The Effect of Calcium Silicate Cement in Remineralization of Artificial Caries Affected Dentine: In Vitro Study

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Abstract

The study aimed to compare the remineralization in artificially caries-affected dentine restored with three different materials. The mechanical strength of dentin was used to assess remineralization effectiveness. Twenty-five human third molar teeth were prepared in class v cavities, and twenty cavities were used to simulate caries-affected dentin using the pH cycling method. Fifteen cavities, simulated as caries-affected dentin, were filled with three groups of different materials (n=5): group 1-calcium silicate cement, group 2-conventional glass ionomer cement, and group 3-resin-modified glass ionomer cement resin and immersed in artificial saliva for one week. The five demineralized cavities without restoration served as the group 4-negative control group, while the five sound cavities served as the group 5-positive control group. All specimens were cut longitudinally, and the nanoindentation test was performed on dentine at the axial wall. The data were analyzed using One-Way ANOVA and the Bonferroni method (α=0.05). All groups had a different modulus of elasticity ($p<0.05$) except for the negative control group, conventional glass ionomer cement, and resin-modified glass ionomer cement, which were not statistically significant. The positive control group had the highest modulus of elasticity at 18.37±1.88 GPa. Calcium silicate cement was the second at 9.68±1.21 GPa, conventional glass ionomer cement at 2.61±0.57 GPa, resin-modified glass ionomer cement at 2.36±0.25 GPa, and the negative control group at 2.11±0.35 GPa respectively. This study found that calcium silicate cement was more effective than glass ionomer cement in remineralizing human dentin.

Keywords: *Calcium silicate cement, Glass ionomer cement, Caries affected dentine, Remineralization*

1. Introduction

The minimal intervention has been an interesting remedy to eliminate the carious tooth structure only on the superficial dentin while conserving the remaining tooth structure. Caries-affected dentin is a partially demineralized layer that was found to be able to spontaneously recover, a process called physiologically remineralization, and accordingly, the caries-affected dentin was left behind (Schwendicke et al., 2016).

Glass ionomers were widely used as a bioactive material. However, glass ionomer cement is a fragile material with low wear resistance and has other limitations. This issue can be resolved by combining strength-increasing substances with the monomer resin. Both resin-modified glass ionomer and glass ionomer have been reported to be effective in remineralization. Remineralization is believed to occur during the ion exchange process of the material (Gao, Smales, & Yip, 2000; Kitasako et al., 2006; Ngo, Mount, Mc Intyre, Tuisuva, & Von Doussa, 2006).

One of the well-known materials is MTA, or Mineral Trioxide Aggregate, which is widely used in the endodontics treatment as a repairing material for dental root perforation, and as a pulp tissue cover material. However, MTA requires a long setting time, which prevents it from being used in a single session, is difficult to apply, and causes dentin coloring (Gandolfi et al., 2015; Parirokh, & Torabinejad, 2010). As a result, in 2009, biodentine was introduced. The main component is tricalcium silicate, which is similar to MTA but forms faster and sets in 12 minutes, and is used in coronal restoration as dentin replacement. Many studies have found that even if all minerals are lost, biodentine can be remineralized by apatite formation, which shows biomimetic remineralization (Kim, Nosrat, & Fouad, 2015; Malkondu, Karapinar Kazandağ, & Kazazoğlu, 2014; Prati, & Gandolfi, 2015).

Although glass ionomer cement has been widely used as a bioactive material, the result is still unsatisfactory. There is still a need to find innovation for a bioactive material that can provide real and sustainable remineralization. Calcium silicate cement has performed a promising result in endodontic treatment therefore the material might be an answer in cariology.

In this study, the remineralization efficiency for 3 material groups was evaluated, which were calcium silicate cement, glass ionomer cement, and resin-modified glass ionomer cement. Dental caries at the cervical root caries was simulated as caries-affected dentin by using a pH cycling method and immersed in the artificial saliva solvent. After that, the remineralization effectiveness was evaluated by analyzing the mechanical strength of the dentin.

2. Objectives

To compare the efficacy of remineralization in artificially caries-affected dentine restored with three different materials: calcium silicate cement, conventional glass ionomer cement, and resin-modified glass ionomer cement. The elastic modulus of dentin was investigated to determine the effectiveness of remineralization

The null hypothesis was that there was no significant difference in remineralization efficiency in artificially caries-affected dentine restored with three different materials.

