In vitro Comparison of Self *versus* Professionally Applied Remineralizing Materials

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Objective: *To compare the effect of 4 remineralizing materials on the incipient artificial carious lesion and its proximal sound enamel when used with fluoride dentifrice.*

Study Design: *Models mimicking proximal contact were made, each of which was placed with an artificial carious specimen in contact with a sound enamel specimen. Each carious specimen was treated with one of four materials: glass ionomer cement (GIC), resin modified glass ionomer cement (RMGIC), 5000 ppm sodium fluoride (F-gel), and casein phosphopeptide amorphous calcium phosphate (CPP-ACP). The GIC and RMGIC specimens were thermocycled. Then all specimens were pH-cycled for 5 days with twice a day soaking in 1,000 ppm NaF dentifrice solution. Specimens were examined by polarized light microscopy and lesion area quantified by image analysis.*

Results: *RMGIC significantly yielded smaller areas of lesion than CPP-ACP and GIC ($p<0.05$). F-gel reduced more area of lesion than CPP-ACP significantly ($p<0.05$). In the associated contact, RMGIC significantly reduced the area of lesion better than CPP-ACP ($p<0.05$).*

Conclusions:*The most effective remineralizing material in reducing the carious lesion areas was RMGIC followed by F-gel, GIC and CPP-ACP. The demineralization inhibitory effects on the associated sound contact enamel followed the same trend.*

Keywords: *remineralization, demineralization, artificial caries, fluoride-releasing material, CPP-ACP, resin modified glass ionomer cement, glass ionomer cement, sodium fluoride gel.*

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INTRODUCTION

Traditionally, caries have been treated by removing the affected tooth structure and restoring the cavities with different dental materials. Management of caries has now shifted to a more conservative approach, which includes early intervention before the lesion cavitates.

When proximal caries is detected and after the risk factors have been modified, the dentist may consider using remineralizing agents to enhance the reversal of proximal lesion due to its difficulty to access by saliva.

One way to prevent and reverse caries is to keep the environment around the lesion in a state that promotes remineralization and inhibits demineralization with the use of materials containing fluoride, calcium phosphate all of which can be applied professionally or by patients themselves where there is adequate compliance.

Glass ionomer cements (GIC) and resin modified glass ionomer cements (RMGIC) are fluoride products for professional application, while F-gels such as sodium fluoride gel can be applied by patients. GIC have the ability to release and reuptake fluoride, which can be especially effective in preventing recurrent caries.[1,2,3] Moreover, GIC adheres very well to enamel and dentin by physicochemical bonding.[4,5]

RMGIC has the ability to recharge and release fluoride like GIC, but in greater amounts[6,7]; it can also bond with tooth structure. Its advantages over conventional GICs include better wear resistance,[8] higher compressive strength and higher fracture strength.[9]

Sodium fluoride gels have high fluoride concentrations and have been shown clinically to reduce the decayed-missing-filled (DMF) index when compared with the use of low fluoride concentration materials.[10] After the use of F-gels, CaF_2 is formed on the demineralized enamel.[11] Hydrogen phosphate ions (HPO^{-2}_4) in saliva are suggested to adsorb to the active sites (kinks) resulting in fluorapatite formation on the surface of calcium fluoride crystals, which helps reduce their dissolution rate.[11,12]

Casein phosphopeptide amorphous calcium phosphate

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(CPP-ACP) consists of casein phosphopeptides (CPP), aggregating with calcium phosphate to form clusters of amorphous calcium phosphate (ACP) in metastable solution[13] preventing calcium phosphate precipitation and resulting in a state of supersaturation with respect to enamel thus depressing demineralization and promoting remineralization[13] throughout the body of a carious lesion.[14] CPP-ACP has been shown to remineralize tooth structures and inhibit the cariogenic mechanism *in vitro*,[15,16,17] in animal studies[18] and *in vivo*.[15,19,20,21] Previous research also reported that once enamel was remineralized by CPP-ACP, it became more acid resistant.[19]

The objective of this *in vitro* study was to compare the effect of 4 remineralizing materials: GIC, RMGIC, F-gel, and CPP-ACP on the reduction of proximal artificial carious lesion area and their ability to decrease the extent of caries formation in intact enamel when used with fluoride dentifrice. The null hypothesis was that there are no differences among the four materials in the remineralization of proximal carious lesions and inhibition of demineralization of associated intact enamel.

