SPIONs in Dentistry: The Development and Application of Magnetic Nanoparticles for Targeted Drug Delivery in the Oral Environment.

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Introduction: Currently, superparamagnetic iron oxide nanoparticles (SPIONs) have been used in targeted drug delivery of genetic material and anti-cancer drugs, but their use in localized drug delivery in the oral environment has not been thoroughly explored. **Objective**: The aim of this study was to explore the possibility of using SPIONs (avg. 20 nm in diameter) as transporters of therapeutic agents into the dentin tubules and pulp chambers of teeth. **Methods**: SPIONs were applied to acid-etched and EDTA treated cementum below the cemento- enamel junction of maxillary and mandibular molars and placed in a magnetic field (10 minutes to 3 hours). Penetrability of the SPIONs was evaluated using three different methods: Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (N=15) of distilled water placed in the pulp chamber before and after application of the SPIONs, Laser-Ablation Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to analyze cross sections of the prepared teeth, and Energy-Dispersive X-ray Spectroscopy (EDX) to analyze samples of paper placed between the teeth samples and the magnet for SPIONs.

Results: Samples analyzed with ICP-OES demonstrated a range from 0% SPION penetration to 33.25% SPION penetration, while ICP-MS demonstrated elevated iron concentrations across the entire cross-section of the tooth (cementum to pulp) compared to controls. Finally, EDX analysis detected the presence of SPIONs on the paper samples

Conclusions: These initial results demonstrate that SPIONs can fully penetrate human teeth when placed on a treated cementum surface and subjected to an external magnetic field, making them an attractive candidate for novel drug delivery applications in the oral environment.

INTRODUCTION

The development of magnetic nanoparticles has led to their use in many novel biomedical applications. Particularly, in the last three decades superparamagnetic iron oxide nanoparticles (SPIONs) have become attractive candidates for targeted localized drug delivery. Much of this research has focused on developing SPIONs for the delivery of anti-cancer drugs and genetic material in gene therapy.[1] SPIONs offer an advantage over other drug delivery systems due to their ability to be targeted to a specific tissue by subjecting the particles to a magnetic field.[2] To date, the possibility of using SPIONs for drug delivery applications in the oral cavity is only beginning to be explored. Much of this research has focused on localized antimicrobial therapy to reduce the unwanted side effects associated with the use of systemic antimicrobials in treatment of severe forms of periodontitis.[3]

In this study, SPION cores (~20 nm) consisting of magnetite (Fe_3O_4) were used to explore the permeability of cementum and dentin as a route for drug delivery into the pulp under influence of an external magnetic field. Highly sensitive spectroscopy characterization tools were employed for detection, such as: Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA- ICP-MS) and Scanning Electronic Microscopy associated with Energy-dispersive X-ray spectroscopy (SEM/EDX). Three experimental conditions were tested: (1) SPIONs guided by magnetic field, (2) SPION infiltration without magnetic field, and (3) a control, with no SPIONs added. Also, four time periods of magnetic field exposure were analyzed for initial experiments: 10 min, 1 hour, 2 hours and 3 hours. The null hypothesis tested was: there is no difference in SPION concentration among the experimental conditions.

MATERIALS AND METHODS

Materials: An iron oxide (II, III), magnetic nanoparticle solution (20 nm avg. part. Size, 5mg/mL in toluene), was acquired from Sigma-Aldrich (Missouri, USA). Fifteen maxillary and mandibular molar teeth (extracted for orthodontic proposes) were obtained from patients and placed in refrigeration in deionized water. Tooth Conditioner Gel (34% phosphoric acid), and 17% EDTA were purchased from Dentsply (Ontario, Canada). 25.4 mm x 3 mm Rare-Earth Magnets (Nd-Fe-B), were purchased from Lee Valley tools (Manitoba, Canada).

Tooth preparation: For ICP-OES experiments, 15 intact human molars were cut horizontally in two parts separating the crown from the roots with a Buehler® IsoMet® Low Speed Saw (Illinois, USA) under water irrigation. For LA-ICP-MS analysis, root trunks were collected by sectioning the collected molars 1 mm above the CEJ and sectioning the roots. These root trunks were then sectioned in half to make two semi-circular samples. Any remaining pulp tissue and periodontal ligament was manually removed. All prepared samples were stored in deionized H_2O.

