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Early dentine remineralisation: Morpho-mechanical assessment

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ABSTRACT

Objectives: The purpose of this study was to evaluate some physical–mechanical and morphological changes of demineralised dentine at early stages of dentine remineralisation.

Methods: Extracted human third molars were sectioned to obtain dentine discs. After polishing the dentine surfaces, three groups were established: (1) untreated dentine – UD, (2) 37% phosphoric acid application for 15 s (partially demineralised dentine – PDD) and (3) 10% phosphoric acid for 12 h, at 25 °C (totally demineralised dentine – TDD). Five different remineralizing fluids were used for 30 min: chlorhexidine (CHX), artificial saliva (AS), phosphated solution (PS), $ZnCl_2$ and ZnO solutions. Atomic force microscopy (AFM) imaging/nano-indentation, surface nano-roughness and fibrils diameter were determined. X-ray diffraction (XRD), energy dispersive elemental analyses (EDX) and high resolution scanning electron microscopy analysis (HRSEM) were applied.

Results: PDD and TDD preserved some mineral contents. After demineralisation and immersion in all solutions, width of nanomechanical properties and fibrils was increased, and total nanoroughness was decreased. Peritubular and intertubular dentine were remineralised.

Conclusion: Mineral exists in PA-demineralised dentine matrix and it is important since it may work as a constant site for further nucleation. The dentine surface remineralisation process may be stimulated as early as 30 min in abiotic conditions, with a pH ranging from 7.0 to 7.5.

Clinical significance: The existence of enzymes and remineralising factors within the dentine matrix may facilitate early dentine remineralisation under favourable conditions. This process should be stimulated by new reparative materials.

1. Introduction

Demineralisation of dentine is the process of removing mineral ions from the apatite latticework leaving the collagen fibres without support except for the water contained within the dentine, decreasing the mechanical properties of the affected tissue.[1] Remineralisation of dentine refers to the proceeding of restoring the inorganic matrix.[2] This remineralisation process physiologically occurs onto demineralised dental surfaces, where the mineral is reabsorbed and damaged crystals are rebuilt.[3] Thus, nanometer sized hydroxyapatite (HA) develops and grows within nucleation sites[1,4] and a primary template of collagen fibrils to re-establish part of the mechanical properties of the tissue and protect them from hydrolytic and enzymatic degradation.[5] Nucleation may

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be enhanced by the presence of interfaces which allow aggregation and densification of the liquid-like prenucleation clusters.[6]

Biomimetic mineralisation imitates the natural process of mineralisation, simulating the natural formation process of mineral crystals on the surface of organic and inorganic matrix without using harsh conditions.[2] It has been shown that dentine, even in the absence of cells, is able to actively participate in tissue reparative processes. It contains matrix-bonded bioactive molecules, enzymes and growth factors that may be liberated and activated through different mechanisms in order to complete reparative processes.[7] Different dentine remineralizing agents have been proposed. Chlorexidine in solution produces digluconate anions which may result in gradual precipitation in the presence of other mono- and divalent cations being in the substrate.[8] Similarly, zinc may not only act as a MMPs inhibitor, but also influencing signalling pathways and stimulating a metabolic effect in hard tissue mineralisation[9] and remineralisation processes.[10] Zinc has also been shown to inhibit dentine demineralisation[11] and somehow facilitates enamel remineralisation.[10]

The sites which need a remineralisation strategy in dentine could be root caries, erosion of cervical area, affected dentine and the exposed collagen incompletely infiltrated.[12] The analysis of the limited literature available on dentine remineralisation at 30 min of immersing demineralised collagen in remineralizing solutions indicates that specific studies on the relationship between the dentine micro-nanostructure and the physicochemical properties of this treated substrate, at this time point, should be performed. The combination of mechanical data with microscopy techniques appears to be a valuable tool to be applied in dentine remineralisation studies.[13]

