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The deposition and imaging of silica sub-micron particles in dentine

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ABSTRACT

Objectives: Sub-micron particles may assist in the delivery of compounds into dentine tubules. The surface interactions of the particles with dentine may prevent them from entering the tubules. The aim of this study is to investigate whether silica particles, treated with surfactants improves dentine tubules occlusion using both artificial and human tooth models

Methods: Spherical silica particles (size 130–810 nm) bearing an encapsulated ruthenium luminescent complex were coated with the following surfactants: Zonyl® FSA, Triton® X-100 and Tween20®. The particles were prepared as 0.004% w/v and 0.04% w/v solutions with deionized water and were applied to the surface of; (1) in vitro model of PET ThinCert™ cell culture inserts; (2) 0.1 mm thick sections of human molar teeth.

Results: Scanning electron and confocal fluorescence microscopy images show that particles without any coating and with TritonX-100 coating had the highest aggregation. Particles with Tween-20 are less aggregated on the surface and show inclusion in the tubules. Particles coated with fluorosurfactant Zonyl show a preference for aggregation at the tubule. With the ThinCert™ membranes high aggregation within the artificial tubules was increased by particle concentration.

Conclusions: The use of silica sub-micron particles on hard dental tissues is dependent on the modification of the surface chemistry of both the particle and the dentine and the employment of the fluorosurfactant may improve tubule occlusion. The use of ThinCerts™ membrane is useful in vitro model to mimic dentinal tubules and observe the ability of particles to occlude small channels.

Clinical significance: The use of silica sub-micron particles on hard dentine tissues is dependent on the modification of the surface coating of the particles. This may influence how particles are incorporated in potential delivery vehicles applied to the dentine surface with the employment of a fluorosurfactant showing promise.

1. Introduction

Dentinal tubules are microscopic channels within the dentinestructure, which traverse from the junction of the protective outer enamel to the internal pulp containing the soft tissues [1]. They are around 1–4 microns in width and formed via the odontoblast cells [2]. There is variation in both arrangement and width of the tubules depending on their position within the tooth [3]. Once the outer enamel structure is breached, exposure of dentine occurs and allows communication through the tubular space to the pulp underneath. This can lead to irreversible damage to the soft tissues of the tooth. Nano- and sub-micron sized particles offer the possibility of a multi-functional dental agent, by both delivering anti-pathogenic or analgesic drugs into the tubules combined with the ability to occlude of the internal space,thus helping to prevent infection or infiltration into the pulp [4–6]. Their size makes them an attractive vehicle for entering into the dentinal tubule and investigators have looked to different types of particles to undertake such a task. These include calcium fluoride [7], combinations of carbonate-hydroxyapatite nanocrystals [8–10] as well as bioactive glass [11,12]. The general aim of these studies is to increase mineral content of enamel and dentine, where the particle acts as a seed for further growth of crystalline structure leading to the closure of the tubules. One approach is that the sub-micron particles may be introduced into a scaffold of collagen and provide a structure for this growth and subsequent mineralisation [13]. Furthermore, antibacterial actives or compounds may be attached to the particles to inhibit or break up bacterial growth.

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A problem with the use of such particles is that they are liable to aggregate [14], thus preventing them from entering a dentinal tubule. Methods of preventing this from occurring include adding surfactants to the particles. However, it is not known how the addition of surfactants influences the movement of particles on the dentine surface and into the tubules. Researchers have used mechanical forces such as cavitation form imploding bubbles to push particles into the dentinal tubules [15]. Observing such activity of the sub-micron particles may be problematical due to the complex surface of dentine and a simple *in vitro* model system may assist in observing how particles behave on a dentine like surface.

We have chosen silica particles for their distinct advantages: availability in sizes ranging for nanometer to submicron without altering the particle surface and their porous structure, which can act as a container for molecules. Most studies involving silica particles in dental applications have concentrated in their property as a composite in biomaterials. We have previously introduced the influence of surfactants of metal complexes attached on the outside of the silica surface [16].

The aim of this research is to investigate the interaction of silica particles with human molar teeth sections, with a particular focus on entering the opening of dentine tubules. We have also used different surfactants as coatings for the particles to address their influence upon the surface interactions of the particle with the dentine surfaces. An alternative *in vitro* models has also been evaluated against dentine slices of human teeth to determine its suitability as a research tool.

2. Materials and methods

2.1. Tooth preparation

Human decay-free lower first permanent molar teeth, which were covered by the United Kingdom Human Tissue Act (HTA), were chosen for the purpose of assessing the entry of sub-micron particles into the tubules. The crown removed and the root sliced in half along the vertical axis using the bone saw. The sectioned roots were ground down using a grinder/polisher with 400/800 papers, to approximately 0.1 mm thick sections measured by a micrometer. This method was used to produce sections with perpendicular tubule openings. Before imaging/nanoparticle application, the sections were etched by a 60 s immersion in a 10% citric acid solution. The sections were then ultrasonicated for 180 s. The sections were dehydrated using successive ethanol baths for ten-minute periods of 50%, 75% then 100% before drying for 1 h in a 60° oven.

