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# The use of acetone to enhance the infiltration of HA nanoparticles into a demineralized dentin collagen matrix

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ABSTRACT

*Objectives.* This study investigates the role of acetone, as a carrier for nano-hydroxyapatite (nano-HA) in solution, to enhance the infiltration of fully demineralized dentin with HA nanoparticles (NPs).

*Methods.* Dentin specimens were fully demineralized and subsequently infiltrated with two types of water-based nano-HA solutions (one containing acetone and one without). Characterization of the dentin surfaces and nano-HA particles was performed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The surface wettability and infiltration capacity of the nano-HA solutions were quantified by means of contact angle measurements and energy dispersive X-ray spectroscopy (EDS), respectively. Contact angle measurements were taken at baseline and repeated at regular intervals to assess the effect of acetone. The P and Ca levels of infiltrated dentin specimens were measured and compared to sound dentin and non-infiltrated controls.

*Results.* The presence of acetone resulted in an eight-fold decrease in the contact angles of the nano-HA solutions recorded on the surface of demineralized dentin compared to nano-HA solutions without acetone (one-way ANOVA, $p < 0.05$). Perfect wetting of the demineralized dentin surface was achieved 5 min after the application of the nano-HA solution containing acetone. Infiltration of demineralized dentin with the nano-HA solution containing acetone restored the lost mineral content by 50%, whereas the mean mineralization values for P and Ca in dentin treated with the acetone-free nano-HA solution were less than 6%.

*Significance.* Acetone was shown to act as a vehicle to enhance the capacity to infiltrate demineralized dentin with HA NPs. The successful infiltration of dentin collagen with HA NPs provides a suitable scaffold, whereby the infiltrated HA NPs have the potential to act as seeds that may initiate heterogenous mineral growth when exposed to an appropriate mineral-rich environment.



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## 1. Introduction

A number of different dentin remineralization strategies have been reported, most of which involve the use of bioactive glass [1,2], fluoride-releasing materials [3,4], casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complexes [5,6], artificial saliva solutions [7], calcium hydroxide [8] and Portland cement [9,10]. The use of engineered nanoparticles (ENPs) has also been examined as an alternative strategy for the management of dental caries and has become the focus of much research in this field. Nano-sized calcium fluoride (n-$CaF_2$) [11], nano-hydroxyapatite (nano-HA) [12,13], nano-sized carbonated apatite (n-CAP) [14], carbonate-hydroxyapatite nano-crystals (CHA) [15], nano-particulate bioactive glass [1] and silica nanoparticles (NPs) [16] are among those ENPs that have demonstrated an increase in the mineral content of enamel and/or dentin. However, a significant challenge when using these materials is to achieve an effective and deep infiltration of the demineralized dentin collagen matrix with the ENPs, while avoiding precipitation of the particles on the surface.

In adhesive restorative dentistry, the infiltration of partly demineralized dentin with resin-based hydrophilic dentin adhesives is also a significant problem. The dentin surface to be infiltrated is kept moist to prevent the surface collagen network from collapsing and thus creating a pathway for the hydrophilic monomers to penetrate. The deep infiltration of the resin monomers into the intra-fibrillar spaces of the collagen matrix network is further aided by the inclusion of volatile solvents in the adhesive that displace the water from the dentin surface [17,18]. Acetone is a clear, colorless, low-boiling, flammable and volatile liquid characterized by rapid evaporation and a faintly aromatic, sweetish odor. It readily mixes with most organic solvents and mixes completely with water. It is naturally produced and disposed of in the human body as a result of normal metabolic processes and is considered a safe ingredient that is commonly found in a range of commonly used products ranging from cosmetics to processed and unprocessed foods; it has been rated as Generally Recognised As Safe (GRAS) substance when present in beverages, baked foods, desserts, and preserves at concentrations ranging from 5 to 8 $mg\,l^{-1}$ [19]. The successful application of this strategy in dentin bonding systems forms the basis for our hypothesis that HA NPs suspended in a water–acetone solution should work in a similar manner and aid a higher concentration and deeper infiltration of the particulates into the collagen network of the demineralized dentin.

Previous work by this group has reported a strategy to increase the mineral content of dentin that has been fully demineralized by acid [16,20]. We have investigated the potential of infiltrating and embedding NPs in the collagen matrix and thus create nucleation seeds for subsequent mineral growth by means of heterogenous deposition of interfibrillar calcium phosphate minerals. The aim of this present study is to investigate the role of acetone, which has been used successfully in dentin bonding systems, to increase the infiltration potential of nano-HA solutions into the demineralized dentin. The hypothesis is that acetone will act as a carrier, enhancing the depth of penetration of HA NPs into the demineralized dentin matrix.

## 2. Methods and materials

### 2.1. Experimental design and specimen preparation

The experimental design involved exposing sound and fully demineralized dentin to two types of nano-HA solutions (one containing acetone and one without) and a distilled-deionised water (DW) solution with no added NPs, which served as a control. After application of the test solutions to the dentinal surfaces, surface wettability was quantified by contact angle measurements to aid interpretation of the role of acetone. Demineralized dentin specimens were also infiltrated with the test solutions. Following a 24 h infiltration, the infiltration capacity of the two nano-HA solutions was tested by means of energy dispersive X-ray spectroscopy (EDS) to determine whether the presence of acetone facilitates the penetration of NPs to the collagen matrix.

Dentin specimens were prepared in the form of discs (1 mm thick) and blocks (length × width × height: 5 mm × 1 mm × 1 mm) from the crowns of sound human premolar teeth that had been extracted for orthodontic purposes as part of routine dental care. Ethical approval for the use of the extracted human teeth was obtained. A total of forty dentin specimens were sectioned (18 discs and 22 blocks) using a low-speed precision blade saw (VC-50, Leco, Michigan, USA) equipped with a diamond wafering blade (Buehler, Dusseldorf, Germany). All dentin specimens were sonicated for 10 min to remove cutting debris and then allocated for morphological characterization of the surface by scanning electron microscopy (SEM), contact angle measurements and EDS analysis. A number of sound dentin specimens were stored in DW at 4 °C and the remaining specimens were fully demineralized in formic acid, in accordance with the demineralization protocol reported by Besinis et al. [20]. In brief, specimens were initially fixed overnight at 4 °C with 3% glutaraldehyde in 0.1 M cacodylate buffer and then rinsed (3 × 3 min) with 0.1 M cacodylate buffer to remove glutaraldehyde before rinsing with DW. Specimens were then fully submersed in 4 N formic acid for 48 h. When the demineralization process was complete, specimens were rinsed with DW (3 × 3 min) to remove the acid and subsequently stored in DW at 4 °C. Fixation of dentin was essential to ensure that the collagen substructure would maintain its morphology during the demineralization process and that would resist shrinkage and any other deformational forces due to the dehydration process [21]. Glutaraldehyde stabilizes the collagen fibrils in biological tissues and induces intra- and intermolecular crosslinks, including crosslinking of the extracellular proteins of the dentin collagen matrix [22].

### 2.2. SEM morphological characterization

Sound ($n = 3$) and fully demineralized dentin ($n = 3$) blocks were prepared for morphological surface characterization by SEM. All specimens were dehydrated with ascending ethanol grades and hexamethyldisilazane (HMDS) to prevent tissue