The surface properties of dentine and their influence on remineralisation have insufficiently been investigated.[2] AFM nano-indentation performed in hydrated dentine, where force and indenter displacement are simultaneously recorded and the elastic modulus and hardness are determined from the load displacement curve, is a suitable method for the determination of the visco-elasticity of the demineralised dentine and its effective remineralisation.[14,15] The mechanical properties determination integrates factors such as microstructure, mineral density and location of mineral within the organic matrix.[16] The mineral phase in collagenous hard tissues such as bone and dentine is classified as intrafibrillar apatites, which are deposited within or immediately adjacent to gap zones of the collagen molecules and extend along the microfibrillar spaces within the fibril; and extrafibrillar apatites, which are located within the intersticial spaces separating the collagen fibrils. Previous studies have shown that intrafibrillar apatites play a significant role in the mechanical properties of mineralised tissues.[13,14]

Therefore, the aim of this study was to investigate some physical, chemical, mechanical and morphologic changes of demineralised dentine specimens after 30 min of immersion in different remineralizing solutions. The null hypothesis that was established is that nanomechanical properties, nanoroughness and morphological characteristics did not differ after immersing partially demineralised dentine samples in some remineralizing solutions.

2. Materials and methods

2.1. *Specimens preparation*

Twenty-four extracted non-carious human third molars were obtained from young patients (20–26 years old) with informed consent from donors, under a protocol approved by the Institution review board. The teeth were stored in 0.1% (w/v) thymol solution at 4 °C and used within one week after extraction. Teeth were sectioned at the cementum–enamel junction to remove the roots, after organic debris/calculus extraction. Mineralised dentine discs (0.75 mm $\pm$ 0.08 mm thick and 6.0 $\pm$ 0.01 mm diameter) were obtained from the mid-coronal portion of each tooth using a diamond saw under water cooling. Dentine discs were polished through SiC abrasive papers from 800 up to 4000 grits (Struers LaboPol-4) followed by final polishing steps performed using diamond pastes (Buehler-MetaDi, Buehler Ltd., Lake Bluff, IL, USA) through 1 μm down to 0.25 μm.

Eighteen specimens were partially demineralised with 37% phosphoric acid for 15 s, at 25 °C (partially demineralised dentine-PDD) following the method that described in Carrilho et al.[17] PDD discs were rinsed in deionised water under constant stirring at 4 °C for 72 h. Dentine discs were dried over anhydrous calcium sulphate (8 h). Specimens were rehydrated in 0.9% NaCl containing 10 U/ml^{-1} of penicillin G and 300 mg/ml of streptomycin for 24 h (pH 7.0).

Fifteen discs were assigned to the following five immersion solutions and placed in Eppendorf tubes: (i) artificial saliva (AS) containing 50 mM Hepes (Applichem, Darmstadt, Germany), 5 mM $CaCl_2$, 0.001 mM $ZnCl_2$, 150 mM NaCl, 100 U/ml^{-1} of penicillin, and 1000 μg/ml^{-1} of streptomycin (pH 7.2); (ii) 40 mM chlorhexidine digluconate in AS (pH 7.4) (CHX); (iii) phosphate solution (PS) containing 50 mM Hepes (Applichem, Darmstadt,Germany), 1.5 mM $CaCl_2$, 0.90 mM KH_2PO_4, 2 ppm sodium fluoride, 100 U/ml^{-1} of penicillin, and 1000 μg/ml^{-1} of streptomycin; (iv) 3.33 mg/ml of zinc chloride was added to the AS (pH 7.0) ($ZnCl_2$); (v) 10 wt% zinc oxide was added to AS (pH 7.0) (ZnO). Specimens were incubated at 37 °C for 30 min. Chemicals used in this study are described in Table 1.

Nine further dentine discs of the following groups were also analyzed to serve as controls: (1) Untreated dentine (UD), (2) PDD, and (3) totally demineralised dentine with 10% phosphoric acid for 12 h at 25 °C (TDD). Discs were rinsed in deionised water for 72 h.[17]

All specimens were polished with 4000-grit SiC abrasive papers and cleaned in an ultrasonic bath (Model QS3, Ultrawave Ltd, Cardiff, UK) containing deionised water [pH 7.4] for 5 min.

2.2. *AFM imaging and nano-indentation*

Three dentine specimens from the untreated dentine (UD) and the totally demineralised dentine (TDD) groups were selected for characterization. Three additional specimens from the partially demineralised dentine (PDD), stored in each of the