The Effect of Biodentine[TM] Application in Affected Dentin Remineralization after Partial Caries Excavation *In Vivo*

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Abstract

Treatment of deep carious lesions is typically accomplished by complete caries removal, risking pulp necrosis. With dental science and technology advancements, dentists can currently perform minimally invasive caries removal by leaving a partial carious lesion.

We evaluated the remineralization of affected dentin after leaving partially infected dentin (Group 1) or removing all infected (Group 2) dentin. Biodentine[TM] was applied to the participants in both study groups and they were evaluated after four weeks. (Ethical Clearance No: 100/EthicalApproval/FKGUI/XII/2016).

Both groups showed radiographically insignificant remineralization from baseline to week four. Between-group comparisons of remineralization were also insignificant. Affected dentin remineralization can occur whether the infected dentin is left partially intact or is completely removed.

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Introduction

Dental caries occurs due to changes in biofilm ecology, from a balanced population of microorganisms to one that is acidogenic, aciduric, and cariogenic. This imbalance develops due to glucose fermentation, which lowers the pH on the tooth surfaces. This causes further demineralization of hard tooth tissue. Based on this understanding, full bacterial removal is not needed because changing the ecological metabolic biofilm balance is usually sufficient to allow for remineralization to occur and stop the carious lesion.[1] Traditionally, carious lesions are treated by removing all of the carious lesion to obtain healthy tissue before restoration.[2] This approach follows the complete caries removal technique of Black in 1908, which was the standard of care at that time.[3,4] After more than 100 years, Black's technique is still in wide use.

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According to research done by Schwendicke in 2016, more than 66% of the clinicians in France and Germany still perform complete caries removal, even though more conservative techniques exist that do not risk the tooth pulp.[5] In 2008, Burrow et al. found that pulp exposure due to excavation after complete caries removal was approximately 53%.[6]

A minimally invasive approach for remediating deep carious lesions was developed to preserve pulp vitality.[7] Vital pulp is needed for the pulp nutritional functions, cell preservation, sensation, and formation of new dentin by odontoblasts or odontoblast-like cells for pulp protection or defense.[8] For deep carious lesions, the remaining dentin is very thin, approximately one-quarter of its normal thickness. Therefore, complete carious lesion removal is not mandatory because the probability of pulp exposure is too high.[2] Research has shown that it is impossible to remove all bacteria in a carious lesion.[9] In 1968, Shovelton and Crone found that 40% of the bacteria remained in dentin tubules after complete excavation of deep carious lesions.[9]

Deep carious lesions feature the following two dentin layers: the infected dentin and the affected dentin. The infected dentin is the outer carious lesion layer, filled with bacteria that cause complete destruction that cannot be repaired. The affected dentin is the inner layer with remaining collagen matrix so that remineralization can occur.[10,11] A minimally invasive approach involves partial removal of the carious lesion from the dentin. There are two techniques for partially carious lesion removal. The first of these is selective removal of the soft infected dentin, which leaves some infected dentin. The second one is selective removal of the firm dentin which leaves only the affected dentin.[12] Partial removal of a carious lesion maintain dentin thickness, compared to that found after complete carious lesion removal. To preserve pulp vitality, a minimal remaining dentin thickness should be of 0.25–0.5 mm from the pulp.[13,14]

In conventional therapy, infected dentin removal that leaves a thin layer of dentin intend to form reparative dentin through dentin remineralization. Conventional remineralization in dentin occurs on top available apatite crystals. If no or only a few apatite crystals are available, remineralization cannot occur. Dentin remineralization is also distinct from enamel remineralization, and crystallization in enamel remineralization cannot be applied to dentin remineralization. Demineralized dentin exposes collagen. More complex remineralization is required for dentin remineralization.

For treatment of minimally invasive carious lesions, a new approach involves leaving infected dentin in the cavity. Due to our understanding of metabolic biofilm balance, bioactive material development also has a role in changing the metabolic balance. In 2014, Atmeh et al. found that calcium silica could trigger dentin remineralization in totally demineralized dentin.[15] Hydroxyl ions can destroy bacteria left in the infected dentin so that the layer under the restoration material can be in a bacteria-free environment. In a bacteria-free environment, the pulp heals and deposits dentin materials.[16] The affected dentin with 10% collagen can also induce remineralization.[11] The biomaterial used in vital pulp treatment must fulfill several criteria, including having the ability to kill bacteria, induce mineralization, and create a bacteria-impermeable environment.[16]

Dentin remineralization with biomaterial application has been researched *in vitro* using different methods. Remineralization starts with the formation of mineralized foci beginning 2 days after the biomaterial application. At 14- and 28-day post-application, mineral deposits form and begin to grow.[17] During observation using a scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX), mineralized spherulites were formed 7 days after the bioactive material application. At 28 days, the silica was no longer detected, and calcium and phosphates ion concentrations were increasing.[18]

Biomimetic biomaterial is used to repair or replace damaged human tissues or cells and is needed for treatment of deep carious lesions. The current biomaterials have bioactive properties that can induce dentin remineralization.

