



Effectiveness of Two Bioactive Restorative Materials in Dental Hard Tissues Remineralization as Indicated by Laser Fluorescence

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ABSTRACT

Twenty extracted human sound premolars were selected and grouped into four groups and two class V cavity preparations, facial and lingual, were prepared at the cervical one-third of the crown of each tooth and extending into the root cementum to make a total of 40 cavities (n=10). The teeth were soaked in a demineralizing solution of pH 4.5 for 72 hours and a tooth surface conditioner was applied to remove remnants of the smear layer. Three laser fluorescence readings were recorded by DIAGNOdent pen for each cavity at enamel margin, dentin floor, and cementum margin. The groups were restored with four different restorative materials Cavit, temporary filling material (control), Ketac-Fil; a conventional glass-ionomer, and two bioactive restorative materials; Glass Carbomer and Biodentine. The restored teeth were stored in mineral water (37°C) for three weeks and brushed twice daily with Tooth Mousse toothpaste. The teeth were longitudinally cut into halves in the middle of the restorations and three DIAGNOdent pen readings were recorded at the same previous sites. Data were collected and statistically analyzed by one-way ANOVA and Tukey's post-hoc-test at ($p < 0.05$). The results showed a significant increase in DIAGNOdent pen readings after soaking in the demineralizing solution in all tooth hard tissues, while a significant decrease was noted after the application of the restorations and storage in the three groups, other than control, indicating that both examined bioactive restorative materials were suitable for enhancing remineralization and subsequently arresting the three-tooth hard tissues carious lesions as the conventional glass-ionomer.

Keywords: Tooth remineralization, Glass-ionomer, Bioactive materials, Laser fluorescence, DIAGNOdent pen

INTRODUCTION

The biocompatibility of dental restorative materials has increasing attention from both dental practitioners and patients who are concerned with a healthier and natural life [1]. Biocompatible materials, in regards to tissue response, are classified as either bioinert or bioactive [2,3]. The bioactive materials interact with the host tissue, in a controlled manner, as they release biologically active ions into the surrounding media at certain levels to be biologically beneficial [4]. New restorative materials have been introduced, which possess bioactive properties by promoting remineralization of the tooth hard tissues through releasing calcium and phosphate ions [4,5]. Remineralization of decalcified enamel has been described as the deposition of the mineral phase in the demineralized defects at the molecular level [6,7]. In addition, remineralization of demineralized dentin has also been proven [8,9]. It is worth here to mention that the bioactivity of the dental restorative materials and their capability to remineralize initially carious dentin is a crucial attribute in modern restorative dentistry for preservation of the dental hard tissues, in order to limit cutting of dentin to an extent just sufficient to prevent the disease progression and to allow healing of the partially diseased dentin.

Among the most popular biocompatible restorative materials are glass-ionomer cement owing to their attractive characteristics, although conventional glass-ionomer cement has low mechanical properties, they are involved in a wide range of clinical applications [4]; because of their biocompatibility [10], and their fluoride release capability [8,11].

They are sometimes argued to be bioactive or not because they release beneficial active ions as fluoride, phosphate, calcium, strontium, and silicon into the surrounding media [8,12].

Two recently introduced bioactive restorative materials were selected to compare their effect in arresting hard dental tissues carious lesions and inducing remineralization to that of conventional glass-ionomer, Glass Carbomer cement (GCP Dental, Mijlweg, Netherlands) which is a new glass-ionomer based restorative material that has been introduced to the dental profession with claims of increased bioactivity [4]; it contains nanofiller powder particles of added fluoroapatite and hydroxyapatite, which are thought to aid their ultimate remineralization of demineralized dental hard tissues [13-15] and Biodentine (Septodont, Saint Maur des Fosses-France) which is another bioactive material [16] that has been introduced as an alternative to Mineral Trioxide Aggregate (MTA) with a comparable ability to induce dentin bridge formation [17,18], moreover, cell viability with tricalcium silicate cement was found to be higher compared to MTA and much more than glass-ionomer cement [19], so that it has increasingly substituted calcium hydroxide in pulp capping due to less caustic effects and higher clinical success rates [20,21], moreover they have high alkalinity; which favors apatite formation [22] and enhances dentin remineralization [23], with improved properties than MTA and shorter setting time; Biodentine is indicated in restorative dentistry for pulp capping [24-26] and as a temporary restoration, as well as, a base under resin composite restorations [24,27,28]. It is a calcium-silicate-based dental cement mainly containing tricalcium silicate, dicalcium silicate, calcium carbonate and calcium oxide giving it the ability of high calcium ion releasing [26,29], furthermore it was suggested that tricalcium silicate may be effective for the treatment of dentin hypersensitivity [30].

