

Effect of Tetracalcium phosphate/Nanomonetite Cement on Dentine Remineralization in Artificial Caries: An *In vitro* Study

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Abstract

Context: Modern concepts of operative dentistry, popularly known as “minimal intervention dentistry,” propose that only the infected dentin should be removed, leaving the affected dentine that has the potential to remineralize.

Aim: The aim of this study was to compare the remineralizing potential of GIC, calcium hydroxide preparations and the novel tetracalcium phosphate/monetite cement (TTCP/M).

Study Design: An experimental *in vitro* inter group randomized control trial.

Materials and Methods: This study consisted of five groups. Group I was a control group-the demineralization of dentin without any remineralization (CGR), Group II was the water suspension of calcium hydroxide sub base (Calxyl) covered with conventional glass ionomer cement (CAG). Group III was light-cured, resin modified calcium ion releasing base liner covered with conventional glass ionomer cement (CLM). Group IV were cavities filled with conventional glass ionomer cement without sub base (GIC) and Group V was the novel tetracalcium phosphate/monetite cement (BPC). Standardized class V cavities were prepared on the buccal and lingual surfaces. Artificial caries like lesions were produced and subsequently restorations of experimental specimens were performed. The depth of demineralization and remineralization were photographed under a polarized light microscope and quantified using a computerized imaging system.

Statistical analysis: The obtained results were subjected to statistical analysis using by the Shapiro-Wilk test for normality ($P>0.05$).

Results: Group V, Biphasic porous ceramics (BPC) showed the highest amount of remineralization (20, 65 μm). The expressive, but very thin layer of remineralization there was found in the group II (Water suspension of calcium hydroxide sub base-11, 51 μm). Group IV light-cured, resin modified calcium ion releasing base liner (CLM) covered with conventional glass ionomer cement there had a less thickness (14, 58 μm) of the remineralization than group III Glass ionomer cement without any sub base-16, 30 μm , but the density of their mineralisation in this group was more marked.

Keywords: Calcium hydroxide; Glass ionomer cement; Hydroxyapatite; Remineralization; Tetracalcium phosphate/monetite cement

Introduction

The conventional treatment of deep caries lesions involves complete removal of carious dentine followed by tooth restoration. In this technique, there is a potential risk of iatrogenic pulp exposure making the course of the treatment less predictable. Alternative approaches have been proposed to preserve pulp sensibility, such as indirect pulp capping [1,2].

To preserve pulp vitality and successfully treat caries profunda, timely and correct diagnosis, effective removal of carious dentin as

well as the use of appropriate medications and adequate restoration of cavity is required. Medications used in the treatment of caries profunda should primarily act on the remaining bacteria but also stimulate odontoblasts to produce tertiary dentin. Calcium hydroxide preparations are still the most commonly used agents in the treatment of deep carious lesions. When applied on the pulpal wall they stimulate odontoblasts to produce reparative dentin as well as kill microorganisms remained in a thin layer of dentin close to the pulp. The high pH (12.5) gives calcium hydroxide an important antimicrobial activity. The bactericidal action of $\text{Ca}(\text{OH})_2$ depends

on the release of hydroxyl ions in an aqueous environment [3]. Glass ionomer cement (GIC) is one such adhesive restorative material developed by Wilson and Kent in 1972. The two most important properties of GIC are adhesion to tooth structure and release of fluoride and other ions. The release of calcium and hydroxide ions facilitate remineralization of the affected dentine and the presence of fluoride ions enables remineralization to occur at a pH of 4.5 instead of the normal pH of 5.5. *In vitro*, GIC has been found to exert an effect on enamel as well as dentin remineralization and to attenuate enamel demineralization in the neighborhood [4]. Hydroxyapatite (HA) has been used successfully in clinical and animal studies for endodontic treatments. Absolute biocompatibility, osseointegration, ability to achieve excellent hermetic seal, formation of chemical bond with the tooth structure, insolubility in tissue fluids, good radiopacity and easy handling characteristics have led to the wide spread use of these materials in the area of endodontic science. Biphasic porous calcium phosphate ceramics, containing active tetracalcium phosphate/monetite cement (TTCP/M) was used in our study [5]. The material is characterized by self-hardening during acido-basic reaction with calcium-deficient hydroxyapatite (CDHA) as a final product after the addition of hardening liquid. The Calcium phosphate ceramics (CPCs) have good bioactivity, non cytotoxicity, osteoconductivity and they can be replaced by new bone because of their bioresorbable properties [6]. The properties of cements are influenced by various parameters such as the particle size of starting cement precursors, the TTCP/DCPA mole ratio, the powder to liquid ratio (P/L) in cement paste, and the chemical nature of hardening liquids and additives. TTCP cements showed better antimicrobial activity than the calcium hydroxide ones and no significant influence on cell proliferation with the increase in alkaline phosphatase (ALP) activity and osteogenesis promotion was verified [5]. The novel material has been showed very successful by the remineralization of the exposed dentin and early enamel demineralization and had comparable remineralization potential as commercial calcium silicate/phosphate (SENSODYNE®) and magnesium aluminium silicate (Colgate) [6,7].

