Remineralized Effect of Chlorhexidine Gluconate as A Cavity Disinfectant on Dentin Carious Lesion Restored with High Viscosity Glass Ionomer Cement

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Abstract

Chlorhexidine gluconate (CHX) is usually used as a cavity disinfectant in atraumatic restorative treatment, but its remineralized effects on contacted carious dentin are not clear. This research aimed to compare the mean mineral density (MD) difference of carious dentin with or without cavity disinfectant CHX before restoring with high viscosity glass ionomer cement (H-GIC) *in vitro*. Selective caries removal to leathery dentin was performed in fourteen extracted primary molars with dentin carious lesion. Then, the samples were randomly divided into 2 groups: Group A (n=7) restored with H-GIC (Equia Forte™) without the disinfectant and Group B (n=7) disinfected the cavity with 2% CHX for 1 minute before restored with H-GIC (Equia Forte™). The samples were scanned using microcomputed tomography and analyzed for the mean MD of the teeth at the baseline and after the restoration. The percentages of the mean MD difference between Groups A (Equia Forte™) and B (2% CHX and Equia Forte™) were 14.59 $\pm$ 9.868 and 30.90 $\pm$ 14.319, respectively. Group B had a significantly higher percentage of mineral gain than Group A at a 95% confident interval (p-value = 0.029, p-value < 0.05). CHX was shown to inhibit the matrix metalloproteinase (MMP) activity, which can keep collagen cross-linking of tooth structure from collagen degradation. The remineralization of dentin is encouraged from the remaining scaffold collagen fibrils that crystalize minerals. The group with 2% CHX as a cavity disinfectant had higher mean mineral densities gain and percentage of the mean mineral density difference than the non-CHX group. Consequently, 2% CHX enhances the remineralization of adjacent dentin of the restoration.

Keywords: *Chlorhexidine gluconate, High viscosity glass ionomer cement, Mean mineral density, Primary molar teeth, Micro-computed tomography, H-GIC*

1. Introduction

At present, the management of dental caries is turned to the new preventive and restorative strategy called 'minimal intervention' (FDI World Dental Federation, 2017), which promotes preserving tooth structure and emphasizing maximum tooth function. One of the management with evidence-based outcomes is selective caries removal that is used in deep caries without any signs and symptoms of pulpal degenerate (American Academy of Pediatric Dentistry, 2019). The concepts of selective caries removal are removing surrounded axial-wall caries and leaving pulpal wall caries in the cavity. The remaining bacteria in deep caries acts as an irritant that promotes the inflammatory process and subsequently induced odontoblast cells to form reactionary dentin. Nevertheless, the severe irritant leads to odontoblast cells' death, which stimulates the dental pulp stem cells or progenitor cells to differentiate odontoblast-like cells to form reparative dentin (Smith et al., 1995). Leaving deep caries nearly pulp motivates dentin to repair itself in addition to avoiding pulpal exposure and maintaining pulpal vitality (Schwendicke et al., 2016).

Selective caries removal is often used in atraumatic restorative treatment (ART), which is a method to manage deep caries by hand instrument for less trauma (Giacaman, Munoz-Sandoval, Neuhaus, Fontana, & Chalas, 2018). ART was developed mainly for treating caries in children living in under-served areas where resources are limited (Dorri et al., 2017). High viscosity glass ionomer cement (H-GIC) is one of the materials that has been used in ART (Bonifácio et al., 2009). ART using H-GIC helps dental treatment easier, faster, and more comfortable than the conventional restorative treatment (Caro et al., 2012). Besides reducing pulpal damage, ART leads to reduce pain experience and gain a better attitude in dental treatment and is more cost-effective than the conventional treatment (Dorri et al., 2017; Luz, Barata, Meller, Slavutzky, & Araujo, 2012; Schwendicke et al., 2018). Therefore, ART is suitable for childhood patients who have severe multiple caries, prevention programs, and controlled disease needs. Even if the selective caries removal method provides a high survival rate, defective restoration, pulpal inflammation, and secondary caries can still cause the failure of ART (van Gemert-Schriks, van Amerongen, ten Cate, & Aartman, 2007).

