Influence of Dental Humidity on Marginal Adaptation of Biodentine Restorations

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Biodentine is a tricalcium silicate cement used as a dentin replacement in restorative dentistry. The aim of this study was to assess the influence of cavity moisture on the immediate sealing and morphology of the interface between Biodentine and the dental tissues. 20 class II cavities were prepared in extracted teeth. 10 cavities were dried using the air spray before restoring with Biodentine and 10 cavities were preserved moist for the restorative procedure. The teeth were subjected to a microleakage test and then the axial sections were observed under optical microscope and scanning electron microscope. In most situations Biodentine provided an immediate good marginal sealing and a tide contact with adjacent tissues in both occlusal and cervical margins which did not seem to be influenced by the moisture of the dental supporting tissues.

Keywords: Biodentine, dental moisture, microleakage, interface morphology

Experimental part
The study groups included 20 freshly intact molars extracted for orthodontic or periodontal reasons. Class II cavities were prepared in either mesial or distal surfaces using a fine diamond pear-shaped bur with high speed and water-spray cooling. The width of the cavity was about one third of the intercuspal distance and the gingival margin was placed bellow the enamel-cement junction. The specimens were randomly divided in 2 groups. 10 cavities were dried using the air spray until the dentin appearance became opaque (group A). The other 10 cavities were kept moist for restoration procedures, only the pooling water being adsorbed with cotton pellets (group B). Biodentine (Septodont) was mixed in the amalgamator according to the instructions and used for restoration. The material was condensed in the cavity with a plugger, using a mylar matrix. After setting, the specimens were stored in distilled water for 24 h; then the excessive material at the margins was removed with extra-fine diamond burs. The apices of all teeth were sealed with a self-adhering flowable composite resin and the external surfaces of each sample were covered with two layers of nail varnish except for the restoration and about 1mm around the tooth-restoration interface. The samples were rehydrated in distilled water for 5 minutes and then they were immersed in 1% methylene blue for 24 hours. Then the specimens were axially sectioned in a mesial-distal direction through the middles of the restorations using a double ended diamond disc in low-speed handpiece.

The images of the microleakages at the enamel margins and cervical margins were registered and scored using an optical Carl-Zeiss AX10 Imager A1m microscope, coupled with a high-resolution digital camera, using Dark Field and Bright Field filters, at 50X magnification. For cervical

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margin the scores for dye penetration were: 0 = no dye penetration; 1 = dye penetration from the cavosurface margin to less than half the length of the cervical wall; 2 = more than half the length of the cervical wall, but not involving the axial wall; 3 = along the whole length of the cervical wall and also involving the axial wall. For the occlusal margins, the scores were: 0 = no dye penetration; 1 = dye penetration from the cavosurface margin to less than half the thickness of occlusal enamel; 2 = more than half of the thickness of enamel, but not involving the dentin; 3 = the whole thickness of enamel and involving the dentin.

The sections were also observed by scanning electron microscopy using a VEGA II LSH (TESCAN) microscope in order to assess the morphology of the interface at the margins of the restorations.

Results and discussions

Results of the microleakage study

Microleakage evaluations are used to estimate the resistance of tooth-restoration interface to the passage of bacteria, fluids, chemical substances, molecules and ions. Despite the controversies related to the clinical significance of such tests [14], the stain penetration is one of the most common method of assessment which can provide important information on possible clinical performance of new restorative materials. Kersten and Moorer found that leakage of methylene blue was comparable with that of small bacterial metabolic product of similar molecular size [15].

The leakage scores and the mean values for each group are listed in Table 1.

Within each group, most of the samples showed a good marginal sealing, with no sign of marginal leakage at cavity margins. Each group recorded only one score of 1 for enamel and for cervical margins respectively. Scores 2 and 3, showing a deep penetration involving the axial wall was not found in any of the specimens regardless the location of the margin and moisture of the cavity (Fig. 1 and 2).

Our results are consistent with previous studies supporting the good marginal sealing ability of Biodentine.

