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Hierarchical structure and mechanical properties of remineralized dentin

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ABSTRACT

It is widely accepted that the mechanical properties of dentin are significantly determined by its hierarchical structure. The current correlation between the mechanical properties and the hierarchical structure was mainly established by studying altered forms of dentin, which limits the potential outcome of the research. In this study, dentins with three different hierarchical structures were obtained via two different remineralization procedures and at different remineralization stages: (1) a dentin structure with amorphous minerals incorporated into the collagen fibrils, (2) a dentin with crystallized nanominerals incorporated into the collagen fibrils, and (3) a dentin with an out-of-order mineral layer filling the collagen fibrils matrix. Nanoindentation tests were performed to investigate the mechanical behavior of the remineralized dentin slides. The results showed that the incorporation of the crystallized nanominerals into the acid-etched demineralized organic fibrils resulted in a remarkable improvement of the mechanical properties of the dentin. In contrast, for the other two structures, i.e. the amorphous minerals inside the collagen fibrils and the out-of-order mineral layer within the collagen fibrils matrix, the excellent mechanical properties of dentin could not be restored.

1. Introduction

Dentin, the most abundant mineralized tissue in human teeth, exhibits superior biomechanical properties. It protects the enamel, the extremely brittle outer layer of the teeth, and inhibits the propagation of cracks into the deeper dentin layers by absorbing the compressive, shear, and tensile forces applied to the teeth (Goldberg et al., 2011; Imbeni et al., 2005; Marshall, 1993). The basic building block of dentin is a mineralized collagen fibril in which the carbonated hydroxyapatite platelets embedded within the interstices of the fibril are roughly aligned parallel to the long axis. This specific structure endows dentin with a remarkable hardness and an optimum modulus (Olszta et al., 2007; Weiner and Wagner, 1998). The correlation between the mechanical properties and the hierarchical structure of dentin has been widely investigated for decades. However, due to the fact that the objects available for investigation are usually confined to altered forms of dentin, there is still a lot of knowledge to be gained.

Conservative dental practice is increasingly focusing on the remineralization of caries lesions by applying solution chemistry, which has always been considered as an ideal and minimally invasive technique to overcome tooth decay (Cate, 2001). Conventionally, a heterogeneous nucleation route is employed to

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initiate the dentin remineralization process, which requires seed crystallites. However, the ultrastructure of dentin, key to its remarkable biomechanical properties, could not be restored by this approach (Koutsoukos and Nancollas, 1981; Vollenweider et al., 2007). In nature, biological systems often take advantage of amorphous minerals as an intermediate phase to eventually achieve an oriented mineralization inside the collagen (Beniash et al., 1997; Mahamid et al., 2008). During this biomineralization, amorphous calcium phosphate (ACP) is temporally stabilized by acidic biomacromolecules (Bar-Yosef Ofir et al., 2004; Blumenthal et al., 1977).

Recently, this strategy has been successfully applied to some extent in dentin remineralization experiments. The presence of casein phosphopeptide-stabilized ACP induced a higher remineralization degree of dentin surfaces (Cao et al., 2013; Oshiro et al., 2007). The mineral content of the demineralized dentin slides was restored by using polyaspartic acid as a polymer-induced liquid precursor agent (Burwell et al., 2012; Thula et al., 2011). Furthermore, the ultrastructure of dentin was demonstrated to be preserved in a phosphate containing fluid with a calcium release system, in which polyacrylic acid (PAA) and polyvinylphosphonic acid (PVPA) were employed as analogues of non-collagenous proteins (NCPs) (Niu et al., 2014; Tay and Pashley, 2008). The biomimetic mineralization strategy is based on two chemical processes: At first, the PAA stabilizes the ACP nanoprecursors, which then precipitate at specific sites on the surface and within the collagen fibrils with the PVPA playing a templating role (Kim et al., 2010). These studies reported the regeneration of the mineral density and dentin ultrastructure. However, so far, the effect of the remineralization process on the mechanical properties of the dentin has not been studied in detail.