2.2. Particle preparation

The particles used in this investigation consisted of spherical silica encapsulated with a luminescent complex, tris-(2,2′-bipyridyl)ruthenium(II) dichloride, represented as SiO_2–Ru [17], with a size of 640 nm were designated as SiO_2–Ru-640 (640 ± 90 nm). The effect of polymer coating did not change the size within the error limit of the particle size analysed. The sizes were determined by Dynamic Light Scattering measurement using a Zetasizer Nano ZS, (Malvern Instruments Ltd., Malvern, UK). Visualization of the particles was performed using Nanosight tracking Analysis instrument (Malvern Instruments Ltd., Malvern, UK). For confocal fluorescence imaging studies, three different sizes were used (particle concentration 0.004% w/v). SiO_2–Ru-130 (130 ± 50 nm), SiO_2–Ru-430 (430 ± 50 nm), SiO_2–Ru-810 (810 ± 160 nm) estimated by the confocal reflectance images. They were prepared as 0.004% w/v solutions by addition of 50 ml deionised water to 2 mg particles (dry weight), followed by ultrasonication for 5 min. For surfactant coated nanoparticle preparations Zonyl® FSA, Triton® X-100 or Tween20® (purchased by Sigma–Aldrich) were added to 0.04% w/v solutions of the SiO_2–Ru-640. The 0.04% w/v solution required addition of 10 ml deionized water to 4 mg particles (dry weight), followed by ultrasonication for 5 min. Additions of the various surfactants were done on 10 ml aliquots of the 0.04% w/v SiO_2–Ru-640. Preparations are therefore as follows:

1. SiO_2–Ru-640: control sample—(no surfactant).
2. Zonyl-SiO_2–Ru-640: (a) 10 ml—SiO_2–Ru + 10 μl Zonyl FSA (1.3 g/mL) (b) 10 ml—SiO_2–Ru + 100 μl Zonyl FSA (1.3 g/mL). The excess of Zonyl FSA in (b) was used to examine if this affected the particle interaction with dentine.
3. Tween20–SiO_2–Ru-640:10 ml—SiO_2–Ru + 10 μl Tween20 (1.095 g/mL).
4. Triton–SiO_2–Ru-640:10 ml—SiO_2–Ru + 10 μl TritonX—100 (1.7 M).

After addition of the surfactants, 1 ml aliquots were transferred to Eppendorfs centrifuge tubes and centrifuged at 6000 rpm for 6 min, the supernatant discarded and the particles re-suspended in deionized water. This wash step was done to remove excess surfactant.

The photophysical properties of the luminescent particles were analyzed using Edinburgh Instruments FLS920 Series fluorescence spectrometer.

2.3. Application of particles to dentine

The particles were dropped on to the tooth surface with no mechanical agitation taking place. For the SiO_2–Ru-640, Zonyl–SiO_2–Ru-640, Tween20–SiO_2–Ru-640 and the Triton–SiO_2–Ru-640 10 μL of the supplied NP solution, was pipetted on to the dentine surface and uniformly spread using the pipette tip. The surface was left to dry completely and then subsequently washed three-times with 10 μL of de-ionised water by pipetting it on to the surface and aspirating it off. Dentine samples were then prepared for imaging.

2.4. Imaging of particles

All of the samples were prepared by mounting onto a carbon coated aluminium stub before splutter coating with gold using a Quorum Emitech K550X splutter coater. A scanning electron microscope, SEM (Zeiss EVO MA 10) was used under high vacuum and at a range of electron acceleration voltages between 5 and 15 eV.

The confocal microscopy studies were performed using a Leica confocal microscope in reflectance and fluorescence modes. Excitation was at 488 nm at approximately 20% power and at 458, 476 and 488 nm at 100% power, with emission collection at 478–498 nm and 580–800 nm, respectively. The samples were mounted on 12 mm diameter carbon stubs (used in SEM) and were attached to a glass slide.

2.5. Thinsert™ preparation and imaging

Polyethylene terephthalate (PET) ThinCert™ cell culture inserts, normally used for cell tissue work, were selected with a pore size of approximately 3 μm in diameter. They consisted of a polystyrene housing containing a PET membrane. Prior to application of the particles, one batch of control membranes was not splutter coated; the second batch had one coat of gold applied and the third batch had two coats of gold applied. The gold splutter coating deposition was for 2 min delivering a thickness of 15 nm. Sub-micron particles were applied to the surface of each batch of membranes with no mechanical agitation taking place. The membranes and particles were splutter coated before SEM imaging in a similar manner to the dentine surfaces.