<table>
<thead>
<tr>
<th>Score</th>
<th>Group A</th>
<th>Group B</th>
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<tr>
<td></td>
<td>Enamel Margin</td>
<td>Cervical margin</td>
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<tr>
<td>0</td>
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<tr>
<td>3</td>
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<td>0</td>
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<tr>
<td>Mean score and standard deviation</td>
<td>0.1 ± 0.316228</td>
<td>0.1 ± 0.316228</td>
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Table 1

MICROLEAKAGE SCORES IN EACH GROUP

Fig. 1. Images of dye penetration at enamel margins

Fig. 2. Images of dye penetration at cervical margins
in cervical margins. In open-sandwich restorations, Biodentine was found to be similar in terms of microleakage and porosity with glass ionomer cements [6, 7]. Another study which evaluated Biodentine overcoated with composite resins in class II open-sandwich restorations reported higher median scores (0.76±0.83 in permanent teeth and 0.60±0.87 in primary teeth) than those recorded in our study, probably because of the thermocycling procedures applied to the specimens. However, the microleakage was lower than that reported for the tested glass ionomer cement [8]. Similar results were reported when quantitative evaluation by glucose diffusion was used to investigate the microleakage. Biodentine and the resin-modified glass ionomer cement allowed similar glucose diffusion at the interface between the restorative material and the dentin walls in experimentally aged open-sandwich restorations [16]. In close-sandwich class I restorations, Biodentine overcovred by composite resin exhibited superior marginal sealing ability and marginal adaptation when compared to MTA and GIC. After 500 rounds of thermocycling, the mean score for Biodentine was 0.00±0.00 whereas for the tested glass ionomer cement, it was 2.00±0.00 [9]. Another study reported that the ability to prevent fluid movement over time was enhanced in acidic environment [17-19]. On the other hand, Camilleri et al. [10] found significant leakage at the interface of dentine with Biodentine when the material was etched with phosphoric acid and covered with composite resins in open-sandwich restorations, while the tested glass ionomer cements displayed no microleakage when used in similar conditions.

As regarding the use as retrograde filling material, one study concluded that Biodentine provided significant better marginal adaptation when comparing to MTA and glass ionomer cement [20], while other studies reported higher microleakage with Biodentine comparing to MTA when analyzing by fluid filtration method [11] and significantly inferior marginal adaptation comparing to MTA and IRM [19].

These contradictory results could be explained by the different methodologies for restoration, storage, and detecting the leakage. The scores we recorded in cervical margins support the good marginal sealing ability of Biodentine, at least on short term, immediately after the placement of the material since only one specimen in each group have showed a minimal leakage.

Very few studies have investigated the interface between the material and the enamel, since Biodentine is not indicated as single material for long-term restorations. However, the indication for pulp capping and for temporary restoration demands some information about the short term sealing ability at the enamel margin. Our results suggested that the sealing at the enamel margin is very good immediately after the restoration placement. This is consistent with the results of a clinical evaluation on the performance and safety of using Biodentine in posterior restorations which found a resistance to marginal discoloration significantly superior to that of composite resin used as control. Moreover, although deficient marginal adaptations were recorded after 6 months, no marginal discoloration occurred [22].

Within the limitation of our study, it might be concluded that Biodentine can provide an immediate good marginal sealing in both occlusal and cervical margins which is not influenced by the moisture of the dental supporting tissues.

Results of the SEM study

Most images obtained by scanning electron microscopy confirmed an acceptable adaptation of Biodentine to the occlusal and cervical margins and walls of the cavities, regardless the degree of tissue moisture (Fig. 3 and 4).

In enamel margins, the adhesive failures seemed to be related to the difficulties of cement adaptation to the irregularities of the dental substrate at the occlusal margins. The consistency of the mixed material was quite high which determined the material to over cover the margin without being in tight contact with the underlying irregularities of unprepared enamel. However, at the interface with the prepared wall, the adaptation seemed to be fine. A good adaptation was noticed for both groups when the sections involved smooth occlusal margin (Fig. 3b and d). In both situations when occlusal microleakage scores had been 1, the sections of the specimens involved occlusal margins that have been in close contact with a deep occlusal fissure, as shown by the SEM images (Fig. 3a and c). The hydration of the substrate seemed to be less important than the smoothness of the dental substrate and proper condensation and modelling of the material.

As regarding the cervical margin, the marginal adaptation seemed to be good (Fig. 4a and c) even if several large pores have been noticed remote from the margins, at the interfaces of some of the specimens (Fig. 4c). The magnitude of the pores indicated a defective condensation.
of the material rather than a porosity issue of the material. Marginal defects involving small fractures of the material at the margins have been observed in the specimens which had previously showed staining infiltration (Fig. 4b) and in several specimens that had recorded 0 scores in the microleakage study (Fig. 4d). In fact, in several specimens where microleakage had not been previously observed, a gap was observed at the interface under SEM evaluation (Fig. 4d).