There are several ways to improve the success rate of ART. First, the selection of proper restorative material is an important concern for ART. Several studies found that bond strength between the restorative material and affected dentin was different compared with normal dentin (Nakajima, Kunawarote, Prasansuttiporn, & Tagami, 2011; Nakajima et al., 1995; Scholtanus, Purwanta, Dogan, Kleverlaan, & Felizer, 2010; Wei, Sadr, Shimada, & Tagami, 2008). Morphological, chemical, and physical characteristics of the affected dentin showed influences on the bonding structure between the affected dentin and material (Nakajima et al., 2011). A low evidence-based study showed that ART using H-GIC had more failure from the restoration in both primary and permanent teeth when compared with the conventional treatment (Dorri et al., 2017). The survival rate in 2-year follows up of single surface ART with GIC was high in both primary and permanent posterior teeth, while the multiple surface restoration showed a medium survival rate (de Amorim et al., 2018). Although H-GIC was not recommended for multiple surface restoration in primary molar in the past (Chadwick & Evans, 2007), currently a new generation of H-GIC such as Equia Forte™ (GC Corporation, Tokyo, Japan) is claimed to be used with cavity class II. Hybrid technology in Equia Forte™ helps increase flexural strength (Moshaverinia et al., 2019), which prevents material deformation against chewing force (Wang, D'Alpino, Lopes, & Pereira, 2003).

Second, the hypermineralized zone of the adjacent dentin to the restoration helps decrease the progression of secondary caries (Hicks, Flaitz, & Silverstone, 1986; ten Cate & Van Duinen, 1995). The hypermineralized zone occurs from the exchange of charged ions between restorative materials and the tooth's structure. (Hicks et al., 1986). The mean mineral density of dentin after restoring with GIC (Fuji VII, GC) is significantly higher than before restoration whereas the mean lesion depth is decreased (Maneekarn, 2016). Comparing between GIC and amalgam restored cavities, teeth restored with GIC showed less recurrent carious lesion (Hicks et al., 1986; Mickenautsch & Yengopal, 2011). H-GIC contains several minerals that promote the hypermineralized zone underneath contacted dentin (Ngo, Mount, Mc Intyre, Tuisuva, & Von Doussa, 2006). Fluoride and strontium ions from H-GIC can penetrate deep into carious demineralized dentin and produce a remineralization process (Ngo et al., 2006).

Lastly, antimicrobial agents help reducing treatment failure in long term by inhibiting the growth of residual bacteria in deep caries (Koruyucu et al., 2015). Several studies suggested using antimicrobial agents as cavity disinfectant before the restoration (Joshi et al., 2017; Y. Takahashi et al., 2006). Chlorhexidine gluconate (CHX) is a well-known antimicrobial agent to be used in ART (Joshi et al., 2017). CHX's property is to eliminate microorganism cell negative-charge membrane with the positive charge. CHX has been shown to reduce *E. faecalis* in deep caries lesion, which was difficult to eliminate and known to induce pulpal and periapical inflammation in long term (Koruyucu et al., 2015; Radman, Djeri, Arbutina, & Milašin, 2016).

Besides the antimicrobial effect, several studies found that using CHX with polyacrylic acid helps create desirable bondability of GIC (Jaidka, Somani, Singh, & Shafat, 2016; Ruiz, Baca, Pardo-Ridao, Arias-Moliz, & Ferrer-Luque, 2013; Sheikh Hasani, Paryab, Saffarpour, Kharazifard, & Shahrabi, 2017; Wadenya, Menon, & Mante, 2011). CHX helps to neutralize the dentin surface that is applied by an acid conditioner (Sheikh Hasani et al., 2017) and also increases the surface energy of the dentin (Sheikh Hasani et al., 2017). However, evidence is not clear about the effect of CHX on the remineralization ability of the affected dentin after GIC restoration (Lugassy et al., 2018). This study aimed to investigate the effect of CHX on the dentin carious lesion after using cavity disinfectant CHX and restoring with H-GIC.

