Carboxymethyl Chitosan/Amorphous Calcium Phosphate and Dentin Remineralization

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Abstract

Carboxymethyl chitosan/amorphous calcium phosphate (CMC/ACP) is a non-collagen protein analogue that can replace the dentin matrix protein 1 (DMP1) function. The aim of this study was to analyze the effects of CMC/ACP in demineralized dentin.

Two groups of four teeth with eight cavities were created. Group 1 teeth were filled with composite resin, whereas group 2 teeth were first applied with CMC/ACP followed by filling with composite resin. Then, all teeth were soaked in phosphate-buffered saline solution and placed in a shaking incubator at 37°C. On days 7 and 14 of incubation, the gray areas of the samples were analyzed using micro-computed tomography. The gray area of group 2 increased on both days 7 (35.171 ± 5.547) and 14 (43.281 ± 4.834), whereas those of group 1 decreased on day 14 (27.100). Thus, CMC/ACP may induce dentin remineralization.

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Introduction

Paradigm shift of caries treatment from the principle of complete caries removal to minimal intervention dentistry aims to maintain pulp vitality by stimulating regeneration. In the concept of minimal intervention dentistry, the dentin portion retained is the affected dentin, which is actually a layer of dentin that is only demineralized with intact collagen and low amount of bacteria.[1]

Remineralization in affected dentin can be categorized based on its location. Remineralization occurring outside collagen fibrils is called extra-fibrillar remineralization (conventional), whereas that occurring inside collagen fibrils is called intrafibrillar or guided-tissue remineralization (GTR). Conventional remineralization occurs when residual apatite crystal persist on the dentin and epitaxially grow or in a top–down manner. On the other hand, GTR is a bottom-up process that can strengthen the mechanical properties of dentin.[2,3] In GTR, non-collagen proteins require dentin matrix protein 1 (DMP1), which function to bind nanosized amorphous calcium phosphate (ACP) to form a stable electrostatic bond. This bond triggers infiltration of apatite crystal into the collagen gap zone and subsequently forms hydroxyapatite (HAP) crystals.

Currently, there are several types of analogous proteins that can replace the role of DMP1, with one being carboxymethyl chitosan (CMC).[4] CMC, a derivative of chitosan, is rich in the carboxyl and phosphate groups and can bind calcium into the CMC/ACP complex. Further, CMC has an affinity for calcium ions due to the abundance of carboxyl groups in it.[5,6]

We analyzed the effect of CMC/ACP on dentin remineralization. Micro-computed tomography (**CT**) was performed for analyzing the presence of gray level change and for the quantitative measurement of dentin mineral concentration with a CT Analyser software using a resolution between 5 and 30 μm.[7,8]

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Materials and methods

This research protocol was approved by the Ethics Committee of Faculty of Dentistry, Universitas Indonesia (approval No. 137/Ethical Approval/FKG/12/2017 with Protocol No. 051301017).

Preparation of CMC/ACP gel

We diluted 2.5 g CMC powder into 40 mL of water and stirred the mixture at 1000 rpm until it was completely diluted to form a soluble CMC gel. Next, 0.498 g of K2HPO4 was added to the CMC gel and stirred at 500 rpm. Finally, a solution comprising 0.555 g of CaCl2 mixed into 10 mL of deionized water was added dropwise into the CMC gel and stirred for 5 min until the formation of a CMC/ACP gel. The CMC/ACP gel was then frozen for 2 h at −80°C and continued to be lyophilized in the freeze drying unit (Snijders, Holland) for 6 h.

Preparation of dental samples

The extracted teeth (n = 4) were immediately immersed in phosphate-buffered saline (PBS) and stored at 4°C until further treatment. Each tooth was prepared with 2 cavities using cylindrical bur on the mesial and distal portions with a depth of 3 mm. The teeth were then cleaned and dried, and the outside area of the cavity was coated with 2 layers of nail polish. All tooth samples were then immersed in 17% ethylenediaminetetraacetic acid (EDTA) solution for 1 week, followed by storage in shaking incubator at 100 rpm/37°C. The teeth were then rinsed with aquabidest for 30 min and soaked in 20 mL of 1 M NaCl solution (pH 7.0) at 25°C for 8 h.

The distal cavities of the samples were filled directly with composite resin (Z350, 3M), while the mesial cavities were applied with CMC/ACP first before the filling with the composite resin. In addition, the roots of the sample teeth were also soaked in PBS and stored in shaking incubator at 37°C.

Analysis

The analysis was performed on days 7 and 14. To assess dentin remineralization, we used micro-CT (type 1173; Bruker, Belgium), with the same cone angle and X-rays doses for all samples. The density of dentin was calculated by analyzing the gray level using CT-an software, where the value is displayed on the computer. The measuring value of the gray level is proportional to the radiopacity and radiodensity. The higher the gray level, higher is the radiopacity and density.

Data analysis

The data was statistically analyzed with SPSS ver. 20 using one-way analysis of variance test.

Results

Micro-CT analysis of the samples evaluating the gray level was calculated using CT-an software. The results of the analysis are referred to several points, as can be seen in Figure 1.

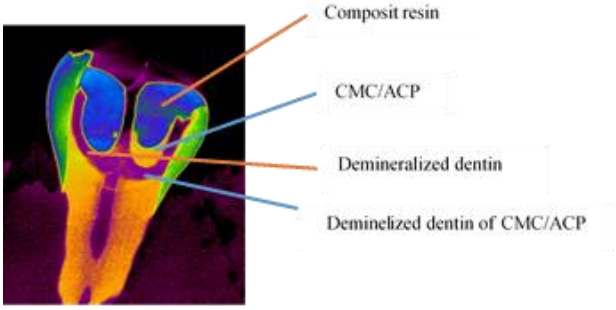

Figure 1. Location of points analyzed by gray level using micro-computed tomography CMC/ACP, carboxymethyl chitosan/amorphous calcium phosphate.