On the other hand, the fluorescence of dental hard tissues has been known for a very long time [31]. Fluorescence is a phenomenon in which light, at a certain wavelength, is absorbed by the tissue and emitted at another wavelength. DIAGNOdent pen (KAVO, Biberach, Germany) employs laser fluorescence to measure early demineralization of tooth hard tissues, as it provides a quantitative and longitudinal assessment of the tooth hard tissues [32]. The device offers a non-invasive reliable caries detecting method as it detects the mineral loss in enamel even before cavitation occurs [33,34].

So that, it was necessary to test these recently introduced two bioactive restorative materials and to compare their tooth hard tissues remineralization potentiality to conventional glass-ionomer, in order to examine their effectiveness in arresting initial caries progression by enhancing tooth remineralization in treatment of acid-induced initial caries-like lesion of tooth hard tissues, through employing a reliable modern caries measuring device as DIAGNOdent pen for evaluation and quantitative comparison between the materials.

MATERIALS AND METHODS

A total of twenty freshly extracted caries-free human premolars, which were extracted for orthodontic reasons were used in the study. The teeth were examined using a magnification lens of 7X to exclude any tooth with structural defects or cracks and were thoroughly washed and scaled to remove blood, mucous, remnants of periodontal ligament and deposits. Teeth were stored refrigerated at 4°C in distilled water containing 0.02% sodium azide for a period not longer than one month [35].

DIAGNOdent pen was calibrated before each testing session, according to the manufacturer instructions, on the supplied ceramic button and then for each tooth where the readings were recorded on its intact enamel at the center of the middle third of the facial surface, after air drying for 3 seconds [36]. Teeth that recorded a value higher than 13 were excluded, as according to the manufacturer instructions; values between zero to 13 are considered healthy enamel.

The teeth were randomly divided into four groups and each tooth received two class V cavities, facial and lingual, to make a total of 40 cavities by means of a diamond stone (Komet; 012 flat-end chuck cylinder) at high-speed and accompanied with copious air/water spray for cooling and the diamond stone was replaced every five preparations. Each cavity was cut in square outline with the occlusal wall placed in enamel approximately 1.5 mm from the cemento-enamel junction and the cervical wall was extended into the roots' cementum 1.5 mm. The depth of the cavities axial wall was kept approximately one mm for standardization of dimensions and to ensure that the pulpal wall was in dentin leaving enough thickness of dentin to cover the pulp.

The prepared teeth were, then soaked in a specially prepared demineralizing solution of pH 4.5, as described by Huang, et al. [37], which consisted of acetic acid solution 50 mM containing 2.2 mM $\text{Ca}(\text{NO}_3)_2$, 2.2 mM KH_2PO_4 and 0.1 ppm

NaF. Teeth were kept in the acidic bath for 72 hours; which pH was checked every 24 hours with a pH meter. After 72 hours, the teeth were thoroughly washed with deionized water and air-dried. The prepared cavities were then treated with Kavitan Kondicioner (SpofaDental, Markova, Czech), a tooth surface conditioner, to remove any remnants of the smear layer and the teeth were washed again with deionized water and dried. Three initial DIAGNOdent pen readings were recorded for each cavity; one in enamel at a distance of 0.5 mm from the middle of the occlusal cavity margin, a second one in dentin at the center of cavity floor and a third one in cementum at a distance of 0.5 mm from at the middle of the cervical cavity margin. The four groups received four different restorative materials as listed in Table 1.