MATERIALS AND METHODS

In this study, the products representing professionally applied materials were GIC (Fuji VII, GC Corporation, Tokyo, Japan) and RMGIC (Proseal, Reliance Orthodontic Products Inc., Itasca, Illinois). Representing self applied product were 5000 ppm sodium fluoride gel (Prevident, Colgate-Palmolive Company, New York, USA) and CPP-ACP (Tooth Mousse, GC Corporation Tokyo, Japan).

Tooth Preparation and Selection, Sectioning

Thirty sound human maxillary premolars and fifteen permanent mandibular molars without cracks, white spot lesions or fillings were collected after extraction for clinical reasons. The use of these teeth satisfied the requirements of the Chulalongkorn University Institutional Review Board (IRB) and informed patient consent was obtained for their use. All teeth were cleaned and cut bucco-lingually into mesial and distal halves. Each half was divided longtitudinally into 4 and 6 specimens for premolar and molar groups respectively, resulting in 12 specimens for each molar and 8 specimens for each premolar. All surfaces of the specimens were coated with nail varnish except for a 2x1 mm^2 window at the same level of the mesial and distal surfaces.

Artificial carious specimens and intact enamel specimens

All specimens were divided into 2 groups. Artificial carious specimens from premolars were immersed in artificial caries solution (Polyacrylic acid 20% 8 ml, Lactic acid 85% 0.88 ml , saturated hydroxyapatite 50 ml , Deionized water 92 ml ,pH 4.8)[22] at 37°C for 84 hours, while intact enamel specimens from molars were kept in deionized water to be used as sound enamel specimens.

Grouping of specimens

In premolars, 30 randomly selected carious specimens from each mesial and distal surface were used as original control, pH-cycling control, and each material applied group. Every group of premolar specimens except the original control group was in contact with intact specimens from molars.