3. Materials and Methods

3.1 Subjects

Twenty-five sound human third molars were prepared and stored in Thymol solution 0.1% at room temperature. The study protocol was reviewed and approved by the ethics committee for reviewing human research projects at Srinakharinwirot University (Certification Number SWUEC/X-008/2565). The sample size calculation from the G*Power 3.1.2 program for one-way analysis of variance (ANOVA). The value of effect size = 1.765475 from the pilot study, error = 0.05, power (1- error prob) = 0.9, and number of groups=5 were used in the computation. The result was 15 samples. The sample size was increased to 5 samples per group (25 samples overall) by adding 30% to each group for sample compensation.

3.2 Sample preparation

1) The cavity was prepared as a class V cavity with a dimension of 4 mm in width, 2 mm in height, and 1.5 mm in depth. Cylindrical bur with a diameter of 1 mm was used (835 010 FG Cylindrical bur, Meisinger, Neuss, Germany). A bur was changed every 5 cavities. The cavities were measured with a dental probe and observed with a stereomicroscope (Olympus SZ61, Tokyo, Japan) to ensure no enamel or pulp was included. Before the cavity preparation, the tooth surface was coated with an acid-resistant varnish (Coat nail varnish, Revlon, North Carolina, USA). The five sound cavities served as the group 5-positive control group and were stored in deionized water.

2) The demineralization simulation for artificial caries affected dentin was done by pH cycling. The teeth with prepared cavities were immersed in the demineralizing solution for 8 hours, alternating with the remineralization solution for 16 hours, for a total of 14 days (Marquezan et al., 2009; Sadoon, Fathy, & Osman, 2020).

3) The demineralization cavities were filled with material in each group according to the instructions from the manufacturer.

Group 1-calcium silicate cement: Cavity restored by calcium silicate, biodentine: Biodentine™ (Septodont, Saint-Maur-des-Fossés, France)

Group 2-conventional glass ionomer cement: Cavity restored by glass ionomer cement, conventional glass ionomer cement Fuji ix type: Fuji ix GP EXTRA (GC, Tokyo, Japan)

Group 3-resin-modified glass ionomer cement resin: Cavity restored by glass ionomer cement, resin-modified glass ionomer Fuji II, light-curing: Fuji II LC (GC, Tokyo, Japan)

Group 4-negative control: the group was subjected to simulated demineralization without any restoration by any material and was stored in deionized water.

The following were the material compositions used in this study, as shown in Table 1.

Table 1 The material compositions used in this study

Material	Manufacturer	Composition
Biodentine™	Septodont, Saint-Maur-des-Fossés, France	Powder: Tricalcium silicate, dicalcium silicate, calcium carbonate and oxide, zirconium oxide, and iron oxide Liquid: calcium chloride, hydrosoluble polymer, water
Fuji ix GP EXTRA	GC, Tokyo, Japan	Powder: Alumino-silicate glass, polyacrylic acid Liquid: Polyacrylic acid, water
Fuji II LC	GC, Tokyo, Japan	Powder: Alumino-silicate glass Liquid: copolymer of Polyacrylic acid, water, HEMA, and camphorquinone

4) All specimens in groups 1, 2, and 3 were stored in the artificial saliva solution (Amaechi, & Higham, 2001) for 1 week.

5) All specimens were cross-sectioned in the long axis through the center of the cavity with a precision saw (Isomet® 1000 Precision Saw, Buehler, Illinois, USA).

6) The specimens were attached to a PVC mold (Diameter 2.5 centimeter) using acrylic resin (PalaXpress® Ultra, Heraeus Kulzer, Indiana, USA). The specimens were polished with silicon carbide abrasive paper of 320, 600, 1200, and 2,000 grit (TOA DCC Waterproof Abrasive Paper, TOA Paint (Thailand), Samutprakan, Thailand) at a speed of 150 rpm and with diamond suspensions of 3, 1, and 0.25 microns (Diamond suspension, Kemet, Kent, UK). The surfaces of the specimens were then cleaned for 10 minutes with an ultrasonic cleaning machine (Ultrasonic cleaning, Derui Shenzhen, China).

3.3 Measurement remineralization by the nanoindentation test

1) The specimens were put on the Nanoindentation testing machine (FISCHERSCOPE®.HM2000, HELMUT FISCHER, Sindelfingen, Germany). The test was conducted in the temperature-controlled cabinet inside a temperature-controlled room at 23±2 degrees Celsius with a relative humidity of 50±10%.

2) A Vickers indenter was used, and only intertubular dentin was tested. The tested areas were randomly selected for 9 positions. The areas at distances of 10 microns, 20 microns, and 30 microns from the material interface at the axial wall were randomly selected for 3 points per the distance to ensure that the dentins were positioned at similar positions and to avoid experimental variations. One sample occupied a total of 18 positions (Figure 1).

