7th day to 22.080 on the 14th day. While in the Biodentine™ group, calcium content increased from 10.2167 on the 7th day to 29.833 on the 14th day. Phosphate levels in the control group and Biodentine™ from the 7th to 14th day increased, but the value is not as high as the value of calcium. This condition can illustrate that in the control group as well as the Biodentine™ group remineralization can occur. However, remineralization in the Biodentine™ group was faster than the control group. Magnitude higher calcium value than phosphate showed that both in the control group and Biodentine™ from the 7th day have started to form ion bonds in the form of hydroxy apatite.

The release of calcium and phosphate ions in the control group between the 7th to 14th day had a significant differences value, whereas in the Biodentine™ group did not significantly (Tables 2 and 3) this showed that the release of calcium and phosphate ions from Biodentine™ as bioactive material release constant. The constant ion release value in Biodentine™ from the 7th to 14th day was caused by silica calcium gel at the time of mixing having hydration correlated with rapid release of calcium ions [18]. Biodentine™ main content is tricalcium silicate with a very small particle size of 2,811 m²/g so that at the time of setting will be easy and quick release of calcium and phosphate ions [12].

To analyze whether conventional remineralization or GTR is done with TEM. The results obtained in the control and Biodentine™ group remineralized GTR, although in the Biodentine™ group much more. This indicates that the physiological GTR process can occur in this study that is in the control group. The continuity of GTR in the control group indicates that the role of DMP 1 of dentin still exists and its minerals are obtained from PBS fluids. While in the group Biodentine™ remineralization process can take place quickly because in addition to utilizing calcium ions from PBS also from Biodentine™ which is a bioactive material that releases many ions dominated by calcium and phosphate ions. By proving that GTR in demineralization dentine can naturally occur, the method in this study can be defined as a standard method for simulating research on vital teeth that resemble a physical process.

Conclusion

Biodentine™ can trigger an increase in Guided Tissue Remineralization (GTR) in dentin.

Financial Support: None.

Conflict of Interest: The authors declare no conflicts of interest.

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