Journal of Dentistry 56 (2017) 112–120

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.intl.elsevierhealth.com/journals/jden

Penetration of sub-micron particles into dentinal tubules using ultrasonic cavitation

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ARTICLE INFO

Article history:
Received 14 October 2016
Received in revised form 14 November 2016
Accepted 19 November 2016

Keywords:
Dental nanomaterials
Sub-micron particles
Tubule occlusion
Ultrasonic scalers
Cavitation
Electron microscopy

ABSTRACT

Objectives: Functionalised silica sub-micron particles are being investigated as a method of delivering antimicrobials and remineralisation agents into dentinal tubules. However, their methods of application are not optimised, resulting in shallow penetration and aggregation. The aim of this study is to investigate the impact of cavitation occurring around ultrasonic scalers for enhancing particle penetration into dentinal tubules.

Methods: Dentine slices were prepared from premolar teeth. Silica sub-micron particles were prepared in water or acetone. Cavitation from an ultrasonic scaler (Satelec P5 Newtron, Acteon, France) was applied to dentine slices immersed inside the sub-micron particle solutions. Samples were imaged with scanning electron microscopy (SEM) to assess tubule occlusion and particle penetration.

Results: Qualitative observations of SEM images showed some tubule occlusion. The particles could penetrate inside the tubules up to 60 μm when there was no cavitation and up to ~180 μm when there was cavitation.

Conclusions: The cavitation bubbles produced from an ultrasonic scaler may be used to deliver sub-micron particles into dentine. This method has the potential to deliver such particles deeper into the dentinal tubules.

Clinical significance: Cavitation from a clinical ultrasonic scaler may enhance penetration of sub-micron particles into dentinal tubules. This can aid in the development of novel methods for delivering therapeutic clinical materials for hypersensitivity relief and treatment of dentinal caries.

1. Introduction

Exposure of dentinal tubules can lead to dentinal caries or dentinal hypersensitivity [1,2]. The causative agent of caries is bacteria which invade the dentinal tubules with a direct path to the pulp of the tooth. The elimination of the bacteria is fundamental in the treatment of caries [2] and this may be achieved by the use of antimicrobials. For treatment of dentinal hypersensitivity, research is directed at reducing the permeability of the dentine, achieved through partially or fully blocking the tubules [3], which may lead to reduced sensitivity and discomfort [4]. The use of tubule blocking agents is a major area of interest within the oral healthcare industry for treating dentinal hypersensitivity [4].

Nanoscale materials have attracted interest as a more efficient method of delivering antimicrobials and remineralisation agents into dentinal tubules [5,6]. Sub-micron particles (SMPs) with diameters between 100 and 1000 nm are considered to be safe and have been shown to cause less inflammation than nanoparticles (NPs) [7]. Both NPs and SMPs have a large surface area to volume ratio, which increases solubility and reactivity. If they are non-agglomerated and dispersed, they can easily enter dentinal tubules as their diameter is smaller than the tubule diameter, which ranges from 2 to 4 μm. The size and reactivity of NPs and SMPs may allow them to be delivered further into dentinal tubules, with an enhanced potential for decontamination, remineralisation and reduced sensitisation compared with contemporary treatment regimens [5]. This may offer advantages such as allowing antimicrobials to reach bacteria deep in the tubules, and also for

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http://dx.doi.org/10.1016/j.jdent.2016.11.006
0300-5712/

increasing the retention time of remineralising and tubule occluding materials.

An effective penetration depth into dentinal tubules remains a key issue for the development of NP and SMP technology. Besinis et al. showed how acetone could be incorporated into nanoparticle solution to act as a carrier to deliver NPs further into dentinal tubules [8]. The potential of the solvent to displace water in the tubules may allow deeper particle penetration. Furthermore, reduced particulate aggregation using surfactants has been reported to improve delivery within tubules, reduce the surface interactions with dentine and increase occlusion of tubules [9].

Cavitation is the generation and collapse of gas or vapour bubbles in a liquid, occurring when the local pressure falls below the saturated vapour pressure, which can occur when an ultrasound field is applied in a liquid [10]. These bubbles can grow to many times their original size and rapidly collapse, releasing high amplitude shock waves and high velocity micro jets [11]. Previous researchers have demonstrated successful penetration of chitosan NPs into dentinal tubules using cavitation [12,13]. However, in these studies the ultrasound was delivered by a large-scale transducer that may not be suitable for a clinical application. An ultrasonic scaler produces cavitation and may provide a more practical method of directing SMPs deeper into dentinal tubules [14,15].

Consequently, the aims of this research are to investigate the impact of cavitation from ultrasonic scalers, combined with acetone, in enhancing SMP penetration into dentinal tubules.