3.4 Data Analysis

From the experiment, data from the 5 groups were compared. From the nanoindentation test, the mean modulus of elasticity of dentin was statistically analyzed and normality-tested with a Shapiro-Wilk test. One-Way ANOVA was conducted to analyze and multiple comparisons using the Bonferroni method (α = 0.05).

4. Results and Discussion

4.1 Results

From the mean value of the modulus of elasticity of the 5 groups, the positive control group had the highest modulus of elasticity at 18.37±1.88 GPa. Calcium silicate cement was the second at 9.68±1.21 GPa, conventional glass ionomer cement was the third at 2.61±0.57 GPa, and resin-modified glass ionomer cement was the fourth at 2.36±0.25 GPa. The negative control group was at 2.11±0.35 GPa. The results were shown in Table 2.

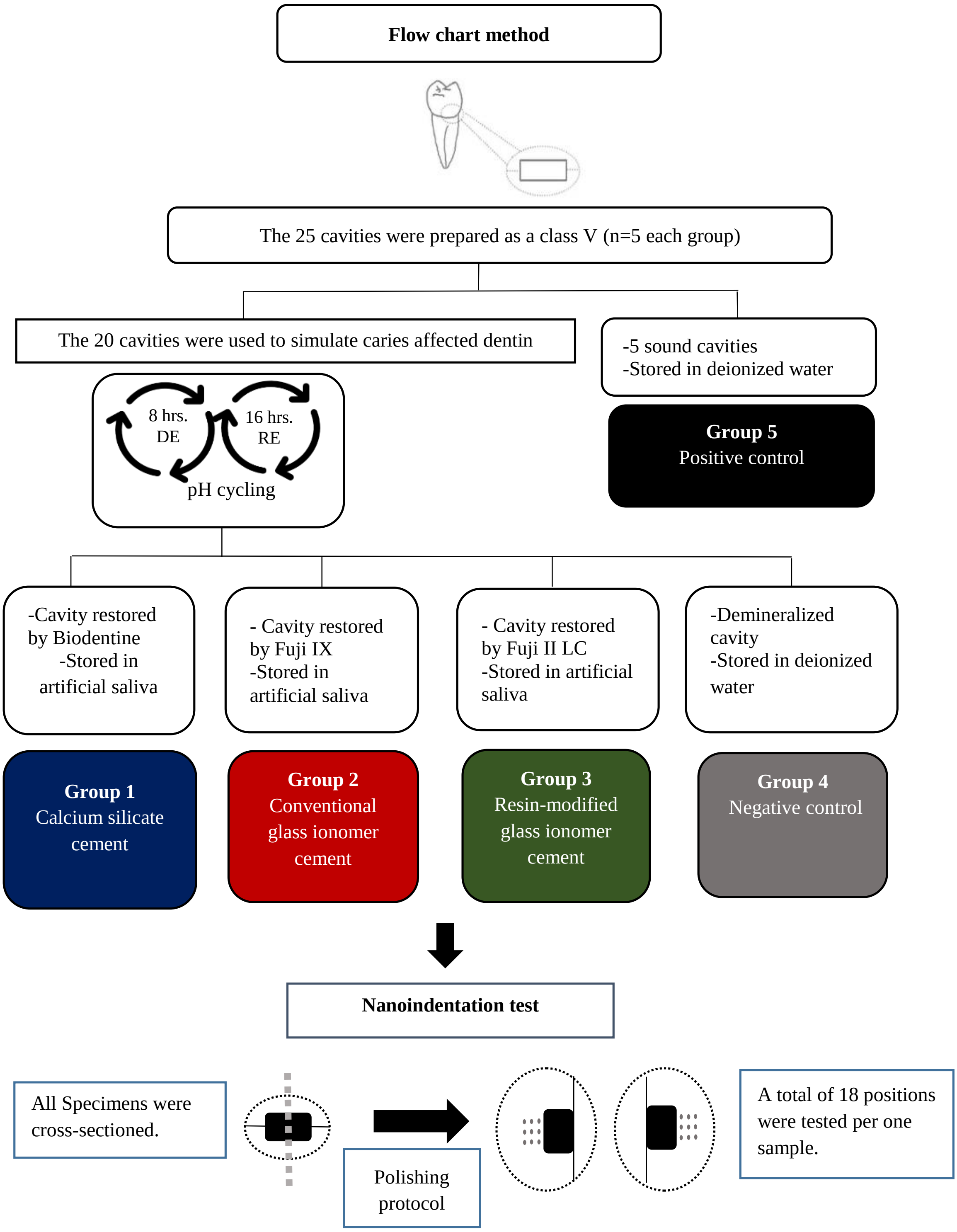

Figure 1 Flow chart method

Table 2 Descriptive Statistics for Modulus of Elasticity of 5 Study Groups

	Quantity	Mean	SD	Min	Max
1.Calcium silicate cement	5	9.68	1.21	8.35	11.53
2.Conventional glass ionomer cement	5	2.61	0.57	2.04	3.31
3.Resin-modified glass ionomer cement	5	2.36	0.25	1.96	2.63
4.Negative control	5	2.11	0.35	1.62	2.58
5.Positive control	5	18.37	1.88	15.77	20.34