The results of the analysis are shown in Table 1. The highest mean value belonged to the CMC/ACP group on day 14 (43.281), while the lowest belonged to demineralized dentin group on day 14 (27.100). CMC/ACP application after days 7 and 14 increased the gray level, while the amount decreased in the demineralized group. Conclusion: Thus, dentin may be remineralized using CMC/ACP.

Table 2 shows the significant differences observed only between the demineralized and

CMC/ACP groups on day 14. Conclusion: Thus, the application of CMC/ACP may remineralize the demineralized dentin.

Group	n	Mean ± SD	95% confidence Interval	*P*-value
Demineralized dentin	4	35.243 ± 3.757	29.263–41.222	0.002*
Demineralized, 7 days	4	29.313 ± 4.569	22.042–36.583	
Demineralized, 14 days	4	27.100 ± 3.016	17.499–36.700	
CMC/ACP, 7 days	4	35.171 ± 5.547	30.344–47.998	
CMC/ACP, 14 days	4	43.281 ± 4.834	35.589–50.973	

*test significance with one-way analysis of variance test; $p < 0.05$
CMC/ACP, carboxymethyl chitosan/amorphous calcium phosphate; SD, standard deviation

Table 1. Mean, standard deviation, and significance gray scale value on demineralized dentin and carboxymethyl chitosan/amorphous calcium phosphate group.

Discussion

The use of samples from teeth extracted for orthodontic purposes and soaked in PBS solution at 4°C in order to maintain the tooth vitality was based on the Chen's method (2017).[4] Demineralization of the samples was performed using 17% EDTA for 7 days, because it is a chelating agent that can bind calcium and form soluble calcium chelates.[9] In the process of rinsing with deionized water, soluble calcium chelates gets dissolved to create demineralized dentin with intact collagen. Finally, the sample was immersed in 20 mL of 1 M NaCl solution to remove the soluble portion and retain the non-collagen protein fixed on the dentin.[5]

We used CMC in this study because of its chitosan-derivative properties, i.e., its richness in the carboxyl and phosphate groups, which enables binding of calcium into the CMC/ACP complex. CMC is biodegradable, biocompatible, easily available in the nature, nontoxic, and antibacterial. CMC can be used in nano-sizes produced from crustacean waste.[10] The CMC/ACP combination assays can serve as non-collagen protein analogs to replace the role of DMP1 lost due to the caries process. In GTR process, DMP1 binds ACP to form stable electrostatic bonds.

We performed the analysis on days 7 and 14 because, on day 7, the process of remineralization begins to occur by forming the octacalcium phosphate bond, while, on day 14, the process to form HAP mineral starts.

Samples analysis was performed using micro-CT because this device has a nondestructive advantage, which does not require cutting of the sample. The thickness of the image slice is kept constant to prevent the occurrence of irregularities due to errors in cutting. The minimum slice thickness can be adjusted by adjusting the X-ray beam; therefore, the slice on the micro-CT can be much thinner than the dentin slices created from a cutting machine. Micro-CT testing demonstrates the ability of X-ray penetration into the remineralized lesions by monitoring the gray level changes by measuring the dentin mineral concentration quantitatively and accurately with a resolution of 5–30 μm.[7]

The results of the analysis shown in Table 1 reveals that the highest mean values were obtained with the CMC/ACP group on day 14 (43.281), while, the lowest value was obtained with the demineralized dentin group on day 14 (27.100). CMC/ACP application after days 7 and 14 resulted in increased gray level, while, in the demineralized group, it resulted in decrease in the gray level. This result shows that CMC/ACP may improve the process of remineralization; this finding is in agreement with that of Chen (2017), who found that CMC/ACP can improve the process of GTR. However, because we used micro-CT here, the form of GTR could not be identified.[7] This trend is consistent with that reported by Burwell,[11] who found that micro-CT imaging can only be used for the analysis of the occurrence of dentin remineralization in general.

In the CMC/ACP group on day 7, the mean value showed a continuing increase until day 14. The increase in the mean value in the CMC/ACP group between days 7 and 14 do not show any significant difference (Table 2), which indicates that the process of remineralization in the CMC/ACP group occurs constantly. Meanwhile, in the control group or demineralized

dentin, the mean value from days 7 to 14 decreased. These results reinforce that the role of CMC/ACP in replacing the DMP1 function is extremely significant, which is supported by the results of our analysis in Table 2. Significant difference was observed (0.004) between the results of the mean value between days 14 of the dentin demineralization group and the CMC/ACP group.

	Demineralized dentin	Demineralized, 7 days	Demineralized, 14days	CMC/ACP, 7 days	CMC/ACP, 14 days
Demineralized dentin	-	1.000	0.364	-	-
Demineralized, 7 days	-	-	-	0.139	-
Demineralized, 14 days	-	-	-	-	0.004*
CMC/ACP, 7 days	-	-	-	-	1.000

*test significance with post-hoc Bonferroni test; $p < 0.05$
CMC/ACP, carboxymethyl chitosan/amorphous calcium phosphate

Table 2. Significance values of the gray scale level between the demineralized dentin and CMC/ACP group

Conclusion

In vitro analysis using micro-CT of dentin remineralization in this study exposed by CMC/ACP demonstrated an increase in the gray level values. It can thus be concluded that CMC/ACP may induce dentin remineralization.

Declaration of Interest

The authors report no conflict of interest.

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