2. Materials and methods

2.1. Dentine specimen preparation & particle application

Intact, caries free, single-rooted human molars and premolars were used in this study. The use of teeth was authorised by a licence from the United Kingdom Human Tissue Act (HTA) (Licencing number: 12313, Clinical Research Network Consortium Reference: BCHCDent355.1548.TB). Teeth were decoronated at the cemento-enamel junction and 2 mm thick longitudinal slices were cut from the external surfaces of the roots using a low speed water cooled saw (Buehler, Isomet, UK). The area of each section was 8 ± 1 mm length, 5 ± 1 mm width. The outer surfaces of the slices were ground using silicon carbide (SiC) paper with grit size P500 until nominally flat, and then polished using P1200 and P4000 grade SiC paper to remove microscale grooves. The tooth slices were then immersed in 10% citric acid (BDH, Poole, England) for 2 min to remove the smear layer and expose the dentinal tubules as described previously [9]. Specimens were subsequently ultrasonicated in an ultrasonic cleaner (In-ceram, Vitasonic) in 200 ml reverse osmosis (RO) water for 10 min prior to imaging using a stereomicroscope (Zeiss PrimoTech, Oberkochen, Germany) (resolution 17 pixels/μm), to determine the direction of the dentinal tubules. Only specimens with dentinal tubules orientated perpendicular to the surface were used in this study to mimic the clinical situation where the tubules on the outer portion of the cervical third of the root become exposed. The specimens were stored in a humidified container prior to testing.

In this study we used silica (SiO_2) sub-micron particles prepared in the same manner as those used by Claire et al. to compare the effect of the cavitation and acetone addition on the particle distribution on the dentine surface. The silica particles were prepared according to a published method [16] containing a luminescent ruthenium complex (tris-(2,2′-bipyridyl)ruthenium (II) dichloride) for introducing a luminescent core for potential luminescence imaging. Dynamic Light Scattering (DLS) was used to estimate the particle size distribution (Zetasizer Nano ZSP, Malvern Instruments, UK). The zeta potential of the particles was also measured (Delsa Nano particle analyser, Beckman Coulter, USA). 1% w/v solutions of sub-micron particles were prepared by adding 0.1 g particles (dry weight) to 10 ml RO water and 10 μl Tween 20 surfactant (Sigma-Aldrich) [9]. The solution was dispersed via ultrasonication for 10 min and then centrifuged at 8000 rpm for 5 min. The supernatant was discarded and the particles were re-suspended in either 10 ml RO water or 5 ml RO water mixed with 5 ml acetone. Solutions were ultrasonicated for 10 min before application to the tooth slices to disperse the particles.

Each particle solution (5 ml) was pipetted into a compartment into 12-well culture plate, and a tooth slice was placed inside (Fig. 1). An ultrasonic scaler (P5 Newtron, Satelec, Acteon, France) with tip 10P was fixed 0.5 mm away from the tooth slice using a translation stage accurate to 10 μm (PT3, Thorlabs, USA) (Fig. 1). The scaler was operated for 20 s at either power 1 (lowest power setting, no cavitation) or power 10 (medium power setting, with cavitation) (n = 6). The same procedure was used for both particle solutions.

2.2. Scanning electron microscopy

Samples were then dried for 1 h in a 60 °C oven and gold-coated (K550X, Quorum Technologies, UK) for SEM (EVO MA10, Carl Zeiss, Germany). After imaging the surface of the samples, they were cryogenically frozen in liquid nitrogen and fractured manually. The fractured specimens were mounted onto SEM stubs using conductive paste (Leit C Plast, Agar Scientific, UK), sputtered with gold and imaged using SEM to determine the depth the particles had travelled into the dentinal tubules. Acceleration voltages of 5–20 kV were used in secondary electron mode for SEM imaging.

A representative dentine slice from SEM measurements was also analysed using focussed ion beam (FIB) SEM (Quanta 3D FEG, FEI, Netherlands). The electron beam was operated at 20 kV with spot size 6–8 and the ion beam was operated at 30 kV. The initial trench was cut using milling parameters of 7 nA at 52°, and subsequent milling was performed at 2 nA, and then, 1 nA at 54°.

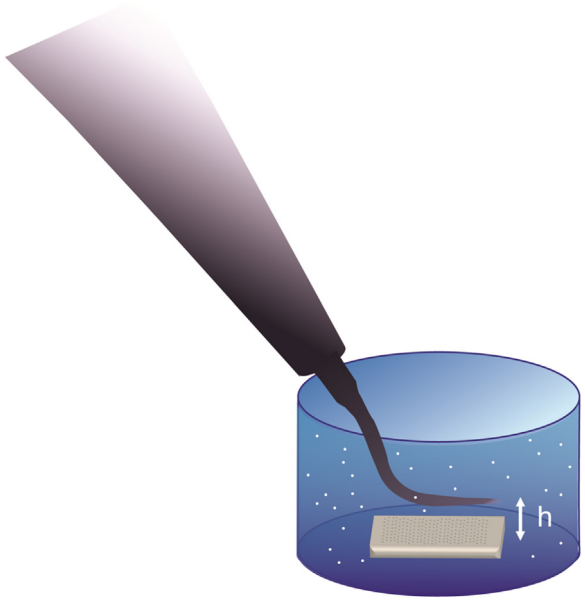

Fig. 1. Schematic of the experimental setup showing the orientation of the ultrasonic scaler. The tip was placed inside a well of a 12 well plate filled with 5 ml particle solution. The dentine slice was placed at the bottom of the well and the scaler was positioned at a fixed height h above the slice (h = 0.5 mm).