



Application of electrophoretic deposition to occlude dentinal tubules *in vitro*

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ABSTRACT

Objectives: This study aims to apply electrophoretic deposition (EPD) for occlusion of dentinal tubules *in vitro* and investigate its effect on tubule occlusion and shear bond strength (SBS).

Methods: Charged mesoporous silica nanoparticles (MSNs) were synthesized and characterized through field-emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), and Fourier transform infrared (FT-IR) spectroscopy analyses. Thirty-nine sensitive dentin specimens were modeled and assigned randomly to three groups with different treatments ($n = 13$ each): group 1, immersion in the MSN suspension; and groups 2 and 3, anodic EPD with the specimen on the negative and positive electrode respectively. The effect of dentinal tubule occlusion was evaluated by dentin permeability test ($n = 10$ each) and FESEM examination ($n = 3$ each). Moreover, 18 specimens were grouped ($n = 6$ each) and treated in the same method. A resin stick was bonded onto each of the specimen using a self-etch adhesive (single bond universal) for SBS testing.

Results: Negatively-charged MSNs were synthesized and characterized as small and well-dispersed particles. After the EPD treatment (group 3), the dentinal tubules were effectively occluded by MSNs, which infiltrated into the tubules at a depth of approximately 7–8 μm and tightly associated with the tubular inwalls. SBS was not significantly different among the three groups ($P > 0.05$).

Conclusions: Synthesized MSNs were deposited into dentinal tubules by EPD treatment without compromising dentin bond strength.

Clinical significance: Application of EPD is a new approach for occlusion of dentinal tubules and exhibits potential in the study of dentin hypersensitivity.

1. Introduction

Dentin hypersensitivity, a prevalent indisposition that makes people seek dental attention, arises from exposed dentin and leads to a short and sharp pain when encountering thermal, evaporative, tactile, osmotic or chemical stimuli [1]. Hydrodynamic theory proposed by Brannstrom [2,3] is the most extensively accepted hypothesis for the etiology of dentin hypersensitivity.

This theory states that obturating the exposed dentinal tubules to stop or lessen the flux is an effective approach for managing dentin hypersensitivity. Researchers have developed various materials, such as resin [4,5], hydroxyapatite [6,7], calcium phosphate [8,9], bioactive glass [10,11], and mesoporous silica nanoparticles (MSNs) [12,13]. These materials are applied mechanically by rubbing or brushing them on a simulated sensitive dentin surface. Electrophoretic deposition (EPD) is also utilized for fine deposition of nanoparticles in the academe and industrial sector [14]. Charged nanoparticles dispersed in a liquid

medium are easily precipitated on the desired material regardless of its surface morphology [15]. EPD is an accurate and controllable deposition process [14]. Owing to the advantages in material precipitation, EPD is supposed to be able to occlude dentinal tubules as well. Nevertheless, to our knowledge, no paper has been published yet regarding the use of EPD on dentin hypersensitivity.

In recent years, MSNs have been broadly studied and applied in the biomedical field due to their stable framework, thermal and chemical stability, large specific surface area, and adsorption capability [16,17]. MSNs have gained increasing attention as an ideal carrier for drug delivery systems [18–20]. In previous studies on dentin hypersensitivity, MSNs were loaded with remineralizing agents such as hydroxyapatite and calcium carbonate to induce occlusion of the open dentinal tubules and applied mechanically to simulated sensitive dentin with favorable outcomes [12,13,21,22]. MSNs can be easily modified as charged nanoparticles with varied zeta potential, which is crucial for application of EPD [14]. Hence, MSNs exhibit potential to be

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precipitated into dentinal tubules in an electric field.

Dentin hypersensitivity often occurs on defective dental hard tissues. After treatment of the sensitive region, the defect often needs to be restored with resin. It's necessary for a desensitizing treatment not to impair adhesive restoration. Therefore, the influence of the electrophoretic desensitization on shear bond strength (SBS) should be evaluated.

In this study, EPD is introduced into the occlusion of dentinal tubules. The objective of this study were (1) to synthesize charged MSNs and apply them with EPD to occlude dentinal tubules and (2) to test the null hypothesis that EPD is not capable of occluding dentinal tubules effectively without compromising SBS.

2. Materials and methods

2.1. Synthesis and characterization of MSNs

MSNs were synthesized based on a previously reported method with slight modifications [23]. Briefly, 251 mg of hexadecyltrimethylammonium bromide (CTAB, J&K scientific, Beijing, China) and 8.5 μL of 26% ammonium hydroxide aqueous solution were added into a 30 mL of deionized (D.I.) water. The solution was stirred at 30 °C for 30 min until CTAB was completely dissolved. Afterward, 196 mg of tetramethyl orthosilicate (TMOS, J&K scientific, Beijing, China) was added to the solution drop by drop and rapidly stirred at 30 °C for 24 h. Then, 330 mg of PEG-silane (Fluorochem, Derbyshire, UK) was added into the solution and the stirring went on for 24 h. The temperature was then increased to 80 °C, and the solution was constantly stirred for another 24 h. The solution was cooled to ambient temperature and placed in a dialysis bag with molecular weight cut-off of 10000 (Yuanye, Shanghai, China). In the next step, the solution was dialyzed in an acid solution containing a mixture of 100 mL of D.I. water, 100 mL of ethanol, and 700 μL of acetic acid for 24 h. The dialysis was repeated three times. The solution was then dialyzed in 2000 mL of D.I. water for 24 h, and the process was repeated three times. The product was filtered through a 200 nm syringe filter (Anpel, Shanghai, China) and stored until use.