ICP-OES Analysis Sample Preparation: The root trunks of prepared teeth were etched with tooth conditioner for 30 seconds, washed with deionized H_2O, and treated with EDTA for 30 seconds. Samples were secured in a small disposable cup by placing the occlusal surface in rope wax (figure 1). The magnet was then placed vertically on the lingual side of the tooth and secured with rope wax. The pulp chamber was filled with 30 µl of milliQ deionized H_2O, and the cup was filled with milliQ deionized H_2O until 3⁄4 to 1⁄2 of the prepared tooth was submersed. 5 samples per time period subjected to the magnetic field (1h, 2h, and 3h) were prepared for a total of 15 samples. After the allotted time, 10 µl of H_2O from the pulp chamber was removed and placed in a test tube containing 4.99 ml of ICP Wash Solution (2% HNO_3 in H_2O, TraceSELECT®) purchased from Sigma-Aldrich (Missouri, USA). Initial 10 µl H_2O samples established a baseline iron concentration in the prepared samples. After the initial 10 µl H_2O samples were collected, 5 µl of the magnetic nanoparticle solution was applied to the etched surface of the prepared teeth with a micropipette, keeping the pulp chamber titled up during the process. The protocol outlined above was repeated, and 10 µl of H_2O from the pulp chambers was collected after the allotted time (1h, 2h, 3h) in the magnetic field and placed in a test tube containing 4.99 ml of ICP Wash Solution.

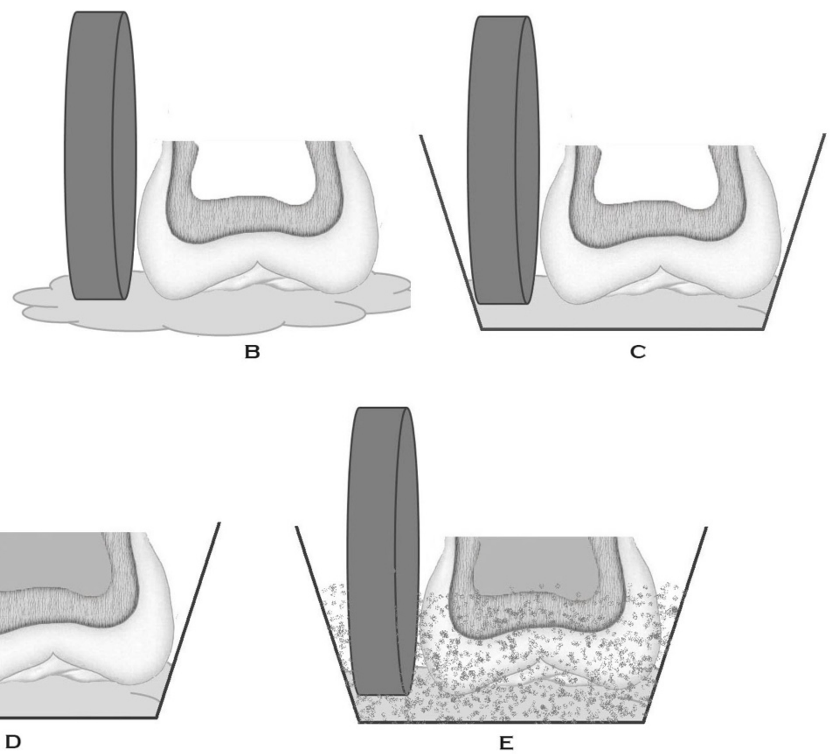

Figure 1. Experimental set-up (A-E) for ICP-OES analysis of SPION penetration of maxillary and mandibular teeth. The rare-earth magnet was placed on lingual aspect of the tooth, while the particles were placed on the treated buccal aspect. Samples were then semi-submerged in distilled water.

LA-ICP-MS Analysis Sample Preparation: Hemisected root trunk samples were removed from storage in deionized H_2O, etched with tooth conditioner for 30 seconds, washed with deionized H_2O, and treated with EDTA for 30 seconds. A 2.5" x 2.5" impermeable iron free mixing pad (3M, Minnesota, USA) was placed on top of a rare-earth magnet with the coated side facing down. The hemisected root samples were placed on top of the mixing pad and then outlined on the mixing pads with a permanent marker. 1 μl of the magnetic nanoparticle solution was applied to the etched surface of the samples with a micropipette. Samples were removed from the magnetic field after 10 minutes and the mixing pads were set aside for SEM/EDX analysis. Controls were prepared following the same protocol except the nanoparticle application was omitted. Samples were then fractured to expose a cemento-pulpal cross-section of dentin and mounted in an aluminum multi-specimen holder for JEOL stubs with autocuring acrylic (Bosworth Co Fastray, Ohio, USA) and is shown in figure 2.