The American Academy of Pediatric Dentistry Guidelines (2014 revision) state that glass ionomer cement (GIC) and matrix trioxide aggregate (MTA) can be used as biomaterials in vital pulp treatment.[19] MTA, which is a calcium silica-based cement, is becoming popular for its bioactive ability.[20] Since 2009, calcium silica-based cement (Biodentine™; Septodont, Inc., Lancaster, PA, USA), specifically made to replace dentin, has been marketed for endodontic application or as a pulp capping material.[21] Biodentine™ contains pure calcium silica particles with a rapid setting time and has produced excellent results during clinical application.

Biomaterial use can trigger classic or non-classic dentin remineralization. During classic remineralization, crystal deposition occurs only in the calcium ion- and phosphate-rich dentin environment. Classic remineralization only produces extrafibrillar remineralization. On the other hand, during non-classic remineralization, biomimetic remineralization occurs so intrafibrilar remineralization can also occur.[22]

Therapies to increase remineralization can be evaluated by observing the teeth mineral quality and quantity changes. Clinicians require diagnostic tools and must perform proper evaluation to monitor carious lesion remineralization. Digital imaging radiography has the ability to increase the diagnostic accuracy and quantitative diagnosis. Digora (The Dental Imaging Company Ltd., Shoreham by Sea, UK) digital radiography can count pixel optical density with sufficient sensitivity to detect slight changes that cannot be seen by the naked eye. In 2012, Nőbrega et al. showed that indirect digital radiography using the Digora instrument is comparable to the direct method for measuring biomaterial gray values.[23]

In our research, partial caries excavations were performed in patients with deep carious lesions and BiodentineTM was applied on the cavities. Before and after treatment, digital radiography was used to measure gray value scores showing the remineralization levels in patients since radiography is one of the most common diagnostic and evaluation tools used by clinicians.

Materials and methods

Consecutive specimens were collected over the course of one month from five adults (18–50 years) which partially infected dentin was left (Group 1), and five adults which all infected dentin was removed (Group 2). The inclusion criteria were no systemic abnormalities, a carious lesion on the first or second premolar and molar with 0.25–1 mm dentin thickness remaining from the pulp, no pulp exposure, a vital tooth, no complaints of sharp pain, ability to assemble a rubber dam, no periapical abnormalities on radiography examination, and direct restoration candidacy.

All subjects were examined according to the inclusion criteria. Then, digital radiographic examinations were performed before treatment using the paralleling technique and bite fixation. The radiograph scanned using Digora for Windows software. The tooth to be treated was isolated using a rubber dam. SableTM and Seek® (Ultradent Products, Inc.) applications were used to show the carious lesion to be excavated. The carious lesion was excavated using one of the two following techniques: leaving some infected dentin close to the pulp (Group 1) and removing all infected dentin (Group 2). After that, 3–4 mm BiodentineTM was applied and closed with Fuji IX GIC (GC Corp, Tokyo, Japan). After four weeks, radiography evaluation was done using the same technique and position.

Results

This study was done using Digora software digital radiography for Windows to measure the carious lesion gray values before BiodentineTM application and after four weeks. Radiographs were taken using the paralleling technique with an indicator cone so there would be no angle difference as well as to facilitate photo interpretation. Increased gray values were observed in both study groups. Data were analyzed using SPSS ver. 20. Mean gray values before and four weeks after BiodentineTM application are shown in Table 1.

	Before treatment		4 weeks after treatment	
Variable	Mean (SD)	Min–Max	Mean (SD)	Min–Max
With infected dentin	124.00 (9.274)	116–137	130.25 (6.397)	125–139
Without infected dentin	157.75 (24.102)	133–187	172.25 (30.369)	141–206

Table 1. Mean Gray Values Before and Four Weeks After BiodentineTM Application.

Normality tests were performed using the Shapiro–Wilk test. Normal data distributions were acquired from both study groups and the data were thus analyzed using paired *t*-tests. The results are shown in Table 2.

		N	Mean (SD)	*p*-value*
Group 1 – With infected dentin	Before	5	124.00 (9.274)	
	After 4 weeks	5	130.25 (6.397)	0.146
Group 2 – Without infected *dentin*	Before	5	157.75 (24.102)	
	After 4 weeks	5	172.25 (30.369)	0.007

*$p < 0.05$

Table 2. Mean Gray Values Before and Four Weeks After BiodentineTM Application.

The baseline data in this results are the grey values before BiodentineTM application. There was no significant difference in the gray values between the baseline and four weeks post BiodentineTM application in Group 1 ($p \geq 0.05$), but there was a significant difference between the baseline and four weeks post BiodentineTM application in Group 2 ($p \leq 0.05$).

Table 3 shows that there was no significant between the groups in gray value escalation ($p \leq 0.05$).

		N	Mean (SD)	*p*-value
Group 1	– Infected dentin group gray value escalation	5	6.25 (5.058)	0.097**
Group 2	– Without infected dentin group gray value escalation	5	14.50 (7.047)	

$p > 0.05$

Table 3. Mean Gray Value Escalation Comparisons Between the Two Groups.