Table 1 Groups and restorative materials used

Group	Restorative Material	Place of Origin
Group-I	Cavit, Temporary Filling Material (Control)	3M ESPE, Seefeld, Germany
Group-II	Ketac-Fil	3M ESPE, Seefeld, Germany
Group-III	Glass Carbomer	GCP, Ridderkerk, Netherlands
Group-IV	Biodentine	Septodont, SaintMaur-des-Fosses, France

Mixing and packing of the cavities were done according to the manufacturer's instructions. For Glass Carbomer restorations, the surface of each restoration was optimized with heat curing by CarboLED CL-02 thermo-cure light-cure high energy lamp (1400 mW/cm², FlashLite 1401, Discus Dental, USA) for 90 seconds and after the initial setting the restorative materials. The groups of Ketac-Fil and Glass Carbomer restorations were covered with a protecting layer of Riva Coat (SDI, Australia), glass-ionomer glaze, to maintain the water balance [38]. The surfaces of restorations in all groups were left without rotary finishing and polishing, as the surface finishing and polishing could affect the amount of fluoride release from glass-ionomer based materials [39]. The restored teeth were stored in a plastic container filled with Vittel, mineral water (Nestle Group, France), which consisted of calcium 240 mg, Magnesium 42 mg, Sodium 5.2 mg, Sulphate 400 mg, Nitrate 4.4 and Bicarbonate 384 mg. Thymol was added (0.1%) to prevent bacterial growth.

The plastic container was placed in an incubator with an adjusted constant temperature of 37°C; to simulate the oral environment for a period of three weeks. The storage medium was changed twice per week. Tooth Mousse toothpaste (GC, Heverlee, Leuven, Belgium) which contains Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) was applied for 30 seconds to the facial and lingual surfaces of each tooth twice daily and brushing was done by means of a soft toothbrush to simulate patient home care and the teeth were washed under running tap water and put back in the storage medium.

After the storage period; the teeth were sectioned longitudinally by means of a diamond disk to be divided into two halves, mesial and distal, at a line bisecting the restorations at the middle to gain access to the dentin at the cavity floor then DIAGNOdent pen readings were recorded at the same previous three sites of enamel at a distance of 0.5 mm from the occlusal cavity margin, dentin at a distance of 0.5 mm under the cavity floor and cementum at a distance of 0.5 mm from the cervical cavity margin.

The results were collected and statistically analyzed using SPSS for Windows (version 22, IBM, Corp., Chicago, IL, USA) employing one-way analysis of variance (ANOVA) and Tukey's post-hoc test ($p < 0.05$).

RESULTS

Comparing the Restorative Materials' Effect, Regardless of the Tissue Type

The DIAGNOdent values recorded for the hard tissues after the three preliminary steps of cavity preparation, acid challenge and the application of the conditioner were considered the baseline values, before the application of the restorative materials while the final values were obtained after allowing for remineralization to occur for three weeks period from the application of the restorative materials.

The analysis of variance showed that the effect of restorative material on DIAGNOdent pen readings of dentin was significant, $F(7,232)=199.3$, $p=0.000$. Groups II, III and IV showed a significant decrease in the laser fluorescence values of DIAGNOdent pen readings of the collective tooth hard tissues; indicating the occurrence of remineralization with the used restorative materials, while Group I, the control group, showed non-significant effect (Table 2). It

is worthy to mention that pooling the readings of the three-tooth hard tissues resulted in a higher standard deviation due to the compositional and optical differences between the tissues. Besides, the difference between Ketac Fil, Glass Carbomer and Biodentine was non-significant, after the designated storage period from the restoration.

