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Research Article

EFFECTS OF POLYASPARTIC ACID ON REMINERALIZATION OF INTRAFIBRILLAR DENTIN AND SIZE OF HYDROXYAPATITE CRYSTALS

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ABSTRACT

Objective: The objective of this study was to analyze the intrafibrillar remineralization process and the size of hydroxyapatite crystals formed.

Methods: The samples were divided into four groups. The control group was immersed in a demineralization solution, whereas the remaining three treatment groups were immersed in a remineralization solution containing polyaspartic acid for 3, 7, or 14 days. The effect of polyaspartic acid on intrafibrillar remineralization during the polymer-induced liquid-precursor (PILP) process was evaluated using transmission electron microscopy, and the size of hydroxyapatite crystals was examined through X-ray diffraction.

Results: Significant differences were detected in the intrafibrillar remineralization between the treatment groups (subjected to remineralization for 3, 7, and 14 days) and the demineralized dentin control group. However, no significant differences were detected in the size of hydroxyapatite crystals between the control and the treatment groups.

Conclusion: Our data suggest that polyaspartic acid enables intrafibrillar remineralization during the PILP process.

Keywords: Dentin remineralization, Polyaspartic acid, Non-collagenous protein.

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INTRODUCTION

Dentin caries is classified into infected dentin and affected dentin [1,2]. Infected dentin is the outermost layer of caries consisting of a mineral matrix and hyrdroxyapatite crystals which are destroyed by a bacterial infection and its byproducts. The damage is irreversible and inhibits remineralization. By contrast, affected dentine is the inner layer that is partially demineralized, but the damage is still reversible. The affected dentine zone can undergo demineralization as it contains healthy collagen fibrils surrounded by hydroxyapatite crystals and collagen crosslinks [2,3]. Dentin remineralization can occur due to the deposition of minerals between collagen fibers [4].

Remineralization can occur through two methods: Conventional remineralization and guided-tissue remineralization (GTR). Conventional remineralization occurs through epitaxial growth of mineral crystals present in demineralized lesions [5], whereas GTR is a method of extrafibrillar and intrafibrillar collagen dentin mineralization in calcium phosphate phase [6]. Non-collagen proteins play a very important role in stabilizing the amorphous calcium phosphate (ACP) formation which does not form aggregates and stays in nano [4,7]. Dentin matrix protein 1 (DMP1), a non-collagen protein with a high affinity for calcium ions, is actively involved in regulating mineral formation by interacting with collagen fibrils through electrostatic bonds and plays a key role in mineral formation in the intrafibrillar and extrafibrillar spaces [8]. However, DMP1 can be damaged by caries, and an analogous material is needed to replace the damaged DMP1. Analogous materials for remineralization must contain multiple phosphate and carboxyl groups that can bind to calcium [7]. Several such analogous materials are known to be able to mimic the function of DMP1 non-collagen protein [4].

Recently, polymer-induced liquid-precursor (PILP) has been developed as a process involved in GTR [2,9,10]. This process is known as a rapid biomimetic remineralization process and uses synthetic anionic polymer materials that can replace the role of non-collagen proteins in intrafibrillar remineralization process [11,12]. These polymers play an important role in the phase of liquid mineral precursors. Fluids easily fill the gaps in collagen fibrils, with the remineralization of collagen fibrils, and the resulting nanodroplets (15–30 nm diameter) diffusing into type 1 collagen intrafibrillar or gap zone [2,13]. Polyaspartic acid, a non-collagen protein analog necessary for the PILP process, is a negatively charged amino acid synthesized using non-toxic and biodegradable materials [4,14].

Remineralization of intrafibrillar collagen can be visualized using transmission electron microscopy (TEM). A TEM is an electron microscope that works by passing electrons through the object of observation and obtains information in the form of images [7,15]. In addition, the average size and crystallinity of a sample can be examined using the X-ray diffraction (XRD) technique [5].

METHODS

The research protocol used in this study (Protocol No. 0512111218) was approved by the Ethics Committee of Faculty of Dentistry, Universitas Indonesia. After extraction, the teeth were immediately immersed in deionized water at a temperature of 40°C. The samples were divided into four groups. Group I (control) represented demineralized dentin (without remineralization), whereas Groups II, III, and IV (treatment groups) were subjected to remineralization for 3, 7, and 14 days, respectively.

In the control group, the dentin block was soaked in demineralization solution (40 ml of 0.05 M acetate buffer containing 2.2 mM calcium phosphate [pH 5]) for 66 h. In the treatment groups, 40 ml of remineralization solution containing 50 mM of Tris buffer, 0.9% NaCl, 0.02% NaN_3, 4.5 mM CaCl2, and 2.1 mM K_2HPO_4 was used. Then, 4 mg of polyaspartic acid powder (molecular weight = 23 kDa, 100 µg/ml;