The morphology and dispersal of the synthesized particles were characterized by field-emission scanning electron microscopy (FESEM) (Sigma, Zeiss, Jena, Göttingen, Germany) and transmission electron microscopy (TEM) (JEM-2100, JEOL, Japan) analyses. Zeta potential was measured through dynamic light scattering (Malvern, UK). Fourier transform infrared spectroscopy (FT-IR) analysis was performed with a Nicolet 5700 spectrometer (Thermo Scientific Inc., Madison, WI, USA) to characterize the functional groups. Spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} .

2.2. Sample preparation and experimental design

Intact human third molars were obtained from healthy patients upon obtaining informed consents under the protocol approved by the Ethics Committee for Human Studies of the School & Hospital of Stomatology, Wuhan University, China. The teeth were preserved in 0.2 wt.% thymol at 4 °C and used within 3 months. Dentin disc specimens were prepared from the collected teeth by using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The teeth were sectioned horizontally beneath the cements/enamel junction, and dentin discs with 1 mm thickness were prepared. After being wet-ground on silicon carbide sandpapers of 600, 800, and 1000 grits, the top surfaces of the discs were etched with 37% phosphoric acid for 15 s to remove the smear layer and simulate sensitive dentin. The discs were then rinsed thoroughly with distilled water.

A customized electrophoretic device was set up to move the charged MSNs toward open dentinal tubules (Fig. 1). Thirty-nine dentin discs were prepared to determine the occlusion of dentinal tubules and randomly assigned into three groups ($n = 13$). In group 1, dentin discs

were fixed on the positive electrode of the electrophoretic device one at a time. The MSNs dispersed in distilled water were placed in a container, and the complete immersion of the disc in the liquid medium was ensured. The disc was immersed for 5 min while the device was turned off. In group 2, the pretreated discs were fixed on the negative electrode one at a time. A new aliquot of MSNs dispersed in distilled water was placed in the container, and the disc was completely immersed. The device was then turned on, and the current intensity was set to 70 μA for 5 min. In group 3, the discs were fixed on the positive electrode, and the remaining steps were similar to that in group 2. After desensitizing treatments, the specimens were rinsed thoroughly with distilled water prior to evaluation.

2.3. Dentin permeability test

After the treatments, 10 of 13 discs were randomly collected from each group and used in dentin permeability test. The device (Fig. 2) used was an apparatus modified based on our previous study [24]. It is composed of a split-chamber element and a water container which mimicked pupal pressure of 20 cm D.I. water. Each disc was firmly fixed within a set of two circular rubber rings, which were sandwiched by Plexiglass blocks, to provide a dentin surface area of 0.38 cm^2 for fluid infiltration. The block close to the pulpal surface of the disc was perforated by an 18-gauge steel tube linking the rest of the device. An air bubble was generated into a glass microcapillary tube, which was horizontally placed and linked the water container and the split-chamber unit. Movement of the bubble under pressure through each dentin disc was recorded in millimeters and transformed into volume flow ($\mu\text{L}\cdot\text{min}^{-1}$). Hydraulic conductance (L_p) was determined by dividing the fluid flow with the simulated pulpal pressure and the available surface area.

L_p was recorded at two time points, that is, after phosphoric acid application and after desensitizing treatment. The permeability of each dentin disc was displayed as a percentage of its maximum permeability which was recorded after phosphoric acid application and set as 100%.

2.4. FESEM observation

The three remaining discs in each group were arranged for FESEM observation. A notch on the pulpal surface of each disc was prepared, and the discs were longitudinally sectioned into halves to observe the horizontal and vertical areas. The discs were rinsed with distilled water and dried. After conductive coating, the effect of obturation was examined by FESEM. From two middle spots of each specimen, micrographs were taken at $\times 2000$, $\times 5000$, and $\times 4000$ magnifications.

2.5. SBS test

An additional 18 specimens were prepared and randomly assigned into three groups ($n = 6$) as mentioned above. After treatment in the container with the electrophoretic device turned on or off, the discs were rinsed with distilled water to prepare SBS specimens. The other surface of the one fixed to the electrode was air dried and applied with universal adhesive (Single bond universal, 3 M, USA) in self-etch mode following the manufacturer's instruction. A resin stick (height = 4 mm, diameter = 2.8 mm) (Charisma, Heraeus, Germany) was built and light cured for 20 s on the middle of each disc. After storage in D.I. water at 37 °C for 24 h, each sample was fixed to a universal mechanical tester (ZY-100 K, Yangzhou, Jiangsu, China) under shear force at a crosshead speed of 1 mm/min until failure. A digital caliper was used to measure the interface area of the transverse section of each sample. SBS (MPa) was then calculated.

2.6. Statistical analysis

Statistical analysis was conducted with IBM SPSS 19.0 software

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