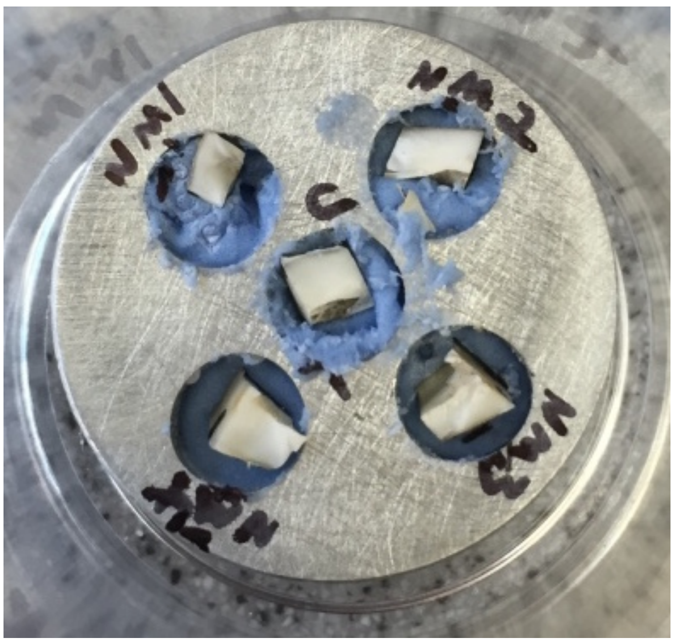

Figure 2. Cross-sections of molars mounted in an aluminum multi-specimen holder for JEOL stubs with autocuring acrylic. External surfaces of the teeth were recorded to ensure proper orientation for LA-ICP-MS analysis. Laser ablation for analysis began on the external cementum surface and ended at the pulp chamber dentin.

SEM/EDX analyses: Analysis was performed using a FEI Quanta 650 FEG Environmental/Scanning Electron Microscope. An Everhart-Thornley detector was used for the SEI, the accelerating voltage for all the materials was 15 kV, and beam working distance around 10 mm; magnifications were in the range of 2.5 k and 50 k. The microscope was equipped for EDX chemical analysis, with an Ultra-thin window EDX detector with the follow parameters: takeoff: 34.7, Amp Time (μs):7.68 resolution (eV): 130. The mixing pads set aside during LA-

ICP-MS experiments were analysed using a backscatter mode to detect SPION presence. EDX-Genesis software was used. The probing depth of the EDX beam is around 1-3 μm.

Results

Fe analysis by ICP-EOS: Analysis of the prepared samples using ICP-OES yielded a broad range of detectable differences in Fe concentrations (from 0.01 ppm to 1.67 ppm increases in Fe). No correlation between increased Fe concentrations in the pulp chamber and variables such as root wall thickness, time in magnetic field, and overall tooth thickness (distance of SPIONs from magnet) could be resolved. **Fe analysis by LA-ICP-MS:** LA-ICP-MS analysis demonstrated higher Fe concentrations between the control and samples where SPIONs were applied to the external cementum surface (fig. 3). A higher and more uniform concentration of Fe across the tooth section was observed in the sample in which the SPIONs were subjected to the external magnetic field in comparison to the sample treated with SPIONs but not placed in the magnetic field. A slight increase in Fe concentration towards the pulpal chamber was observed in the control. Both samples in which SPIONs were applied to the cementum surface demonstrated significant Fe penetration up to 200 μm into the tooth samples.

Fe analysis by SEM/EDX: High magnification (9k x) SEM surveying followed by EDX analysis was conducted on the impermeable paper mixing pads, including a positive and negative control. A positive EDX result for Fe can be seen in figures 4 and 5.