Table 2 Mean collective three hard tissues DIAGNOdent pen readings before and after restorative materials' application

Material	Stage	Mean	Std. Deviation	p-Value
Cavit	Before Restoration	66.8	12.3	0.928
	After Restoration	63.1	14.4	
Ketac Fil	Before Restoration	72	15.8	0.000*
	After Restoration	10.6	7	
Carbomer	Before Restoration	74.1	11.3	0.000*
	After Restoration	10.7	5.9	
Biodentine	Before Restoration	69	16.1	0.000*
	After Restoration	11.5	4.8	

*There was a significant difference

Comparing the Effect of the Examined Restorative Materials Separately on Each of Enamel, Dentin, and Cementum

The analysis of variance showed that the effect of the different restorative materials on DIAGNOdent pen readings of enamel was significant, $F(7,72)=127.8$, $p=0.000$. So that groups II, III and IV showed a significant effect of the used restorative materials on their laser fluorescence readings, while the control group showed a non-significant effect of a cavity (Table 3).

Table 3 Enamel DIAGNOdent pen mean readings and standard deviation before and after restoration and allowing for remineralization

Group	Material	Stage	Enamel Readings	p-value
Group I	Cavit	Before Restoration	52.9 ± 6	0.215
		After Restoration	47.4 ± 6.3	
Group II	Ketac Fil	Before Restoration	57.5 ± 6.9	0.000*
		After Restoration	8 ± 5	
Group III	Glass Carbomer	Before Restoration	63.5 ± 7.5	0.000*
		After Restoration	10.2 ± 5.4	
Group IV	Biodentine	Before Restoration	52.1 ± 9.1	0.000*
		After Restoration	11.1 ± 5.9	

*There was a significant difference

Also, the analysis of variance showed that the effect of restorative materials on DIAGNOdent pen readings of dentin was significant, $F(7,72)=919.1$, $p=0.000$. Also, only the control group showed a non-significant effect of Cavit on the dentin readings, while the three other groups showed a significant difference (Table 4).

Table 4 Dentin DIAGNOdent pen means readings and standard deviation before and after restoration

Group	Material	Stage	Dentin Readings	p-value
Group I	Cavit	Before Restoration	80.2 ± 3.9	0.998
		After Restoration	79.2 ± 6	
Group II	Ketac Fil	Before Restoration	91.8 ± 3.5	0.000*
		After Restoration	7.4 ± 0.8	
Group III	Glass Carbomer	Before Restoration	85.4 ± 7.4	0.000*
		After Restoration	6.6 ± 1.9	
Group IV	Biodentine	Before Restoration	87.2 ± 3.9	0.000*
		After Restoration	8.9 ± 1.4	

*There was a significant difference

Finally, the analysis of variance showed that the effect of restorative materials on DIAGNOdent pen readings of cementum was significant, $F(7,72)=192.4$, $p=0.000$. Again, the groups II, III and IV showed a significant effect of the restorative materials on the laser fluorescence readings of cementum, while the control group showed the non-significant difference (Table 5).

Table 5 Cementum DIAGNOdent pen mean readings and standard deviation before and after restoration

Group	Material	Stage	Cementum Readings	p-value
Group I	Cavit	Before Restoration	67.3 ± 4.3	0.428
		After Restoration	62.8 ± 5.2	
Group II	Ketac Fil	Before Restoration	66.6 ± 6.6	0.000*
		After Restoration	16.3 ± 8.8	
Group III	Glass Carbomer	Before Restoration	73.3 ± 5.9	0.000*
		After Restoration	15.3 ± 6	
Group IV	Biodentine	Before Restoration	67.6 ± 6.8	0.000*
		After Restoration	14.4 ± 4.7	

*There was a significant difference

The analysis of variance showed that the effect of three weeks of storage period after the application of the restorative materials on DIAGNOdent pen readings of tooth tissues was significant, $F(14,225)=345.64$, $p=0.000$.