Recently, we demonstrated that dentin remineralization in the presence of the additive PAA is a step-by-step process, during which ACP is initially formed in the collagen matrix, is then transformed into hydroxyapatite and proceeds towards the surface (Wang et al., 2013). Based on these previous results, in this study, dentin remineralization was performed in the presence of two different remineralization media, i.e. a supersaturated tris-buffered calcium phosphate solution with and without 500 μg/ml of the additive PAA, respectively. Dentin with three different hierarchical structures was obtained after exposure to the two media and at different stages. Nanoindentation experiments were performed to determine the mechanical behavior of the remineralized dentin. Furthermore, we compared the hierarchical structures and the mechanical properties of these remineralized dentin slides. In the presence of PAA, the incorporation of nanominerals into the acid-etched demineralized organic fibrils was achieved, resulting in a remarkable improvement of the mechanical properties of dentin. On the other hand, in the absence of PAA, the restoration of the excellent mechanical properties of dentin could not be achieved due to the formation of a mineral layer that was out-of-order.

2. Materials and methods

2.1. Preparation of the remineralization media

$CaCl_2$, Na_2HPO_4 and PAA (average M_w=1800) were purchased from Sigma-Aldrich (USA). The stock solutions of $CaCl_2$ (0.25 M), Na_2HPO_4 (0.15 M), and PAA (10 g/l) were prepared using triply-distilled water. All solutions were filtered using 0.22 μm Millipore membranes (USA) prior to use. The two different remineralization media were prepared by titrating a 30 ml Na_2HPO_4 solution (12 mM) into equal volumes of the $CaCl_2$ solution (20 mM) for 30 min under constant stirring. The phosphate-containing solution was prepared using the Na_2HPO_4 stock solution. The latter was prepared by dissolving a calculated amount of NaCl (Aladdin, China) and Tris buffer (AMRESCO, USA) in an aqueous solution of $CaCl_2$ (20 mM) diluted from the $CaCl_2$ stock solution. The required volume of PAA (0 or 500 μg/ml) was added to the $CaCl_2$ solution prior to titration. To ensure that the pH value and the ionic strength of the remineralization solution were similar to the corresponding values of physiological fluids, the pH was adjusted to 7.4±0.1 by using a 1 M NaOH solution. Finally, sodium azide (0.02% (w/v)) was added to the remineralization solutions to prevent bacterial growth. The remineralization solutions had the following final concentrations: 10.0 mM calcium, 6.0 mM phosphorus, 60 mM Tris buffer, 200 mM sodium chloride, 0.02% (w/v) sodium azide, and either 0 or 500 μg/ml PAA. The tris-buffered solution (TBS) for the control experiments was prepared simultaneously and following the procedure described above for the preparation of the remineralization solution except that the TBS did not contain any PAA, Calcium (Ca) and Phosphate (P).

2.2. Dentin remineralization

Twenty recently extracted non-carious human third molars, collected with the patients' consent, were obtained under a protocol reviewed and approved by the Medical Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. The teeth were stored in a 0.1% thymol solution for one month prior to the experiments. Dentin slides with dimensions of approx. 10.0 mm × 8.0 mm × 1.5 mm (length × width × thickness) were prepared by making parallel cuts perpendicular to the longitudinal axis of each tooth using a slow speed diamond saw (Isomet 1000, USA) under water cooling. The surface of each dentin slides was then polished with 600- and 2000-grit silicon carbide paper under running water. A groove was generated on each of the dentin slides in the halving line using a dental turbine equipped with a diamond bur. Out of these samples, sixteen slides were demineralized, i.e. etched with 37% phosphoric acid (Aladdin, China) at room temperature for 10 s, to create a mineral-free collagen matrix layer of 3 μm thickness on the dentin surface. Eight demineralized slides were then cultivated in the remineralization solution in the presence of PAA. The specimens were randomly divided into two groups (n=4), which were extracted from the media after 7 (PAA-7) and 30 days (PAA-30), respectively. Another four demineralized slides were cultivated in the remineralization solution not containing PAA for 30 days (no-PAA). Both of the two cultivating glass vials were capped to prevent evaporation of the solution and incubated in a themostated water bath (37 °C) to simulate physiological conditions. During the remineralization process, the pH value was monitored daily and kept constant at 7.4±0.1 by adding a certain amount of a 1 M NaOH solution, if necessary. For the control experiments, the other eight dentin disks were divided into two groups (n=4). The non-