International Journal of Applied Pharmaceutics

ISSN - 0975 - 7058 Vol 12, Special Issue 2, 2020

Research Article

EFFECT OF POLYMER-INDUCED LIQUID PRECURSOR PROCESS CONTAINING POLYASPARTIC ACID ON INTRAFIBRILLAR DENTIN REMINERALIZATION (MICRO-COMPUTED TOMOGRAPHY ANALYSIS)

NORMA AVANTI[1], NILAKESUMA DJAUHARIE[2]*, ANGGRAINI MARGONO[2], RATNA MEIDYAWATI[2], CUT THYSA[1]

[1]Conservative Dentistry Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. [2]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. Email: nila.setyopurnomo@gmail.com

Received: 09 October 2019, Revised and Accepted: 30 June 2020

ABSTRACT

Objective: Caries treatment can be performed by minimal intervention, i.e., by removing the infected dentin and leaving the affected dentin and then inducing remineralization in the affected dentin. The affected dentin still consists of collagen cross bonds. Polymer-induced liquid precursor process is a guided tissue remineralization method that aims to remineralize intrafibrillar and extrafibrillar dentin by adding polymers that are similar to non-collagen protein. One of the non-collagen protein analog materials is polyaspartic acid. The aim of this study was to evaluate the remineralization of dentin on the demineralized dentin surface after immersed in remineralization solutions containing polyaspartic acid as a non-collagen protein analog.

Methods: Human premolar teeth extracted for orthodontic purposes were divided into four groups, namely, one control and three treatment groups. Teeth in the control group were immersed only in the demineralization solution containing acetate buffer (pH 5.0, 66 h). Teeth in the three treatment groups were immersed in acetate buffer (pH 5.0, 66 h) and then continue to immersed in the remineralization solution containing polyaspartic acid for 3, 7, and 14 days. Remineralization was evaluated by micro-CT.

Results: Remineralization appeared on the demineralized dentin surface, characterized by an increase in the grayscale index, after immersion in the remineralization solution containing polyaspartic acid. Significant differences were observed in the mean grayscale index values among the four groups.

Conclusion: Polyaspartic acid has the potential to induce dentin remineralization.

Keywords: Polyaspartic acid, Polymer-induced liquid precursor process, Non-collagen protein analog, Dentin remineralization.

 DOI: http://dx.doi.org/10.22159/ijap.2020.v12s2.OP-45

INTRODUCTION

The conventional treatment for carious lesions consists of extracting all the carious tissue to obtain healthy tissue before restoration [1]. In deep carious lesions, the remaining thickness of the dentin is very less, with only 25% of the thickness of normal dentin [1]. Removing all the infected dentin could risk causing pulp exposure [1]. At present, there has been a change in the concept of caries treatment with minimum intervention, i.e., by removing the infected dentin and leaving the affected dentin. Therefore, a new understanding of the principle involved in the removal of caries is to eliminate the infected dentin and leave the affected dentin and then induce remineralization so that the growth of carious lesions can be stopped [2]. The affected dentin has collagen cross bonds that are important in remineralization, because apatite crystals can bind to these cross bonds [3,4].

Dentin remineralization is a more complex process than enamel remineralization, which is because, in the enamel, carious lesions still have residual mineral crystals, whereas in dentinal caries, there are no residual mineral crystals [5]. Extrafibrillar and intrafibrillar remineralization of type 1 collagen matrices is important for improving the mechanical properties of dentin [5].

Remineralization of dentin can be induced using conventional methods and also by guided tissue remineralization (GTR). In conventional remineralization, the process of remineralization depends on epitaxial growth over existing apatite crystals [6]. In contrast, the GTR method uses the principles of nanotechnology and biomimetics to achieve intrafibrillar and extrafibrillar remineralization of the collagen matrix, without the presence of remaining apatite crystals [6].

Today, several methods of GTR have been developed, such as the polymer-induced liquid precursor (PILP) process using polymers as a non-collagen protein analog. The polymers often used in this method are polyaspartic acid, polyacrylic acid, and polyvinyl phosphonic acid [5]. Gower was the first to conducted research on intrafibrillar remineralization using a PILP process containing polyaspartic acid [5]. Polyaspartic acid used as a non-collagen protein analog in the PILP process can bind to calcium ions because it contains several carboxyl groups and can stabilize amorphous calcium phosphate (ACP) in solutions to form amorphous nanoprecursors [7]. This PILP process uses the concept of formation of nanodroplet precursor ACP.

It can enter rapidly into the gap zone, which can be a basic step in the process of intrafibrillar remineralization [8]. Polyaspartic acid is widely used as a non-collagen protein analog due to its similarity to amino acids in the body, and hence, it is not toxic and also biodegradable [9].