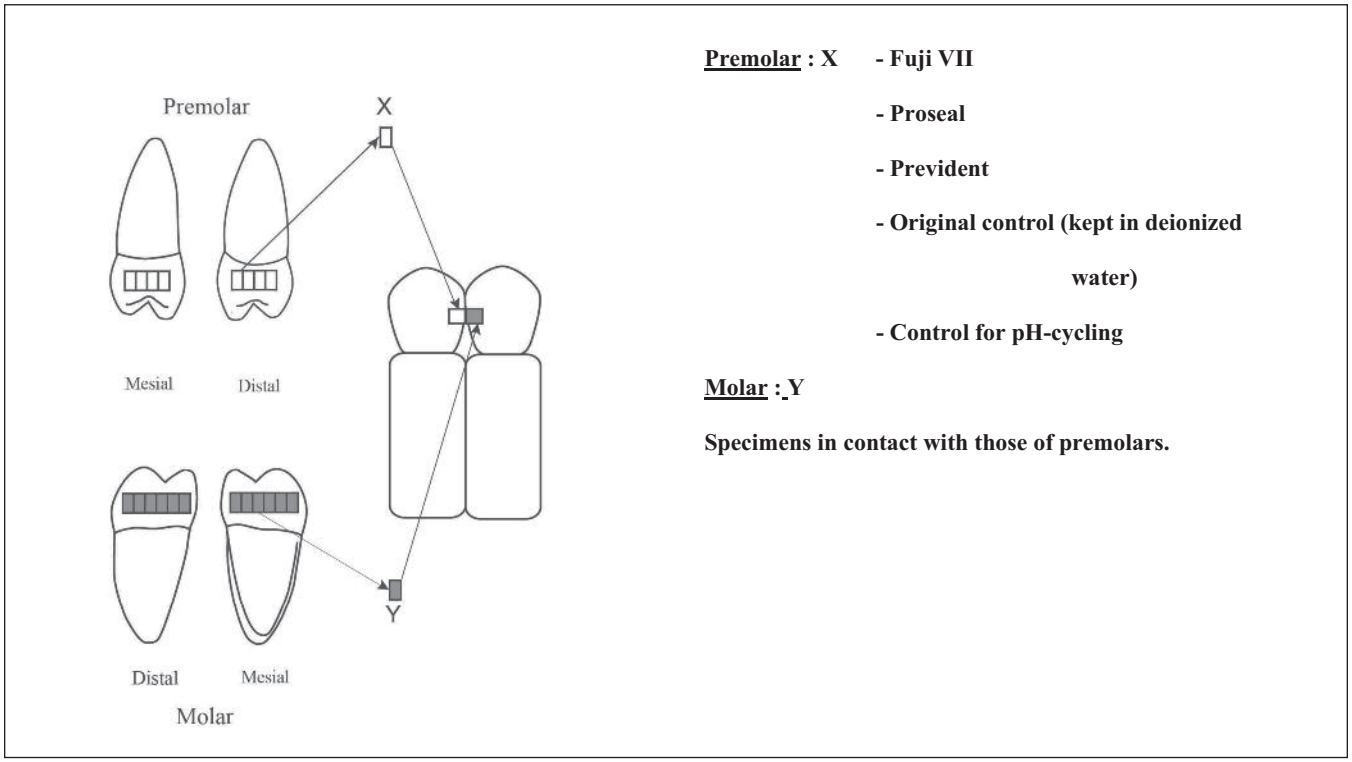

Figure 1. A specimen from a premolar was placed in contact with another specimen from a molar in the proximal contact model.

Each material was applied according to the manufacturers' instructions. Eight mm^3 of F-gel and CPP-ACP were applied with proxabrush to specimens, while GIC or RMGIC were painted to cover 2x1 mm^2 surface of the specimens.

Placing specimens in proximal contact

One carious premolar specimen was placed in contact with another intact molar specimen within the 1mm. slots of 2 epoxy premolar resin models in contact (Fig. 1). The position of both specimens is right below the contact point where caries usually occurs. A total of 30 treated specimens and 30 intact specimens were used to assemble proximal contact models for each treatment condition. 30 specimens were used as original controls and 30 pairs were used as pH-controls for each proximal surface. (Fig. 1)

Thermocycling and pH-cycling model

Three, thirty pairs of models treated with GIC or RMGIC and their pH-cycling controls were immersed in artificial saliva and then thermocycled at 5°C and 55°C with 1 minute dwell time for 500 cycles while 90 pairs of models treated with CPP-ACP or F-gel and their pH-cycling controls were immersed in artificial saliva at 37°C for the same length of time. Then all models went through a pH-cycling process (Table 1), soaking twice a day in fluoride dentifrice solution for 5 days. The process of pH-cycling was divided into 2 periods. The first period contained GIC, F-gel and their control. The second period contained RMGIC, CPP-ACP and their control.

Embedding of specimens

After the pH cycling, the coronal areas of models containing the specimens were embedded in resin cylinders and then each specimen was cross-sectioned at the mid-point of the window with a hard tissue cutting machine (LEICA SP 1600, Nussloch Germany). The sections were approximately 100 μm-thick.

Calculating the lesion area

The sections were analyzed for carious lesions with polarized light microscopy (9300 MEIJI, Saitama, Japan). The pictures of the lesions were captured with an Axio Camera then adjusted to 100% contrast by Adobe Photoshop software for the localized carious lesion region. Then, the areas of lesions were calculated with Image Pro Plus software (Image Pro Plus software, version 405) (Media Cybernetics Inc., Silver Spring, MD, USA). The delta values for remineralization were calculated from the differences in areas of lesions between the experimental specimens and the pH-cycling controls.