2. Objectives

To compare the percentage of the mean mineral density difference of carious dentin at the baseline and after cavity disinfecting with chlorhexidine gluconate and restoring with H-GIC *in vitro*.

3. Materials and Methods

3.1 Subjects

The study protocols were approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU2020-043), and were also approved the notification from the Institutional Biosafety Committee of the Faculty of Dentistry, Chulalongkorn University (DENT CU-IBC 032/2020). A sample size calculation from G*Power 3.1 program was 6.25 samples per group, with an additional 10% of each group for sample compensation, the sample size became 7 samples per group (14 samples in total). Fourteen human primary molars were collected from the Pediatric department clinic of the Faculty of Dentistry, Chulalongkorn University. The inclusion criteria were primary molar extracted teeth with dentin carious lesion with or without pulpal exposure. If the carious lesion was exposed to the dental pulp, exposure size must less than 1x1 mm^2 after selective caries removal. If the carious lesion was not exposed to the dental pulp, the lesion must invade the dentin from visual examination. The remaining tooth structure must be more than 1/3 of the crown, and the roots of the teeth must be at least 1 mm in length.

3.2 Specimen preparation

Fourteen extracted primary molar teeth with carious lesion were stored in 0.9% sodium chloride solution and 10% formalin solution at room temperature for at least 2 weeks following the study of NawrockaA A. et al. (NawrockaA & Łukomska-Szymańska, 2019). The teeth were cleaned with pumice, rinsed in deionized water, and dried with tissue paper. For the horizontal guide plane, all the teeth were prepared by cutting the cusp of the teeth like a flat surface with a slow-speed cutting machine. Then, the teeth were embedded in a resin block and attached with dental pink wax. The carious lesion was removed from all teeth with ART method using only a spoon excavator, rinsed with water, and dried with sterile cotton pellets. Removing carious lesion was followed by the selective caries removal to leathery dentin. All teeth were measured the mean mineral density of dentin by micro-CT as the baseline.

All teeth were allocated into 2 groups of 7 samples each. The teeth were labeled numbers before sampling. A random number table was used for collecting the sequence of the sampling. The odd numbers were grouped to Group A (H-GIC (Equia Forte™)) whereas the even numbers were grouped to Group B (2% CHX and H-GIC (Equia Forte™)). After sampling, the samples were stored in artificial saliva.

The samples in Group A were treated with dentin conditioner 20 seconds before restored with Equia Forte™ and coated with petroleum jelly. The samples in Group B were prepared the cavity with 2% CHX liquid for 1 minute by micro brush according to previous studies (Ersin, Uzel, Aykut, Candan, & Eronat, 2006; Joshi et al., 2017; Kim, Kim, Choi, & Kim, 2011). Then, the cavity was treated with dentin conditioner 20 seconds before restored with Equia Forte™ and coated with petroleum jelly. All samples were stored in the artificial saliva at 37 degrees Celsius for 24 hours before micro-CT scanning.

3.3 Assessment of the micro-CT scan and data analysis

All of the samples were scanned by the micro-CT after restored with H-GIC. The micro-CT was set at a resolution of 1024 x 1024 megapixels comparing with hydroxyapatite mineral density 1200 mg per cm^3, 70 kVp, and 57 µA.

The scanned interested area included the area from the first slide of the occlusal surface to the first slide of the roof of the pulp chamber (figure 1(A)). Results of scanning were showed as sections in the horizontal plane (figure 1(B and C)). Then, the interested area was drawn anti-clockwise to measure the internal of the interested area.