Discussion

A deep carious lesion is one that leaves only one-quarter of the normal dentin thickness, with risk of pulp exposure on excavation. The condition of the pulp in deep carious lesions can include reversible or irreversible inflammation. To lower the risk of pulp exposure, selective carious lesion excavation can be performed.

Selective carious lesion excavation can be accomplished by several methods such as two-visit (stepwise) or one-step excavations. One-step carious lesion excavation is differentiated from stepwise excavations by incomplete removal to firm dentin, which leaves affected dentin, and incomplete removal to soft dentin which leaves infected dentin and affected dentin.

In this research, one-step excavation was performed, leaving only affected dentin, and some infected and affected dentine. Both methods were chosen to preserve the pulp from exposure. Based on the study of Schwendike et al., one-step selective excavation is associated with a lower risk of pulp exposure compared to stepwise excavation.[1]

The difficulty with the selective excavation technique is determining how much of the carious lesion must be removed. To resolve this problem, we used a caries indicator dye that could differentiate between infected and affected dentin; selective excavation limits could thus be set clearly, thereby reducing the risk of this research.

After selective excavation, we applied a remineralization-inducing material. Several calcium silica-based bioactive materials are believed to induce remineralization. We used the pure silica-based material Biodentine™. Remineralization using Biodentine™ *in vitro* was associated to Biodentine™ calcium ion release. Gandolfi et al. found that within 0–3 hours, Biodentine™ released more than 90 parts per million (ppm) of calcium ions, which was a higher rate than the 24 ppm for MTA ProRoot.[24] Another physical advantage of Biodentine™ is its smaller particle size (<1 μm), which can increase its reaction with the body fluid.[24,25] The smaller particle size can result in remineralization probability because intrafibrillar remineralization can occur.

Biodentine™ is a dentine replacement material that demonstrates a great interface layer with dentin. In 2014, TEM examination showed that an interface formed inside dentin tubules after Biodentine™ application, and that amorphous calcium phosphate (ACP) formed after four weeks.[26]

Table 2 shows that both groups demonstrated increased gray values four weeks after Biodentine™ application, even though the change did not rise to the level of statistical significance in Group 1. The non-significant results in Group 1 could have occurred because of the short control period (four weeks after treatment). Given that some infected dentin was left, a longer time may have been required to achieve remineralization in the infected dentin.

Residual infected dentin is not a problem, especially with regard to the development of more carious lesions. Increased gray values, as identified using digital radiography, confirmed the stoppage of the carious process and slight dentin remineralization. This is consistent with research showing that ecological changes in carious lesions can completely stop the lesions, even without completely eliminating all cariogenic bacteria.[27–30] On the other hand, Group 2 demonstrated statistically significant gray value escalation. The results from both groups indicated that remineralization occurred in deep carious lesions, even when affected dentin was left after Biodentine™ application.

In 2015, Prati and Gandolfi used calcium silica bioactive cement soaked in simulated body fluid and analyzed the specimens using SEM/EDX from a biological perspective.[18] They found that after 28 days, apatite increased over time. We therefore evaluated the dentin remineralization levels at four weeks using Digora™ Optima pixel gray values.

Statistically, there was no significant different in gray values between the two groups (Table 3). Groups 1 and 2 demonstrated comparable remineralization. Our results were similar to those of Gandolfi et al. in 2011, who studied *in vitro* remineralization using bioactive materials.[20] The authors stated that the calcium ions that were released from the materials induced remineralization. That study used materials that were 2–20 μm in size and found that new apatite crystals formed and bonded with dentin. Importantly, the crystals were not simply deposited on the surface of the lesion.[20]

This finding is also was supported by a 2015 study by Cao et al., who used an analog method to stabilize ACP nano-precursors and deliver them into the dentin collagen matrix. However, the authors used an analog because of difficulties extracting and purifying non-collagen proteins.[31]

By assuming that collagen and non-collagen proteins such as Dentin Matrix Protein (DMP1 and DMP2) remain in the dentin, intrafibrillar remineralization might occur because there is a regulator for the nucleation process and mineral growth. Non-collagen proteins can be regulated *in vivo* during dentin biomineralization.[31]

The dentistry paradigm in caries treatment can change. Because of the availability of bioactive materials that can induce remineralization, ecological changes can be made without eliminating bacteria-causing caries. By using an alkaline bioactive material, the acidity in the carious lesion can be resolved and, due to the high pH, dentin remineralization can be triggered.

Conclusions

Remineralization occurred in the affected dentin of the deep carious lesion that left infected dentin close to the pulp four weeks after Biodentine™ application; however, no significant changes were seen on radiography. Remineralization occurred in the affected dentin of the deep carious lesion with removal of all infected dentin four weeks after Biodentine™ application. Remineralization occurs in deep carious lesion-affected dentin four weeks after Biodentine™ application, whether the infected dentin is partially left or removed completely.

Declaration of Interest

The authors report no conflict of interest.

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