Comparing the Final Results to the Initial Pre-Treatment Tooth Readings

Cavit control group showed a significant difference between the initial pre-treatment mean DIAGNOdent readings and the final after restoration values for the three examined tooth tissues; indicating the lack of enough remineralization. While the other three Groups II, III and IV showed a non-significant difference between the initial sound tooth readings and the final records for enamel and for dentin indicating a significant effect of the restorative materials on the laser fluorescence readings of the two tissues to approach their initial sound tissues readings; after three weeks from the application. But in the case of cementum, although showing a significant improvement in remineralization with the three materials, it did not reach its sound status readings after the storage period of time, that it recorded initial sound reading (Table 6).

Table 6 Mean DIAGNOdent pen comparison of initial sound and end result readings

Tissue	Initial	Group I (Cavit)		Group II (Ketac Fil)		Group II (Carbomer)		Group II (Biodentine)	
	Mean	Final Mean	p-Value	Final Mean	p-Value	Final Mean	p-Value	Final Mean	p-Value
Enamel	7.5 ± 2.1	47.4 ± 6.3	0*	8.0 ± 5.0	1	10.2 ± 5.4	0.863	11.1 ± 5.9	0.45
Dentin	9.5 ± 2.9	79.2 ± 6.0	0*	7.4 ± 0.8	0.986	6.6 ± 1.9	0.819	8.9 ± 1.4	1
Cementum	8.7 ± 2.7	62.8 ± 5.2	0*	16.3 ± 8.8	0*	15.3 ± 6.0	0.001*	14.4 ± 4.7	0.009*

*There was a significant difference between the values before and after the experiment

DISCUSSION

Immersion of teeth in the demineralizing solution for 72 hours showed an increase in DIAGNOdent pen readings; indicating demineralization [33,34]. Glass-ionomer surface coating glaze which was applied for glass-ionomer based restorative materials, as the surface coating was reported not to inhibit restorative materials' release of fluoride [40,41].

The storage medium used in the current study was mineral water, as described by Lippert, et al. [42], who investigated the remineralization of softened enamel surface and compared utilizing two different storage solutions; artificial saliva and mineral water and they found that remineralization of human enamel occurred equally in both media. The storage period was three weeks after the application of the restorative materials because of the previous work of researchers concerning glass-ionomer, as it was reported that glass-ionomer was found to show an initial fast burst of fluoride release on the first day of restoration; followed by a sharp decrease starting from the second or the third day after ap-

plication [43,44]. Then fluoride release was found to diminish gradually over a period of three weeks to reach a long-term of low-level sustained release [45-47].

DIAGNOdent pen was employed to measure laser fluorescence as an indication of the degree of tooth hard tissues remineralization and it showed reliable results in all steps of the current study before and after acid challenge, as well as, after tooth surface conditioning, which was considered the baseline after demineralization and the final readings after allowing remineralization for three weeks. DIAGNOdent laser fluorescence was employed by many kinds of research and most of them found it to be a reliable method for detecting the degree of hard tissue mineralization [48-53], moreover, Reis, et al. [54], reported that the accuracy of DIAGNOdent under *in vitro* conditions was higher compared to that *in vivo*. So, the reliability of DIAGNOdent encouraged many researchers to use it alone and to rely solely on the device readings in their researches; without employing any other confirmatory method [48-51,55,56]. While Jayarajan, et al. [57], used scanning electron microscopy as an adjunctive method for measuring the enamel remineralization and the findings showed matching results, but Moriyama, et al. [58], compared surface microhardness and cross-sectional microhardness to DIAGNOdent values and reported that there was a negative correlation between them; which is logic because the DIAGNOdent readings when small indicate higher mineralization status and consequently higher hardness numbers. Moreover, Diniz, et al. [59], examined fluorescence-based devices including DIAGNOdent and found them to be effective in monitoring non-cavitated caries-like lesions on smooth surfaces and confirmed their ability to differentiate between sound and demineralized enamel and reported the presence of moderate correlation between laser fluorescence and surface microhardness. Also, many researchers compared DIAGNOdent results with other methods, as Emami, et al. [60], who found that the amount of mineral loss caused an increase in DIAGNOdent readings that was correlating with the results obtained from microradiographs, moreover Aljehani, et al. [61], reported a correlation between lesion depth determined by histopathology and transverse microradiography to DIAGNOdent readings, also Al-Khateeb, et al. [62], used chemical analysis of the mineral content and microradiographs to validate laser fluorescence in diagnosis of early enamel caries and they reported the presence of a significant correlation. However, Rodrigues, et al. [63], found no correlation between DIAGNOdent values and surface microhardness although they agreed that they found it to be effective in detecting the demineralization of enamel.