No correlation could be made between the degree of the moisture of the cavity and the presence of gaps at the interface. Since these defects were not always related to stain penetration, they could occur after the storage of the specimens in the staining solution, being the consequences of the material friability and sensitivity to desiccation during the cutting and vacuum procedures. Therefore, these drawbacks of Biodentine could not be made responsible for the leakage in this experiment, however these characteristics could result in defective sealing in long term experimental conditions.

Most of the studies investigating the morphology of the interface between Biodentine and cavity walls. Another SEM study found that the mean diameter of internal gaps was significant lower comparing to glass ionomer cement and lower, even not statistically significant when comparing to MTA [9].

Several explanations have been proposed for the good marginal sealing ability of Biodentine. The nano structure of the material allowing the material to spread onto the surface and the slight expansion could also contribute to its improved sealing ability [16, 23, 24]. These qualities might compense the high consistency of the material and explain the SEM images that we found at the occlusal margins involving irregular deep fissures.

Another hypothesis suggested that the sealing ability of the material is related to specific properties involving ion release and ionic changes with the environment. Biodentine was found to produce a high pH and to release calcium and silicon ions, creating a mineral infiltration zone along dentin-cement interface and tag-like microstructures [25, 26]. Furthermore, Biodentine showed apatite formation after immersion in phosphate solution [27]. Numerous attempts have been made to produce materials which might induce or release hydroxyapatite [28, 29]. Biodentine showed wider Ca- and Si-rich dentine areas and larger incorporation depth comparing to MTA in the presence of phosphate buffered solutions [30]. Another study found Biodentine crystals firmly attached to the underlying dentine surface and an interfacial layer similar to that formed by MTA, after storage in artificial saliva [31].

Most of the studies investigating the morphology of the interface between Biodentine and dental tissues found an excellent adaptability of the material to the underlying dentin whether they attributed the effect to ionic exchange or micromechanical adhesion. Our images did not reveal the infiltration zone or the presence of tag-like microstructures. Some of the reasons could be related to the preparation of the specimen, which involved cutting procedures resulting in smear layer formation on the samples surfaces and storage in distilled water which might exert a corrosive effect [32]. However, our results support the hypothesis of an intimate contact between Biodentine and the dental tissues, resulting in good sealing at the margins and walls of the restoration.

The porosity of the material has been considered a critical factor in leakage formation [33]. Porosity is an intrinsic characteristic of tricalcium silicate-based cements and occurs as a result of the spaces between the unhydrated cement grains. These spaces are filled with water once the materials hydrates and the hydration products fill these gaps. However, if too high content water-to-cement ratio is used during mixing, excess water eventually dries of and leaves voids that are not filled by hydration products [12]. On the other hand, the dry storage of Biodentine resulted in gaps at the dentin-material interface allowing the passage of the fluorescent microspheres [12], which might suggest that in case of restorative procedures when the material should be kept dried, formation of porosity and gaps might occur, leading to bacterial passage at the interface [34]. Both overwetting and desiccation of the material have been proven to be detrimental to the qualities of the material. Contrary to these facts, in our study the moisture of the supporting dental tissues did not seem to influence the hydration of the material during setting and the quality of sealing and interface between the material and the dental tissues. Neither moist nor dry cavities had significant detrimental effects on the sealing ability and marginal adaptation. These are however preliminary results. The different degrees of dental hydration that we
Conclusions
Biodentine restorations showed a good marginal sealing at occlusal and cervical margin irrespective to the moisture of the supporting tissues. Most images obtained by scanning electron microscopy confirmed an acceptable adaptation of Biodentine at the margins and walls of the cavities, although minor fractures and pores of the material were noticed. No significant differences have been noticed between the restorations applied in wet cavities comparing to dried cavity. Few images showed gaps at the interface. However, these seemed to the related mainly to the preparation of the samples.

Within the limitations of this study, the moisture of the prepared cavity did not seem to influence the quality of sealing and interface between Biodentine and the dental tissues. Neither moist nor dry cavity had significant detrimental effects on the sealing ability and marginal adaptation of the material to the dental supporting tissues.

References
6. 2015, p. 351.
7. 2017, p. 82.