Statistical Analysis

Statistical Analysis was performed with Sigmastat 2.03 software. Mean values and standard deviations of lesion areas for each group were calculated. The paired Students t-test was used to analyze the difference between the original lesion controls from both proximal surfaces to ensure that there was no statistically significant difference, thus allowing comparison of the results from both surfaces. As the pH-cycling was processed from 2 periods, the difference between the lesion area of the pH-cycling control group in both periods was analyzed by the paired Students t-test to ensure that the 2 periods were not different in mimicking the oral environment. One way repeated measurement of variance (ANOVA) and Turkey's multiple comparison were used to analyze the differences among the lesion areas underneath various materials and the demineralized enamel adjacent to

Table 1. pH-cycling procedure

period	duration	Procedure
1	1 minute	Soak in fluoride dentifrice solution (Fluoride dentifrice mixed with deionized water 1:3 M/V)
	5 minutes	Immerse in deionized water
	3 minutes	Remove from deionized water. Apply CPP-ACP to appropriate group
	30 minutes	Immerse in artificial saliva (Magnesium chloride 0.07 g, Calcium chloride 0.199 g, Potassium hydrogen phosphate, 0.439 g,Sodium carboxymethyl cellulose 6.0 g, Sodium fluoride 0.005 g, Sorbitol 70% 36 g, Sodium benzoate 2.4 g, Deionized water 1200 ml)
	5 minutes	Immerse in deionized water
	5hours 26 minutes	Immerse in demineralizing solution (Calcium 2.0 millimolars, Phosphate 2.0 millimolars, Buffer acetate acid, 0.075 molars, pH 4.3)
	5 minutes	Immerse in deionized water
	1 minute	Soak in fluoride dentifrice solution
2	5 minutes	Immerse in deionized water
	3 minutes	Remove from deionized water Apply CPP-ACP or Prevident to appropriate groups.
	30 minutes	Immerse in artificial saliva
	5 minutes	Immerse in deionized water
	16hours45minutes	Immerse in remineralizing solution (Calcium 1.5 millimolars, Phosphate 0.9 millimolars, Potassium chloride 150 millimolars, Trisbuffer 0.1 molars, pH 7.0)
	5 minutes	Immerse in deionized water

Table 2. Mean values of lesion area and delta mean of control group and each experimental group.

Types of materials	Lesion under materials (mm^2±SD)	Delta mean lesion area under materials (mm^2±SD)	Lesion adjacent to materials (mm^2±SD)	Delta mean lesion area adjacent to materials (mm^2±SD)
Mesial baseline control	0.092±0.029	-	-	-
Distal baseline control	0.092±0.029	-	-	-
pH-cycling control period 1	0.119±0.026^{B}	-	0.100±0.025^{A}	-
pH-cycling control period 2	0.120±0.018^{B}	-	0.101±0.021^{A}	-
Proseal	0.058±0.011^{C*}	0.061±0.023^{C}	0.049±0.008^{C*}	0.050±0.023^{C}
Prevident	0.061±0.010^{C,D*}	0.059±0.022^{C,D}	0.054±0.010^{C,D*}	0.047±0.026^{C}
Fuji VII	0.070±0.016^{D,E*}	0.049±0.028^{C,D}	0.053±0.010^{C,D*}	0.047±0.027^{C}
CPP-ACP	0.078±0.016^{E*}	0.042±0.027^{D}	0.058±0.010^{D*}	0.043±0.025^{C}

Different letters in each column indicate statistical significance among groups (p<0.05)

* indicates statistically significant differences compared with the pH-cycling control. (p<0.05)

materials. The differences in the net gain in areas of lesions (delta means) among experimental groups were analyzed by one way ANOVA and Multiple comparison. Paired Student t-test was used to analyze the differences between the lesion areas of the pH-cycling control groups and the lesion areas of each experimental group.

RESULTS

There was no statistically significant difference between the original lesion controls from both proximal surfaces and between the lesion area of the pH-cycling control groups from both periods and thus all results could be compared (p>0.05).

Treatment with each of the remineralizing materials yielded statistically significantly smaller lesion areas when compared with the pH-cycling controls (p<0.05). In carious specimens, RMGIC was most effective in promoting remineralization, followed by F-gel, GIC and CPP-ACP respectively, with a statistically significant difference between RMGIC vs CPP-ACP, RMGIC vs GIC and F-gel vs CPP- ACP (p<0.05). For delta mean analyses, we found a statistically significant difference only between RMGIC and CPP-ACP (p<0.05) (Table 2).

For intact enamel specimens adjacent to the materials, results indicated that all of the four remineralizing materials statistically significantly reduced lesion area when compared with the pH-cycling controls (p<0.05). RMGIC was also the most efficient in inhibiting demineralization, followed by GIC, F-gel and CPP-ACP respectively. There was a statistically significant difference between RMGIC and CPP-ACP only. The delta means of lesion areas adjacent to the four materials did not show any statistically significant differences.