A previous study conducted by Nudelman *et al*. using horse tendon collagen reported remineralization on day 3 [10]. Therefore, our research was conducted to determine whether dentin remineralization could occur on day 3 in demineralized tooth specimens.

Various methods are available for assessing the effectiveness of remineralization in teeth, for example, by SEM, TEM, and micro-CT.

The advantages of micro-CT scanning are that it is non-destructive, the thickness of the slices is constant, and irregularities caused due to physical cutting can be avoided. Micro-CT is often used to analyze mineral concentrations of teeth [11].

The aim of this study was to analyze the occurrence of dentin remineralization in the PILP process containing polyaspartic acid on days 3, 7, and 14, which was examined by micro-CT.

MATERIALS AND METHODS

Materials

A total of 16 sample groups were evaluated in this study. Each sample group consisted of four teeth. The samples were human premolar teeth that have been extracted for orthodontic purposes, the inclusion criteria being fresh extracted premolar teeth with no caries, restoration, fractures, or defects in the crown and roots. The teeth must have also never received endodontic treatment. Demineralization solutions were contained 2.2 mM $CaHPO_4$ $2H_2O$ and 50 mM CH_3COOH (pH 5.0). Remineralization solutions were prepared by mixing polyaspartic acid powder with ingredients as described by Burwell *et al*. [8] and Nurrohman *et al*. [12]. The polyaspartic acid powder used in this study was obtained from Alamanda Polymer (Alabama, USA. CAS#34345-47-6). The remineralization solution was prepared using 50 mM Tris buffer with 0.9% NaCl, 0.02% NaN_3, 4.5 mM $CaCl_2$, 2.1 mM K_2HPO_4, and 100 µg/mL of 27 kDa polyaspartic acid.

Methods

Freshly extracted premolar teeth were stored in deionized water at 4°C with a maximum storage duration of 14 days. Dentin blocks measuring 4.5 mm in length and width and 2 mm in thickness were cut from the midcoronal region of the selected teeth perpendicular to the tubule direction. The specimen surfaces were ground using SiC abrasive paper with 320–1200 grit and then polished using an aqueous diamond suspension (particle sizes 6.0, 3.0, 1.0, and 0.25 microns). The specimen surface was coated with nail polish varnish to prevent demineralization, except in the opened area (2.5×2.5 mm).

The tooth samples were divided into four groups, as follows: Control and treatment groups at 3, 7, and 14 days. Teeth in the control group were immersed in the demineralization solution, whereas teeth in the treatment groups were immersed in the demineralization solution and then in the remineralization solution containing polyaspartic acid. The treatment groups were divided into three categories based on immersions in the remineralization solutions for 3, 7, and 14 days.

All samples including both control and treatments groups were immersed in demineralization solution for 66 h. After the formation of artificial carious lesions, the samples were rinsed with distilled water and soaked in the remineralization solution at 37°C, except those in the control group.

Then, the samples were immersed in 40 ml of remineralization solution with pH 7.4 at 37°C under continuous shaking for 3, 7, and 14 days, except samples in the control group. The pH of the PILP solution was 7.4 at the beginning of the remineralization experiment, and it was 7.1–7.2 at the end of the study.

Remineralization was observed on days 3, 7, and 14. The samples were prepared for analysis by micro-CT.

RESULTS AND DISCUSSION

Evaluation of this study using micro-CT type sky scans 1173 with data viewer software, N Recon and CT analyzer. The results of the examination are represented as numerical data as the grayscale index. The occurrence of remineralization can be observed by measuring the changes in the depth of artificial carious lesions (demineralized dentin). Changes in the depth of artificial carious lesions occur due to the addition of minerals starting from the bottom of the lesion which is indicated by the grayscale index of the teeth in the demineralized dentin group and in the treatment group after remineralization through the process of PILP containing polyaspartic acid on days 3, 7, and 14.

The mean value of the lesion depth in the demineralized dentin group compared to that in the remineralization group with the PILP process containing polyaspartic acid on days 3, 7, and 14 is depicted in Fig. 1.

Table 1 shows the results of descriptive analysis of the depth of the artificial carious lesions in the demineralized dentin group and the results after immersion in remineralization solutions containing polyaspartic acid on days 3, 7, and 14.

The demineralization solutions commonly used in research are acetate buffer, lactate buffer, and EDTA [13]. Acetate buffer and lactate buffer produce more carious lesions resembling natural carious lesions. Acetate buffer produces artificial carious lesions that are deeper than those produced by lactate buffer at the same pH. In this study, dicalcium phosphate dihydrate was added to the acetate buffer demineralization solution to resemble normal salivary content containing calcium and phosphate ions so that it would later produce artificial carious lesions with images resembling natural carious lesions [8,14,15].