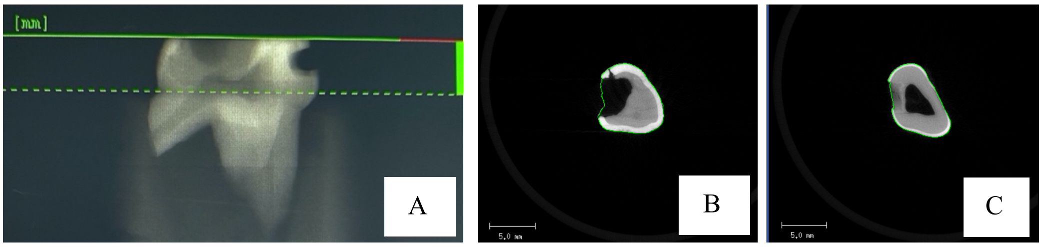

Figure 1 (A) The micro-CT scanned area, which the upper line shows the first occlusal section that was set as the first section for interpreting data, (B) the first slide of the occlusal surface, and (C) the first slide of the roof of the pulp chamber

The cavity was 3D constructed according to the selected slides. The number of selected slides was maintained in the same tooth for comparing mineral density after treatment in each tooth (figure 2). The contrast setting for the analysis in each sample was determined from the difference in density between H-GIC and dentin. Therefore, the H-GIC restored samples were analyzed before the baseline samples. The contrast setting was the average value determined by two calibrated examiners. To eliminate the study bias, intra-rater reliability and inter-rater reliability with Intraclass correlation coefficients (ICCs) have to be greater than or equal to 80% agreement. The mean mineral density of each sample was calculated by the micro-CT programs (Micro-CT Ray Version 4.2 and Micro-CT Evaluation Program Version 6.6) according to the contrast setting. The percentage of the mean mineral density difference between before and after the restoration was calculated using the previously published equation as described (Nakata, Nikaido, Nakashima, Nango, & Tagami, 2012).

$$\% \text{ mean MD difference} = \frac{(\text{Mean MD after restoration - Mean MD baseline}) \times 100}{\text{Mean MD baseline}}$$

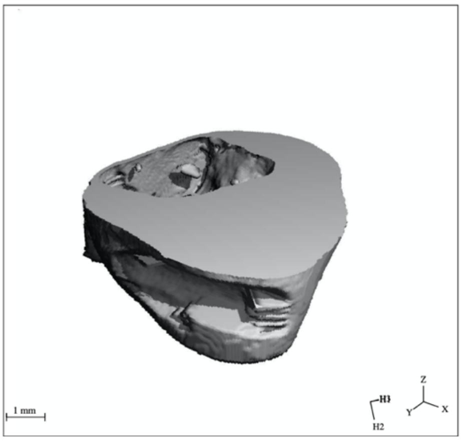

Figure 2 The 3D constructed of the interested area

3.4 Statistical analysis

Shapiro-Wilk test and Levene's test were performed to test the normality and homogeneity of the variance of the mean mineral density of the carious dentin. A comparison of the percentage of the mean mineral density difference between before and after the restoration in the same group was analyzed by pair t-test and between groups by independent t-test. The intra-rater reliability and inter-rater reliability with Intraclass correlation coefficients (ICCs) were greater than or equal to 80% agreement. All of the analyses were performed at a 95% confident interval and SPSS statistic 22.

4. Results and Discussion

4.1 Results

A total of 14 samples were included in the study. The mean mineral density (± SD) at the baseline of 7 samples in Group A (Equia Forte™) was 811.26 ± 90.519 mgHA/ccm and 7 samples in Group B (2% CHX and Equia Forte™) was 707.83 ± 98.042 mgHA/ccm. No significant difference was detected between the mean mineral density at the baseline of the two groups at a 95% confident interval (*P* = 0.063).