The aim of this study was to compare the remineralizing potential of GIC, Calcium hydroxide preparations and Non porous hydroxyapatite (HA) ceramics.

Materials and Methods

Thirty human premolars extracted for therapeutic reasons were used in this study. The teeth used in experiment were extracted for orthodontic reasons and with the informed consent of patient (Protocol No: 088/2005 PH/CEP). Only teeth that were free of caries and restorations and showed no evidence of white spots or cracks on the buccal or lingual surfaces were selected. After extraction, the teeth were polished with pumice on a prophylactic brush, autoclaved and immediately stored in cold distilled water at 4°C until use. Standardized class V cavities (one on the buccal and one on the lingual surface of each tooth) were prepared with high speed diamond flat cylinder burusing water as a coolant. The cavity prepared was 3 mm wide, 2 mm high and 1.5 mm deep, and it was placed parallel to the cement-enamel junction (CEJ), with the preparation extending 1 mm above the CEJ. The bur was replaced after every 5th preparation. Each cavity was measured with a periodontal probe to ensure uniform size. Artificial caries like lesions were created on the exposed cavities by suspending all teeth in an artificial caries system for 2 days (50 ml per sample). The caries solution consisted of 2.2 mM Ca²⁺, 2.2 mM PO₄⁻³ and 50 mM acetic acid at a pH of 4.4. The solution was kept at a temperature of 37°C under constant circulation [8]. After 2 days, the teeth were removed from the artificial caries system. Each tooth was sectioned longitudinally in the occlusogingival direction to get one buccal and one lingual half. In this way 60 samples with empty cavities were created, randomly divided into 5 groups:

- I. CGR-Control group, the empty demineralised cavities without filling.
- II. CAG-Calcium hydroxide sub base+GIC, the water suspension of calcium hydroxide sub base (Calxyl) covered with conventional glass ionomer cement (Calxyl+Ionostar plus Voco).
- III. CLM-Calcimollic Voco, light-cured, resin modified calcium ion releasing base liner covered with conventional glass ionomer cement.
- IV. GIC-Glass-ionomomer cement, cavities filled with conventional glass ionomer cement without sub base (Ionostar plus Voco).



Figure 1: The own novel Biphasic porous calcium phosphate ceramics, containing an active tetra calcium phosphate/monetite mixture (TTCP/M) was used in our study.