The process of remineralization with PILP involves the addition of anionic polymers to the remineralization solution to maintain the ACP, which is generally unstable. The presence of polymers can improve the stability of the liquid precursor ACP in the collagen. The ACP nucleation develops and matures into nanocrystal apatite along with the collagen intrafibrillar space through a non-classical crystallization model. Then, the intrafibrillar hydroxyapatite growth induces intrafibrillar and extrafibrillar remineralization between the closest collagen fibrils [11].

The PILP process contains anionic polymer macromolecules that can mimic the role of non-collagen proteins so that they can stabilize the solution by interacting with calcium and phosphate ions to form a nanodroplet with a diameter of approximately 10–30 nm, which is capable of diffusing into type 1 intrafibrillar collagen [10].

The mechanism involved in changing the structure of calcium phosphate ions indicates the presence of a bond between phosphate ions and carboxyl polymer groups to bind with calcium ions so that it can trigger the formation of polymer-coated nanodroplets. The formation of globular aggregates occurs because of the ability of the polymers to bind to several ions. Calcium ions also act as bridges between polymers. Calcium phosphate aggregation occurs very rapidly and then collects together to form a crystal structure in the form of an apatite rod with a stable shape [11].

ACP precursors that are stabilized by negatively charged polymers in the form of nanodroplets interact with positive charges along with the collagen molecule and then stimulate compaction and nucleation of ACP.

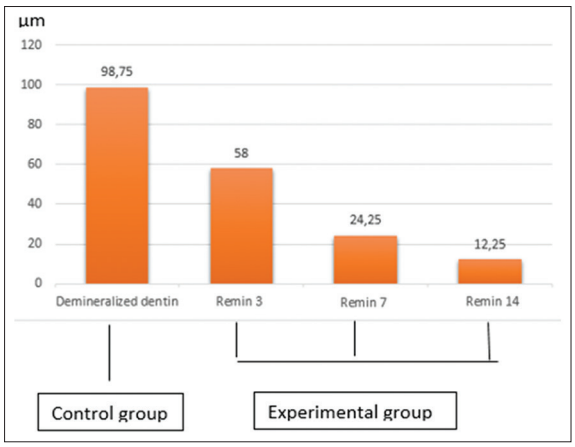

Fig. 1: Graph depicting the mean depth of lesions

Table 1: Results of descriptive analysis of the depth of artificial carious lesions in the demineralized dentin and remineralization dentin groups on days 3, 7, and 14

Variable	Minimum value	Maximum value	Mean	Standard deviation	p-value
Demineralized dentin	71.00	145.00	98.75	16.81	0.393
[a]Remin 3 days	46.00	65.00	58.00	4.53	0.264
[a]Remin 7 days	19.00	37.00	24.25	4.30	0.034
[a]Remin 14 days	10.00	15.00	12.25	1.03	0.572

[a]Remin: Remineralized group

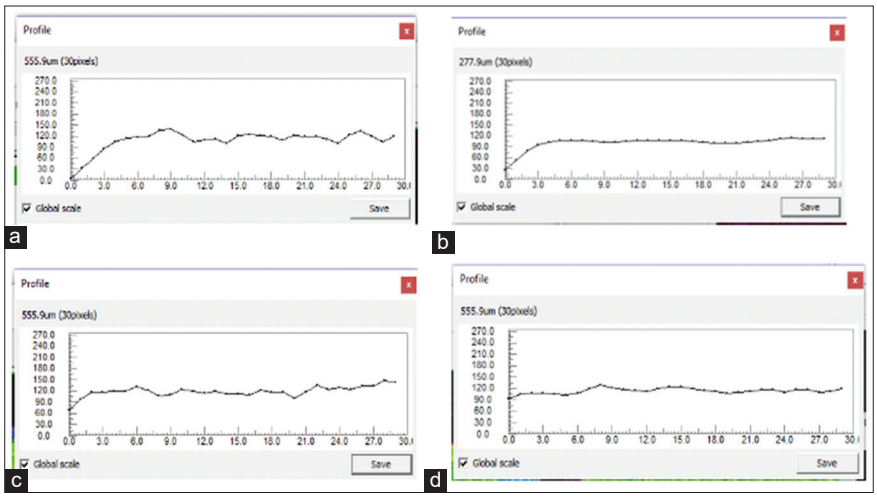

Fig. 2: Graph of the mineral content shown by the results of micro-CT analysis marked by the grayscale index. (a) Demineralized dentin group, (b) 3-day remineralization group, (c) 7-day remineralization group, (d) 14-day remineralization group. The mineral content is indicated by the grayscale index

As shown in Fig. 2, there is a significant difference in changes in the depth of lesions between the groups, which indicates that remineralization has occurred in the demineralized dentin after immersion in remineralization solutions containing polyaspartic acid on days 3, 7, and 14.

