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Infiltration of demineralized dentin with silica and hydroxyapatite nanoparticles

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ABSTRACT

Objectives. The management of demineralized dentin resulting from dental caries or acid erosion remains an oral healthcare clinical challenge. This paper investigates, through a range of studies, the ability of colloidal silica and hydroxyapatite (HA) nanoparticles to infiltrate the collagen structure of demineralized dentin.

Methods. Dentin samples were completely demineralized in 4 N formic acid. The remaining collagen matrix of the dentin samples was subsequently infiltrated with a range of nanoparticulate colloidal silica and HA solutions. The effectiveness and extent of the infiltration was evaluated by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS).

Results. Silica nanoparticles have the ability to penetrate dentin and remain embedded within the collagen matrix. It is suggested that particle size plays a major role in the degree of dentin infiltration, with smaller diameter particles demonstrating a greater infiltrative capacity. The infiltration of demineralized dentin with sol–gel HA nanoparticles was limited but was significantly increased when combined with the deflocculating agent sodium hexametaphosphate. The use of acetone as a transport vehicle is reported to enhance the infiltration capacity of sol–gel HA nanoparticles.

Significance. Collagen infiltrated with HA and silica nanoparticles may provide a suitable scaffold for the remineralization of dentin, whereby the infiltrated particles act as seeds within the collage matrix and given the appropriate remineralizing environment, mineral growth may occur.

1. Introduction

Dentin demineralization results from exposure to acids, from bacterial origin (caries) or dietary/gastric sources (dental erosion). Among the diseases and chronic conditions affecting populations throughout the world, dental caries is among the foremost in prevalence; the consequences of which involve not only physical health but also the economic, social, and psychological well-being of individuals. Dental erosion is a more modern affliction, causing gradual but irreversible loss of tooth structure. It affects primarily the adolescent population worldwide with a reported international incidence of up to 20% of the population and greater national incidences in specific regions [1].

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The management of dental caries involves a range of different strategies, all of which have their inherent limitations: (a) prevention by controlling dietary habits, oral hygiene, plaque control and the use of topical fluoride; dependent on total patient compliance, (b) chemical or mechanical caries removal techniques; that are destructive of tooth tissue, and (c) techniques that enhance remineralization of the mineral content of the tooth; aimed at preserving the demineralized collagen matrix, but at an early stage of development and use.

This paper concerns itself with the latter of these three strategies; the remineralization of dentin. Remineralization of dentin affected by caries or dietary/gastric acid is the process of restoring minerals to the tooth structure. A recent study of caries pathology using synchrotron-based X-ray scattering, confirms that while bacterial acids dissolve the inorganic structure of dentin; the collagen network remains unaffected, enabling the development of future caries treatments that re-mineralize the dentin [2]. Different dentin remineralization strategies have been investigated, most of which focus on the use of bioactive glass [3,4], fluoride-releasing materials [5–7], casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) complexes [8–12], artificial saliva solutions [13], calcium hydroxide [14] and Portland cement [15,16]. An alternative strategy, that is becoming the focus of much research in this field, is the use of nanoparticles for the management of dental caries. Nano-sized calcium fluoride (n-CaF_2) [17], nano-particulate hydroxyapatite [18–20], nano-sized carbonated apatite (n-CAP) [21], carbonate-hydroxyapatite nano-crystals (CHA) [22] and nano-particulate bioactive glass [4] are among those nano-materials that seem to increase the mineral content of enamel and/or dentin. A significant challenge with the use of these materials is to achieve an effective and deep infiltration of the intact demineralized dentin collagen with the particles, while avoiding precipitation on the surface. This paper investigates the novel use of nanoparticles (synthetic calcium hydroxyapatite and colloidal silica) to infiltrate the collagen structure of demineralized dentin as part of a dentin remineralization strategy.

Synthetic calcium hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] is chemically and biologically similar to the mineral component of human bone and teeth. It is one of few materials that are classed as bioactive [23,24], meaning that it will support bone in-growth and osseo-integration without breaking down or dissolving, when used in orthopedic, dental and maxillofacial applications. Dentin is comprised of 70% inorganic materials, 20% organic materials, and 10% water by weight. The inorganic phase of dentin includes trace amounts of calcium carbonate, zinc, fluoride and magnesium but HA is principal inorganic component [25]. Thus, synthetic HA is considered a logical mineral compound to substitute the natural mineral constituent of dentin.

Colloidal silica consists of a stable dispersion of very fine silica particles which are amorphous rather than crystalline. They remain in a colloidal suspension due to their low mass and the electrostatic interactions between the particles and the dispersion medium [26]. The particles are practically insoluble in the dispersing medium which is usually distilled water. The application field of colloidal silica is fairly broad and includes use in the refractory industry, high temperature binders, investment casting, catalysts, abrasion resistant coatings, carbonless paper, increasing friction, as an abrasive, anti-soiling, surfactant or absorbent. Silica is also used in the food industry as a food additive [27] (e.g. anti-caking agent E551). The colloidal nature of the silica solution makes it an attractive mineral for collagen infiltration with the hope that it will penetrate into the demineralized collagen matrix without precipitating on the surface [3,4].

Table 1 – Specifications of the Ludox® colloidal silica solutions.

Specifications	Ludox® CL	Ludox® HS-30
Lot number	2007850480	2006850568
Particle charge	Positive	Negative
Particle size (nm)	12	12
Silica (as SiO_2) (wt%)	30.0[a]	30.1
pH (25 °C)	3.9	9.8
Stabilizing counter ion	Chloride	Sodium

[a] Concentration includes $SiO_2 + Al_2O_3$.

The aim of this study was to assess the ability of HA and silica nano-particulate solutions to infiltrate demineralized dentin, that retains its collagen structural integrity. Our hypothesis is based on the premise that nanoparticles in a colloidal suspension may better infiltrate the demineralized collagen network of dentin and not precipitate on the surface. Thereafter, once the particles [hydroxyapatite (HA) and silica] have infiltrated the demineralized dentin they will remain embedded in the subsurface collagen matrix that acts as a scaffold retaining the particles. Subsequently, given the right environment, the particles may act as a seed for the remineralization of the dentin.

2. Methods and materials

The materials investigated were HA and silica nanoparticles. Two different nano-HA solutions were used; one made by the research group using the sol–gel technique and a commercially available formulation manufactured according to the precipitation technique (sintering temperature: 1100–1200 °C; batch number: P275S, Plasma Biotal Ltd., UK). The second is referred to as ‘control nano-HA’. Two colloidal silica solutions were used; one electronegative (Ludox® HS-30, Grace Davison, UK) and the other electropositive (Ludox® CL, Grace Davison, UK) due to an alumina surface coating (Al_2O_3). Both colloidal silica solutions contain 12 nm particles and are commercially available products characterized by the manufacturer. The specifications for the two colloidal silica solutions are shown in Table 1.

The in-house nano-HA solution was made from a powder that was synthesized using the sol–gel technique, as mainly described by Liu et al. [28]. The final product was characterized with X-ray diffraction (XRD) using a Philips PW1825/00 X-ray diffractometer; the data acquired were analyzed with STOE WinXPOW 2.1 and Traces 4.0 software (Fig. 1). In the powder form the HA nanoparticles have a natural tendency to form agglomerates, which were broken down by ball milling with zirconia balls. The resulting HA powder was mixed with distilled water (DW) and then DW was allowed to evaporate in an electric chamber furnace at 90 °C. HA powder was then