After this, the modulus of elasticity was tested by a normality test using a Shapiro-Wilk test. It was found that the P-value was more than 0.05, or the modulus of elasticity was normally distributed. Thus, a one-way ANOVA was conducted to analyze and compare the mean value for the 5 groups. The result showed a P-value <0.001, which was under 0.05. Accordingly, the mean value of the modulus of elasticity in at least 1 pair within the 5 groups was different and statistically significant at 0.05, as shown in Table 3. Thus, multiple comparisons using the Bonferroni method would be analyzed.

Table 3 One-way ANOVA

	Sum of Squares	df	Mean Square	F	*P*-value
Between groups	1005.731	4	251.433	227.816	<0.001*
Within groups	22.073	20	1.104		
Total	1027.805	24			

** Statistically Significant at 0.05*

From these multiple comparisons, it was found that all groups had a different modulus of elasticity, except the negative control group, conventional glass ionomer cement, and resin-modified ionomer cement, which were not statistically significant according to Table 4.

The comparison of modulus of elasticity was presented as a bar chart. The dentins in the positive control group had the highest modulus of elasticity. After demineralization, the modulus of elasticity for the negative controlled group decreased. After remineralization with calcium silicate cement, the modulus of elasticity increased significantly when compared to the negative control group. For the remineralization with conventional glass ionomer cement and resin-modified glass ionomer cement, the modulus of elasticity was higher, but not statistically significantly higher, when compared with the negative control group as shown in Figure 2.

Table 4 Modulus of Elasticity Paired Comparison Analysis

	Conventional glass ionomer cement	Resin-modified glass ionomer cement	Negative control	Positive control
Calcium silicate cement	7.07 (<0.001*)	7.32 (<0.001*)	7.57 (<0.001*)	-8.69 (<0.001*)
Conventional glass ionomer cement	-	0.25 (1.000)	0.50 (1.000)	-15.76 (<0.001*)
Resin-modified glass ionomer cement		-	0.25 (1.000)	-16.01 (<0.001*)
Negative control			-	-16.26 (<0.001*)

*Data presented in mean difference (P-value), paired comparison by Bonferroni method * Statistically significant at 0.05*

4.2 Discussion

This study aimed to measure the remineralization efficiency of 3 groups of materials. The results showed a difference in the remineralization efficiency. Therefore, the null hypothesis was rejected. When caries-affected dentin was simulated, the dentin was demineralized and had a lower elastic modulus than normal dentin. Only the calcium silicate cement group showed a statistically significant increase in elastic modulus. The result of this study ensured that only calcium silicate cement could stimulate remineralization and restored the mechanical strength of dentin.

