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Remineralization potential of fully demineralized dentin infiltrated with silica and hydroxyapatite nanoparticles

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ABSTRACT

Objective. This study investigates the potential of a novel guided tissue regeneration strategy, using fully demineralized dentin infiltrated with silica and hydroxyapatite (HA) nanoparticles (NPs), to remineralize dentin collagen that is completely devoid of native hydroxyapatite.

Methods. Dentin blocks were fully demineralized with 4 N formic acid and subsequently infiltrated with silica and HA NPs. The remineralizing potential of infiltrated dentin was assessed following a twelve week exposure to an artificial saliva solution by means of TEM, EDS and micro-CT. Measurements were taken at baseline and repeated at regular intervals for the duration of the study to quantify the P and Ca levels, the mineral volume percentage and mineral separation of the infiltrated dentin specimens compared to sound dentin and non-infiltrated controls.

Results. Infiltration of demineralized dentin with nano-HA restored up to 55% of the P and Ca levels at baseline. A local increase in the concentration of calcium phosphate compounds over a period of twelve weeks resulted in a higher concentration in P and Ca levels within the infiltrated specimens when compared to the non-infiltrated controls. Remineralization of demineralized dentin with silica NPs by immersion in artificial saliva was the most effective strategy, restoring 20% of the P levels of sound dentin. Micro-CT data showed a 16% recovery of the mineral volume in dentin infiltrated with silica NPs and a significant decrease in the mineral separation to levels comparable to sound dentin.

Significance. Demineralized dentin infiltrated with silica NPs appears to encourage heterogeneous mineralization of the dentin collagen matrix following exposure to an artificial saliva solution.

1. Introduction

Remineralization of partially demineralized dentin affected by caries or dietary/gastric acid is the process of restoring minerals to the hydroxyapatite's latticework structure. While bacterial acids dissolve the inorganic structure of caries affected dentin, the collagen network in early-stage caries and in parts of extended carious lesions remains unaffected enabling the development of future caries treatments that remineralize the dentin [1]. Different dentin remineralization strategies have been reported, most of which seek to

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restore partially demineralized dentin, focusing on the use of bioactive glass [2,3], fluoride-releasing materials [4,5], casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complexes [6–8], artificial saliva solutions [9], calcium hydroxide [10] and Portland cement [11]. Today, the science of engineered nanoparticles (NPs) offers alternative strategies for the remineralization of acid-affected enamel and dentin, either as a direct replacement of lost minerals or as a carrier for ions (Ca, P and F) that are released following particle dissolution. Nano-sized calcium fluoride (n-CaF_2) [12], nano-particulate hydroxyapatite (nHA) [13,14], nano-sized carbonated apatite (n-CAP) [15], carbonate-hydroxyapatite nano-crystals (nCHA) [16], nano-particulate bioactive glass [3] and nano-precursors of amorphous calcium phosphates [17] are among those nano-materials that have been reported to increase the mineral content of enamel and/or dentin. Notwithstanding, there is conflicting evidence on the efficacy of these various nano-particulate materials used as remineralizing agents for artificial carious lesions, that is most probably related to different methodologies; dentin models, demineralization techniques, type of remineralizing agents used, time of application, demineralization and remineralization cycles together with the inherent difficulty associated with analyzing the results, especially when these are of a modest order. A challenge remains in achieving effective structural and mechanical reconstitution of the remineralized dentin, which may be attributed to an imperfect arrangement of the newly deposited mineral within the demineralized tooth matrix [3].

A further approach using nano-particulate technology for the remineralization of partially demineralized dentin is the development of a guided tissue remineralization strategy as reported by Tay and Pashley [11,17], where they achieved intrafibrillar and interfibrillar remineralization of a 5 μm thick layer of demineralized dentin. This concept is based on the formation of an amorphous calcium phosphate phase within the collagen fibrils of demineralized dentin from first the reaction of Portland cement with water and subsequently with a phosphate containing fluid to create apatite nanocrystals and subsequently larger crystals.

In this investigation we present and assess a novel guided tissue regeneration strategy to remineralize dentin that is fully demineralized and thus completely devoid of native hydroxyapatite. Nucleating NPs embedded within the remaining collagen matrix may encourage heterogeneous mineralization of the dentin collagen matrix following immersion in an artificial saliva solution. The hypothesis being, that NPs in the inter- and intra-fibrillar collagen spaces will reduce the energy barrier required for the formation of a cluster of inorganic ions; further aided by immersion in an artificial saliva solution. We have created, and verified using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS), nano-particulate scaffolds within the collagen matrix of demineralized dentin using nHA (primary particle size < 5 nm) and 12 nm colloidal silica (SiO_2) NPs [18]. The aim of this study is to assess the effectiveness of this strategy as a mechanism for the remineralization of fully demineralized dentin following immersion in artificial saliva.

2. Methods and materials

2.1. Specimen preparation

Blocks of human dentin ($n = 117$) of an oblong shape were obtained from the crowns of intact premolar teeth that had been extracted for orthodontic purposes. Ethical approval for the use of extracted human teeth was obtained in accordance with guidelines from the University of Sheffield. A low-speed precision blade saw (VC-50, Leco, MI, USA) with a diamond wafering blade (Buehler, Dusseldorf, Germany) was used to section the specimens (5 mm × 1 mm × 1 mm). The blocks were sonicated for 10 min to remove cutting debris and then fixed overnight at 4 °C with 3% glutaraldehyde in 0.1 M cacodylate buffer. Fixed samples were rinsed (3× 3 min) with 0.1 M cacodylate buffer to remove glutaraldehyde. Specimens were subsequently sub-divided into different experimental groups as per Table 1. Nine of the samples remained stored in cacodylate buffer at 4 °C and assigned as 'sound control' samples. The remaining samples ($n = 108$) were fully demineralized by storing specimens in 15 ml glass vials containing 4 N formic acid for 48 h. Preliminary experiments were conducted to identify formic acid as the optimal acid to employ and also the time required to achieve complete demineralization of the dentin samples without damaging the non-mineral components of dentin. Other demineralizing agents considered (e.g. phosphoric acid, EDTA) were found to be either too aggressive or too mild (data not shown). After the demineralization process was complete, demineralized dentin specimens were rinsed with distilled water (3× 3 min) to remove the acid; in accordance with a previously reported protocol [18]. The demineralized dentin specimens were then equally and randomly sub-divided in four groups ($n = 27$). The specimens of the first group were assigned as 'non-infiltrated controls', whereas the specimens in the remaining three groups were infiltrated with nano-particulate solutions as per Table 1.

The three groups of 'infiltrated specimens' were stored separately in glass vials containing 15 ml of the respective infiltration solution at room temperature (25 °C). Specimens were fully immersed and the containers were kept under continuous low speed rotation (4 RPM) to avoid possible particle precipitation. Following a 24 h infiltration process, the demineralized dentin specimens infiltrated with NPs (dd-NPs) were removed from the solutions and blot dried. Three specimens from each infiltrated sample group and from the non-infiltrated controls were prepared to be examined by TEM. An equal number of specimens were also prepared for EDS ($n = 12$) and micro-CT ($n = 12$) analysis to determine baseline reference values in triplicate. The remaining dd-NPs specimens and non-infiltrated controls were transferred to new 50 ml polystyrene universal containers (one container per group) containing the artificial saliva solution (Table 3). The artificial saliva was not renewed during the study period to allow monitoring of the remineralization potential in a close system and also to avoid accidentally rinsing the initial infiltration material. pH measurements were taken at regular intervals to ensure that the pH remained stable.