The percentage reduction in area of carious lesions under each material compared to post pH cycling controls ranged from 35-53%, and the percentage reduction of new lesion area in the adjacent sound contact enamel specimens compared to post-pH-cycling controls varied from 42-50% (Table 3)

Table 3. Percentage reduction in area of lesion under materials and the percentage reduction of new lesion area adjacent to materials compared with pH-cycling control.

Types of materials	Percentage reduction in area of lesion under materials		Percentage reduction of lesion area adjacent to materials compared with pH-cycling control	
	Distal side	Mesial side	Distal side	Mesial side
Proseal	-	53.04	50.48	-
Prevident	48.91	-	-	46.72
Fuji VII	-	40.87	46.71	-
CPP-ACP	35.14	-	-	42.18

DISCUSSION

The present *in vitro* study has demonstrated that RMGIC has greater efficacy in promoting remineralization both on the carious specimens and the intact enamel specimens than GIC. These results concur with the data from previous studies, which found that RMGIC released higher amounts of fluoride than normal GIC.[6,7] RMGIC contain hydroxyethyl methacrylate (HEMA), which increases dissolution of polymer matrix from the material, hence allowing RMGIC to release higher amounts of fluoride than normal GIC in the early dissolution stages.[23] The setting reaction for GIC is an acid-base reaction involving fluoride-containing glass and a polyacid. This reaction results in fluoride release and formation of a silica gel surface matrix that in turn acts as a barrier, obstructing further elution of fluoride from the material. In contrast, RMGIC contains more monovalent metal ions that cannot cross-link with the ion matrix, hindering the formation of a closely bonded silica gel barrier, thereby providing a looser matrix for water transportation and fluoride release.[6] Furthermore, RMGIC has a lower powder/liquid ratio than GIC, which allows for better dissolution of fluoride from materials and its diffusion to the environment than GIC.[7] Also, RMGIC shows lower microleakage than GIC.[24,25,26,27] The greater microleakage with GIC may result in the concentration of fluoride in the gap between the material

and tooth surface being less than with RMGIC. Microleakage may also allow acid to diffuse easily from the environment to the enamel surface at the material-tooth interface, promoting greater demineralization with GIC than RMGIC.

CPP-ACP was the least efficacious treatment in both preventing demineralization of the intact enamel specimens and in remineralizing the artificial carious specimens despite the fact that CPP-ACP has been reported to have additional effects in the prevention of demineralization when used with 1100 ppm fluoride dentifrice, the same concentration used in our present study.[14,28] This additive effect may be less than the anticariogenic effect of materials with high fluoride concentrations or a continually fluoride-releasing materials. Furthermore, the present study was performed in the absence of an oral microbial environment or plaque accumulation on the tooth surfaces which may have resulted in decreased CPP-ACP adherence to tooth surfaces since CPP-ACP has been suggested to be incorporated into plaque by binding to bacterial cell surfaces and to the intercellular plaque matrix.[22] Moreover, a recent study reported that the rate of remineralization by CPP-ACP increased with a decrease in pH from 7 to 5.5.[29] We used approximately 17 hours of remineralizing period (pH7) to mimic the oral condition after their risk factors have been modified. In continuous low pH condition, the CPP- ACP may yield better results.

The results of this study can be clinically applied in cases of non-compliant patients; the dentist may choose to separate the teeth with an elastic band and then after etching, use an extra-fine brush to apply a thin layer of liquid RMGIC on the carious lesion then light cure the material instead of relying on the patient to use self-applied fluoride. Alternatively, RMGIC can also be dabbed on to the matrix band, which is then slid through the open contact area and adapted to the proximal contour of the tooth before being light-cured.

CONCLUSION

In this study we found that all of the 4 remineralizing materials yielded less lesion area than control groups indicating the potential of the materials to be used for the reversal of caries in non-cavitated initial lesions. These *in vitro* studies should now be corroborated in further *in vivo* studies to fully assess the application of these materials for clinical use.

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