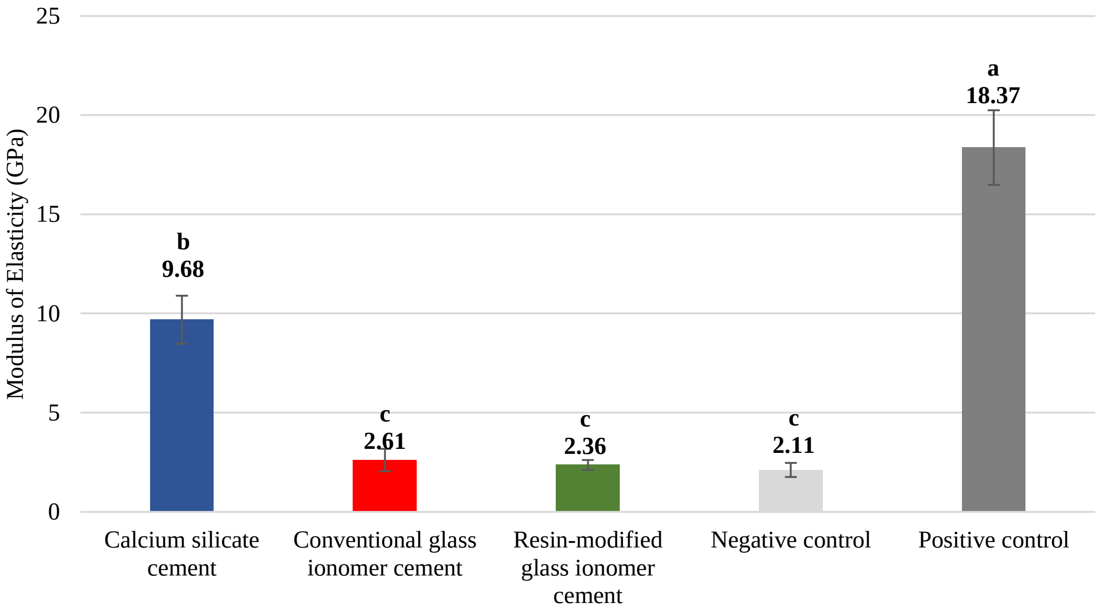

Figure 2 Mean Value of Modulus of Elasticity for 5 Groups of DentinsGroups labeled with the same letters do not differ significantly ($p > 0.05$)

Glass ionomer cement groups, both conventional and resin-modified glass ionomer cement were found to have a remineralization effect as they could restore the hardness (microhardness) of caries-affected dentin when compared to resin composite (Aykut-Yetkiner, Simşek, Eronat, & Ciftçioğlu, 2014). But when compared to the new novel calcium silicate cement, it was discovered that only calcium silicate cement could cause remineralization and restore the mechanical strength of dentin.

The result from this study was conformed with a previous study (Schwendicke et al., 2019) that compared the remineralization efficiencies of calcium silicate cement and glass ionomer cement as measured by microhardness and mineral content. In that study, both glass ionomer and calcium silicate cement were reported to provide some mineral content, but glass ionomer cement was found to be incapable of restoring dentin strength in a significant way when compared to calcium silicate cement. It could explain that calcium silicate cement could form a carbonate apatite formation layer between material and dentin (Jefferies, 2014; Schwendicke et al., 2019).

Furthermore, a study comparing the remineralization effect of glass ionomer and calcium silicate cement in complete demineralized dentin found that glass ionomer cement cannot form new apatite when compared to calcium silicate cement by transmission microscope electron microscopy. There was no ultrastructural evidence of mineral deposits other than the uptake of GIC-specific ions (Kim et al.,2010).

It was explained that glass ionomer cement and calcium silicate cement had different material compositions and properties; glass ionomer cement was acidic and undergoes remineralization via ion exchange, whereas calcium silicate cement had alkaline properties and apatite formation properties, which may provide a better condition for mineral return and reconstruction (Kim et al.,2010; Watson, Atmeh, Sajini, Cook, & Festy, 2014).

It has been shown that when the dentin loses minerals, the dentin loses its mechanical strength (Bertassoni, Habelitz, Marshall, & Marshall, 2011). As a result, the number of minerals alone may not accurately represent the quality of remineralization. Furthermore, in this study, we used the nanoindentation technique, which is a highly accurate method. Because teeth were small organs, there was a difference in the amount of minerals found in each area around the intertubular dentin and peritubular dentin (Marshall, Jr, Marshall, Kinney, & Balooch, 1997). The nanoscale hardness test could be used to choose a location in the dentin region between the dentinal tubules (Joves, Inoue, Sadr, Nikaido, & Tagami, 2014). Therefore, the intervention used in this study was trustworthy.

The remineralization process in the dentin was still complicated and remains unclear, such as in the case of the classical ion-based crystallization concept, which was epitaxial growth with an accumulation of residual crystallites when the dentin was partially demineralized and the non-classical ion-based crystallization concept, which was biomimetic remineralization in natural teeth even when all minerals were lost. Many studies designed an experiment that simulated the biomimetic remineralization process by using a biomimetic analog such as aspartic acid, polyacrylic acid (PAA), or polyvinyl phosphonic acid (PVPA), then immersing in a biomimetic analog acting as a remineralization catalyst (He et al., 2019; Kim et al.,2010). In clinical use, this might be a limitation as the saliva did not have those components. Accordingly, in this study, we designed the experiment to be similar to a clinical situation. The dental caries was simulated as caries-affected dentin by the pH cycling method that affects partial remineralization and aging in the artificial saliva without a remineralization catalyst.

This study was designed as a laboratory experiment, and it had limitations, including the inability to simulate real human caries-affected dentin and the need for additional research, such as a clinical study. This study focused solely on mechanical strength; additional research into ultrastructure is possible.

5. Conclusion

Within the limitation of this study, it was concluded that calcium silicate cement was more effective than glass ionomer cement in remineralizing human artificial caries affected dentin.

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