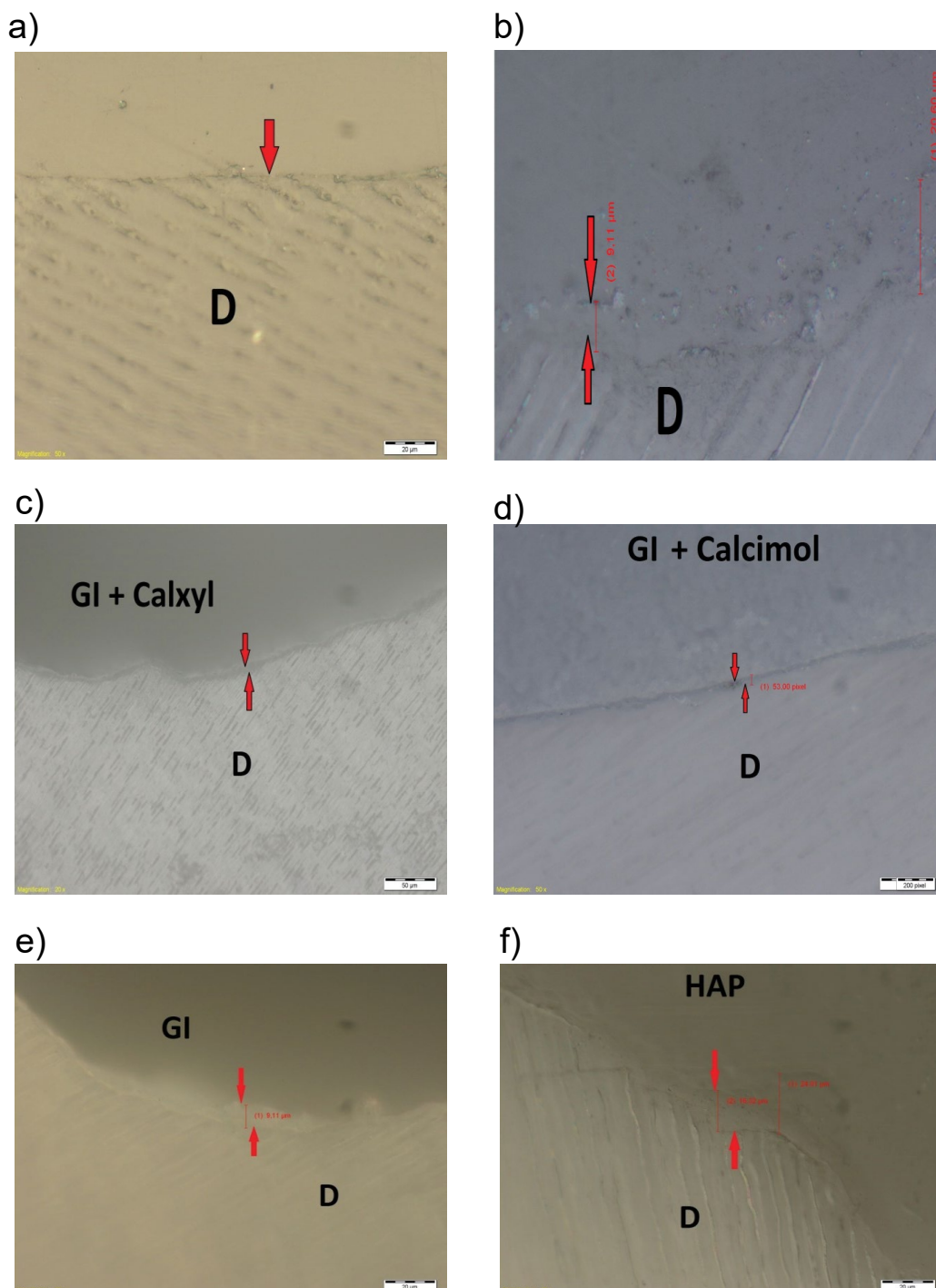


Figure 2:

a: The empty cavity without filling and demineralisation.

b: I. Group (control group): The empty demineralised cavity without filling. The area of demineralisation is demarcated by the arrows.

c: The expressive, but very thin layer of remineralisation of dentine in the group II. (The water suspension of calcium hydroxide sub base (Calxyl)) covered with conventional glass ionomer cement (Ionostar plus Voco).

d: Group III. Calcimol LC Voco (Light-cured, resin modified calcium ion releasing base liner) covered with conventional glass ionomer cement (GI). The higher thickness of the remineralisation than by the previous group, but the density of the remineralisation is less marked.

e: The cavity filled with conventional glass ionomer cement (GIC-group IV) without sub base). The thick layer of remineralization with less density.

f: The cavity filled with the novel TTCP/M ceramics (V.Group-BPC). The expressive thickness of the remineralisation with the similar density as the sound dentine (D).

V. BPC-Biphasic porous calcium phosphate ceramics, the novel material was prepared by sintering of transformed tetracalcium phosphate-monetite cement at Slovak academy of sciences, Košice (Figure 1).