According to Gower (2008), ACP can diffuse into liquid collagen through capillary action, [10] whereas Nudelman *et al*. (2010) stated that the infiltration of ACP into collagen is aided by the interaction of the charge between the complex of minerals and certain areas of collagen fibrils. This interaction occurs between the negatively charged complexes of minerals and the positively charged C-telopeptide region in the gap zone [16].

Polymers have 16 times higher ability to bind calcium than that in the absence of polymers [17]. The fluidic nature of liquid ACP makes it easier to fill the intrafibrillar space [18]. Polymers can also produce structures similar to the natural structure of dentin [19].

Polyaspartic acid plays a role in inhibiting the nucleation of apatite in mineralized solutions and stabilizing the formation of the liquid-like precursor ACP.

According to Gower (2008), the liquid-like precursor ACP infiltrates into collagen fibrils through capillary action and later turns into apatite crystals. Polyaspartic acid contains a carboxylic group that plays a key role in crystal growth and directs the synthesis of calcium phosphate [19].

Polyaspartic acid is a negatively charged polyamine acid. It is a polyanionic electrolyte with a carboxylic group that is bound to amino acids so that it can significantly bind calcium ions. These polymers have an essential role in the mineral precursor phase. According to Krogstad (2015), remineralization of PILP is caused due to the presence of ionic bonds in the polymer. The interaction of polymers with minerals produces interactions between the complex of negatively charged minerals and the positively charged C-terminus end in the gap zone, followed by the infiltration of ACP into fibrils [11]. ACP is the initial solid phase of remineralization that can turn into octacalcium phosphate or hydroxyapatite minerals [20].

In the present study, the examination of the depth of artificial carious lesions on day 3 after immersion in the remineralization solutions containing polyaspartic acid revealed that the depth of the lesion was reduced compared to the depth of the lesion in the demineralized dentin group. In a previous study conducted by Krogstad (2017), aggregate nanodroplets measuring 4–5 nm in size were formed in 30 min and turned into nanorod within 24 h [11].

Nudelman *et al*. (2010) stated that within 24 h mineral precursors can enter into the gap zone and diffuse along the collagen fibrils. Furthermore, within 72 h, ACP can turn into apatite crystals along the collagen fibrils in the tendon [10].

However, Burwell *et al*. (2012) reported that intrafibrillar remineralization had begun within 7 days, but it was not perfect, and complete intrafibrillar remineralization of collagen fibrils had occurred at 14 days [8,16].

In this study, after immersion in remineralization solutions containing polyaspartic acid on the 7th day, there was a reduction in the depth of the artificial carious lesion. This indicates that an increasing amount of minerals enter the base layer of the lesion. Calcium and phosphate ions assemble themselves into stable particles known as prenucleation clusters. In the presence of polyaspartic acid polymers as non-collagen protein analogs, these prenucleation clusters condense into fluidic ACP precursors (with a size of 10–30 nm) that are capable of diffusing into the type 1 collagen intrafibrillar space; polyaspartic acid polymers that stabilize these ACP precursors are negatively charged and interact with

the positively charged side of the collagen molecule and then later induce solidification and nucleation of ACP in the collagen [17]. The depth of the artificial carious lesions on day 14 was noticeably reduced compared to that in the remineralization group at 3 and 7 days.

In this study, after 3, 7, and 14 days of immersion in the remineralization solutions containing polyaspartic acid, the micro-CT images showed that the lesion depth was reduced, marked by an increase in the grayscale index, in the demineralized dentin group. Furthermore, the micro-CT images demonstrated the recovery of minerals starting from the deepest part of the lesion and producing complete remineralization after 14 days.

This was indicated by an increase in the grayscale index in the base layer of the artificial carious lesions on the micro-CT images, based on which it can be stated that the process of remineralization occurs in a bottom-up direction [8].

For clinical applications, it is being developed through incorporating polymeric acid as analog non-collagenous acidic proteins and contributes setting reaction in bioactive ceramic material to obtain biomimetic and bioactive dental restorative for *in situ* remineralization [21].

A limitation of this study is that the strength of remineralization is not yet clear. Further research is needed in this regard.

CONCLUSION

This study examined the remineralization of intrafibrillar dentin collagen using PILP process containing polyaspartic acid on days 3, 7, and 14 in comparison with demineralized dentin using micro-CT images. Remineralization was characterized by a reduction in the depth of artificial carious lesions as analyzed by an increase in the grayscale index on the micro-CT images on day 3. On days 7 and 14, remineralization continued to occur and was characterized by a reduced lesion depth and an increased grayscale index.

AUTHORS' CONTRIBUTORS

All the authors have contributed equally.

CONFLICTS OF INTEREST

Declared none.

FUNDING

Nill.

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