The mean mineral densities after the restoration ± SD were 925.97 ± 90.890 mgHA/ccm and 916.07 ± 63.049 mgHA/ccm for groups A and B, respectively (*P* = 0.817). The comparison of the mean mineral density gain of Group A and Group B were significant difference; Group A's mineral gain = 114.71 ± 75.910 mgHA/ccm while Group B's mineral gain = 208.23 ± 75.473 mgHA/ccm (Pair t-test, *P* < 0.01). The mean % mean MD difference between the two groups were 14.59 ± 9.868 and 30.90 ± 14.319 (*P* = 0.029) (Table 1). There was normal distribution between the mean % mean MD difference between groups A and B (Levene's test for equality of variances; *P* = 0.166).

Table 1 shows the mean percent MD difference between group A and B

GROUP	A: Equia™	B: CHX-Equia™	*P*
Mean mineral density baseline	811.26 ± 90.519	707.83 ± 98.042	0.063
Mean mineral density after restoration	925.97 ± 90.890	916.07 ± 63.049	0.817
Mean mineral density gain	114.71 ± 75.910	208.23 ± 75.473	<0.01*
Mean % mean MD difference	14.59 ± 9.868	30.90 ± 14.319	0.029$^{\alpha}$

*significant difference ($p < 0.01$) both 2 groups, $^{\alpha}$significant difference ($p < 0.05$)

4.2 Discussion

The results from this study showed that H-GIC promoted remineralization to the adjacent affected dentin and also found that the group that used 2% CHX as cavity disinfectant had a higher mean % mean MD difference. This effect is related to some CHX properties, in which CHX acts as a barrier to the dentin surface by neutralizing the dentin surface which is applied by an acid conditioner (Sheikh Hasani et al., 2017).

The composition of dentin has mineral phase, organic matrix, and water. The mineral phase is 70 wt% of hydroxyapatite and the organic matrix is 20 wt% of type I collagen (Goldberg, Kulkarni, Young, & Boskey, 2011). Pathogenesis of dentin carious lesion originates from dissolved minerals when pH is critically decreased. Then, bacterial acid production stimulates dentin degradation, owing to dentin-embedded and salivary matrix metalloproteinases, and cathepsins activation (Takahashi & Nyvad, 2016). CHX has been shown to inhibit the matrix metalloproteinase (MMP) activity, which can keep collagen cross-linking of the tooth's structure from collagen degradation (Carrilho et al., 2007; Kim et al., 2011). Accordingly, the remineralization of the dentin is encouraged from the remaining scaffold collagen fibrils that crystalize minerals (Bertassoni, Habelitz, Marshall, & Marshall, 2011; Kim et al., 2011). Demineralized dentin relates to decreased elastic modulus due to deformable organic network (Bertassoni, Habelitz, Kinney, Marshall, & Marshall Jr, 2009; Chien et al., 2016; Liang et al., 2015). Extrafibrillar mineral, intrafibrillar mineral, and protein triple helix are organic structures surrounding the collagen fibrils, which create collagen fibrils to elastic behavior and resist to the demineralization. Intra- and extrafibrillar mineral also have self mechanical recovery into likely normal dentin structure, though dentin is partially demineralized. When the collagen fibrils are reincorporated with minerals, the remineralization is promoted again (Bertassoni et al., 2009).

Our study corresponded with the previous study that investigated the remineralization of dentin through elastic modulus (Kim et al., 2011), which showed that the elastic modulus of the CHX treated group on demineralized dentin block was more than the non-CHX group. Besides, the higher concentration of CHX, the more elastic modulus was found. Therefore, the application of CHX on demineralized dentin is effective in promoting the remineralization of deep residual caries.

5. Conclusion

According to our findings, the group that used 2% CHX as cavity disinfectant with H-GIC restoration had higher mean mineral density gain and percentage of the mean mineral density difference than the group with H-GIC restoration alone. Consequently, 2% CHX enhances the remineralization of the adjacent dentin of the restoration.

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c) Department of biochemistry, faculty of dentistry, Chulalongkorn University.

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