The results of the current study showed significant decrease in DIAGNOdent pen readings after three weeks period of storage after the application of the restorative materials; indicating that significant remineralization occurred in all tested materials except for the Cavit control group, in which the non-significant improvement in DIAGNOdent pen readings depended only on the effect of the toothpaste which contained Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) and sodium fluoride.

The conventional glass-ionomer group showed a significant decrease in DIAGNOdent pen readings indicating remineralization of all the tooth hard tissues, as the release of fluoride, calcium and phosphate ions provides glass-ionomer with the potentiality of remineralizing the carious tooth tissues [8], where ion exchange compensates the demineralized tissues ions and thus induces remineralization of hydroxyapatite crystals [64].

Also, nano-filled Glass Carbomer induced a significant degree of remineralization, but with a non-significant difference from the conventional glass-ionomer. This similarity in materials results could be explained by the study of Zainuddin, et al. [13], as they characterized glass-ionomer cement and Glass Carbomer using magic angle spinning nuclear magnetic resonance spectroscopy and their results showed that the apatite in Glass Carbomer was not fluorapatite but largely hydroxyapatite, which was partially consumed during the cement formation reducing hydroxyapatite availability for remineralization. In the same context, Mitra, et al. [40], found the fluoride release of a nano-filled resin-modified glass-ionomer, Ketac Nano, was similar to that of conventional glass-ionomer.

Also, the results showed that Biodentine induced a significant degree of remineralization, as these cement releases calcium over a long duration [29]. In addition, Biodentine was reported to release silicon ions into the underlying dentin [65]. Saito, et al. [66], demonstrated that silicate (silicon and oxygen) to be a stronger inducer of remineralization of dentin matrix than fluoride, calcium or phosphate. In agreement with this finding Atmeh, et al. [67], found Biodentine to cause remineralization of demineralized dentin, as indicated by two-photon fluorescence microscopy with tetracycline labeling and they confirmed their results by Raman Spectroscopy and backscattered electron SEM imaging, but they reported that Biodentine induced calcium phosphate mineral formation within the dentin matrix more than

glass-ionomer cement and their best results were obtained when stored in phosphate-rich medium and after a storage period of eight weeks.

It is worth here to mention that in the current *in vitro* study the lack of oral environment and salivary biofilm, to offer a state of calcium and phosphate ions supersaturation condition for the remineralization, did not prevent the remineralizing restorative materials from producing significant remineralization. But the results showed that the storage period of three weeks was suitable for enamel, dentin to reach their initial mineralization state as indicated by their laser fluorescence, while cementum although showing significant remineralization but did not reach its initial sound state.

CONCLUSION

With limitations of the current *in vitro* study, the followings may be concluded:

- The two examined bioactive restorative materials were found to have the ability to arrest initial carious lesions of all tooth hard tissues and inducing remineralization
- Tricalcium silicate cement was found to have the same remineralizing effect of fluoride-containing glass-ionomer cement
- The addition of nanofiller powder particles of fluorapatite and hydroxyapatite to glass-ionomer was not found to increase its remineralizing capability over the conventional cement
- Three weeks of storage after the application of the restorative materials was a sufficient period for enamel and dentin to approach their preoperative initial mineralized status, while cementum has not reached its initial sound state readings in the same period
- DIAGNOdent pen was found to be an effortless and helpful quantitative method in recording the tooth mineralization status

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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