The restored teeth (groups II-V) were then stored in artificial saliva at 37°C for 30 days at pH 7 with constant circulation, which was changed every 48 h. The artificial saliva consisted of 20 mM NaHCO₃, 3 mM NaH₂PO₄ and 1 mM CaCl₂ [9]. At the end of 30 days, the specimens were removed from the artificial saliva [9] and were mounted on acrylic blocks. At the end of 30 days, the specimens were mounted on acrylic blocks for sectioning. A section of 100 µm thickness was obtained by cutting through the center of the cavity using a Silverstone Taylor hard tissue microtome. The sections were mounted in glass slides to be viewed under polarized light microscopy using an Olympus dual stage polarized light microscope (model BX-51; Dual mount Corporation, Minneapolis, MN, USA). Photomicrographs were made at 50 x magnification. The demineralized areas were quantified with a computerized imaging system, the Image Pro Plus.

The artificial lesion was quantified at three points. Lesion depth was measured from the surface of the lesion to the depth of the lesion, at D1, D2 and D3. Final lesion depth for each section (in µm) was taken as the average of the three representative measurements from the surface to the depth of the lesion.

Statistical Analysis

The results obtained were subjected to statistical analysis using Student's paired and unpaired "t" test, one way ANOVA and post hoc Tukey's test.

Results

The measurements were analyzed by the Shapiro-Wilk test for normality ($P>0.05$) and Kolmogorov-Smirnov test for equal distribution ($P>0.1$). Data were normally and uniformly distributed; parametric tests were used for further analysis.

Descriptive statistics provided the mean remineralization values for each group (Table 1, Graph 1). One-way analysis of variance was used to evaluate the significance of differences between groups at a level of difference of 0.0001 (Table 2). The Tukey multiple comparisons test revealed significant differences among groups (Table 3). Significant differences were found between the specimens of group V-BPC (biphasic porous calcium phosphate ceramics) and group III-CLM (Calcimollic Voco) and group IV-GIC-glass ionomomer cement. In addition, the high significance was found between group IV-GIC-glass-ionomomer cement and control group and no statistically significant difference was found between this group and group III (CLM). The polarized light microscopy of the remineralization layers of each groups are shown in figures 2a-2f.

Discussion

It is generally accepted that GICs bond chemically to tooth substrates through ionic bonds with calcium ions of apatite. Glass-ionomer requires continuing research, particularly in relation to its ability to enhance remineralization of demineralized tooth structure. The ion exchange adhesion to both enamel and dentine is the best method of adhesion available and is limited only by the physical properties of the glass-ionomer. Therefore, these properties need to be enhanced, particularly increased resistance to fracture as well evaluated, whether the remineralizing potential of GIC can be enhanced with the addition of zinc acetate [10]. Incorporation of zinc acetate to glass ionomer at 2% w/w was effective in enhancing the remineralization property and fluoride release of the cement without affecting its setting time and shear bond strength [8]. It was observed that GIC with 2% zinc acetate showed the highest amount of remineralization, followed by GIC with 1% zinc acetate. Enhanced remineralization in the presence of zinc is in accordance with the previous studies conducted by Ten Cate, et al. [9], Lippert, et al. [11] and Lynch, et al. [12]. The most likely explanation for the enhanced remineralization in the presence of combination of zinc and fluoride is the smaller increase in surface porosity during remineralization, as zinc retards the crystal growth at the surface

Table 1: Descriptive analysis showing the average values of remineralization and mean value of difference in the remineralization among experimental groups.

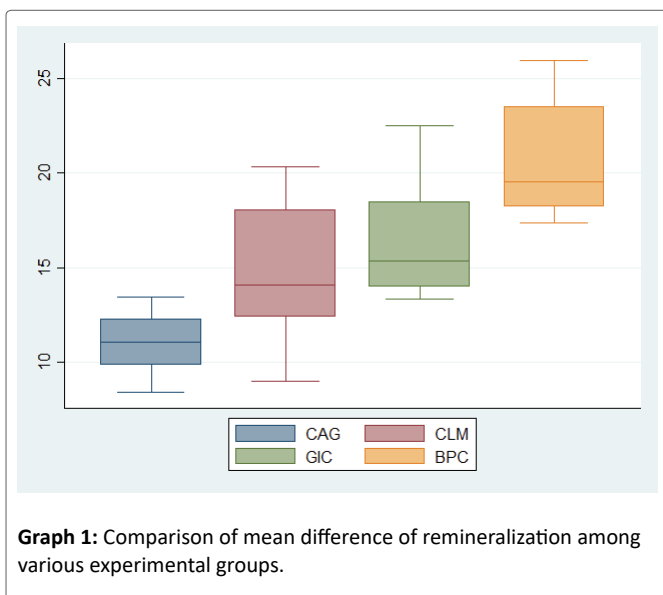
Materials	Obs	Remineralisation (µm)	max	min	Mean difference	skewness	kurtosis
CAG	12	11.519	13.46	8.44	1.519007	0.1019456	1.966818
CLM	12	14.58833	20.32	8.99	3.718425	0.0693552	1.956387
GIC	12	16.30167	22.49	13.35	2.893726	0.8535218	2.677297
BPC	12	20.65667	25.95	17.36	3.013679	0.4685457	1.721832
Total		15.63667	25.95	8.44	4.492011	0.3689244	2.306401

Table 2: One-way analysis of variance of differences between groups.

Source	SS	df	MS	F	Prob>F
Between groups	578.883667	3	192.961222	22.98	0
Within groups	369.4898	44	8.39749545		
Total	948.373467	47	20.1781589		

Table 3: The results of the significant differences between selected groups.

Material	Contrast	St.Err	t	p	[95% Conf. Interval]
CLM vs CAG	3.588333	1.18304	3.03	0.02	.429611 6.747056
GIC vs CAG	5.301667	1.18304	4.48	0	2.142944 8.460389
BPC vs CAG	9.656667	1.18304	8.16	0	6.497944 12.81539
GIC vs CLM	1.713333	1.18304	1.45	0.477	-1.445389 4.872056
BPC vs CLM	6.068333	1.18304	5.13	0	2.909611 9.227056
BPC vs GIC	4.355	1.18304	3.68	0.003	1.196278 7.513722



thereby facilitating ingress of more ions. Thus, zinc allows preferential deposition of mineral in the deeper parts of the lesions enhancing lesion body remineralization. However, plain GIC also showed some amount of remineralization, as supported by previous studies. Also, silica released from the GIC restoration could have a mineralization promoting effect, as it has been reported from crystal growth studies of hydroxy apatite [13]. It is a well-known fact that in aqueous solution the tetra calcium phosphate hydrolyses on two products-calcium deficient hydroxy apatite and calcium hydroxide [14]. The calcium hydroxide can interact with acid calcium phosphates like monetite or brushite to final hydroxy apatite phase. The acido-basic reactions between basic and acidic calcium phosphates in the form of aqueous slurries were successfully utilized to prepare calcium phosphate self-hardened bone cements [15]. The origin microcrystalline tetra calcium phosphate/monetite bio cement mixture was synthesized in a more active form by *in situ* partial transformation of TTCP to nanomonetite with final Ca/P in mixture equal to 1.67 (the same like in hydroxyapatite). It was observed that the fine nano crystalline hydroxy apatite precipitates are formed during the transformation of TTCPM mixtures, which represent nuclei for further precipitation of the calcium phosphates at supersaturated conditions related to hydroxy apatite in artificial saliva [16]. In our previous study we found that the new fluoride tooth paste formulation with bio active tetra calcium phosphate/monetite calcium phosphate mixture effectively occluded dentine tubules and showed good dentine remineralization potential under de/remineralization cycling [6]. The combination of calcium hydroxide and GIC was

recommended by Mount, et al. [17]. With the dentin-thick less than 0.5 mm they prefer the water suspension of calcium hydroxide, with a width of dentin more than 0.5 mm the calcium-salicylate cement is recommended. The similar results were published by Maltz, et al. [18] and Warfänger, et al. [19].

Conclusions

In our study we found that the new material with bioactive tetra calcium phosphate/monetite calcium phosphate mixture showed good dentine remineralization in standardized class V cavities under de/remineralization cycling.

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