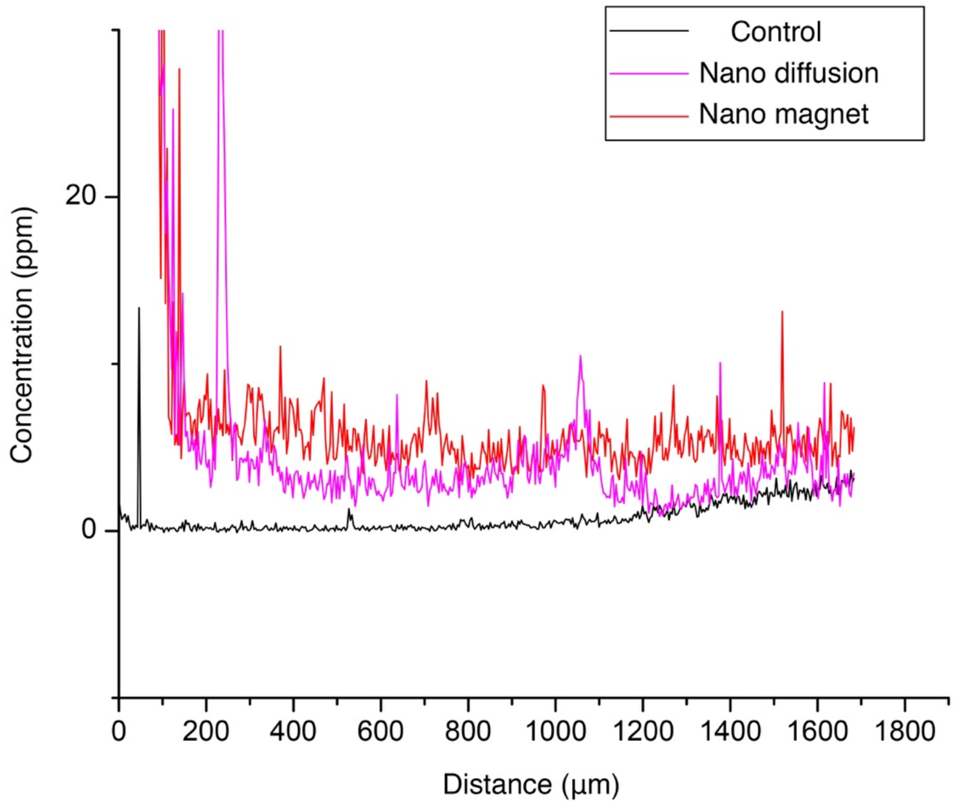

figure 3. LA-ICP-MS analysis of tooth samples (n=3). Laser ablation of the samples were conducted directionally in a cementum to pulpal dentin orientation. Nano diffusion represents the tooth sample with SPIONs loaded onto a treated root surface but was not placed in a magnetic field.

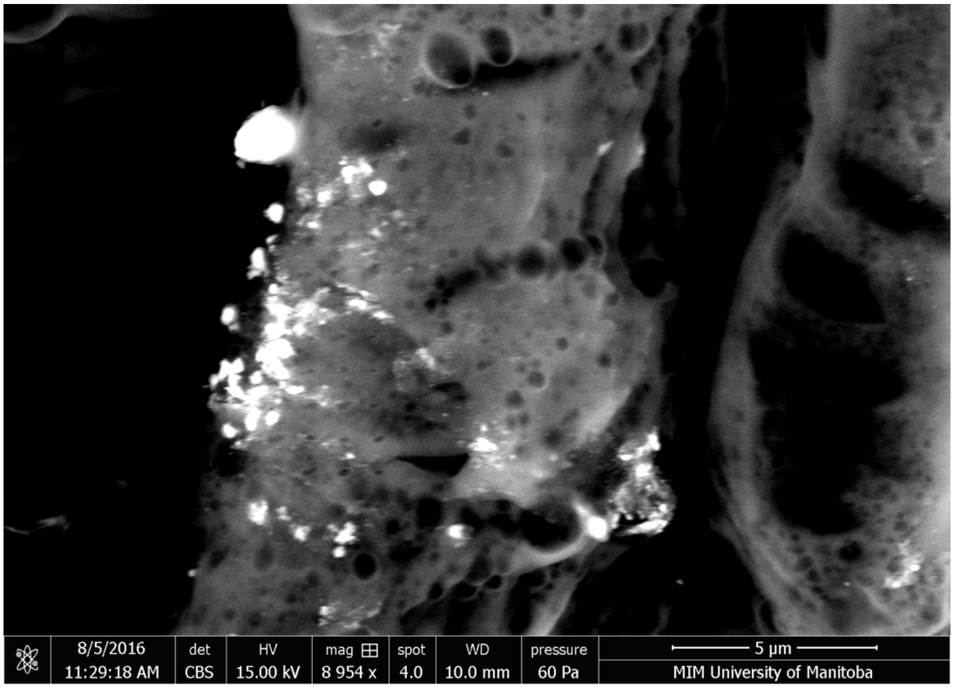

Figure 4. SEM image of nanoparticle agglomeration on paper fiber in mixing pad. Mixing pads were placed between the teeth samples and the magnet. Scale bar 5 μm.

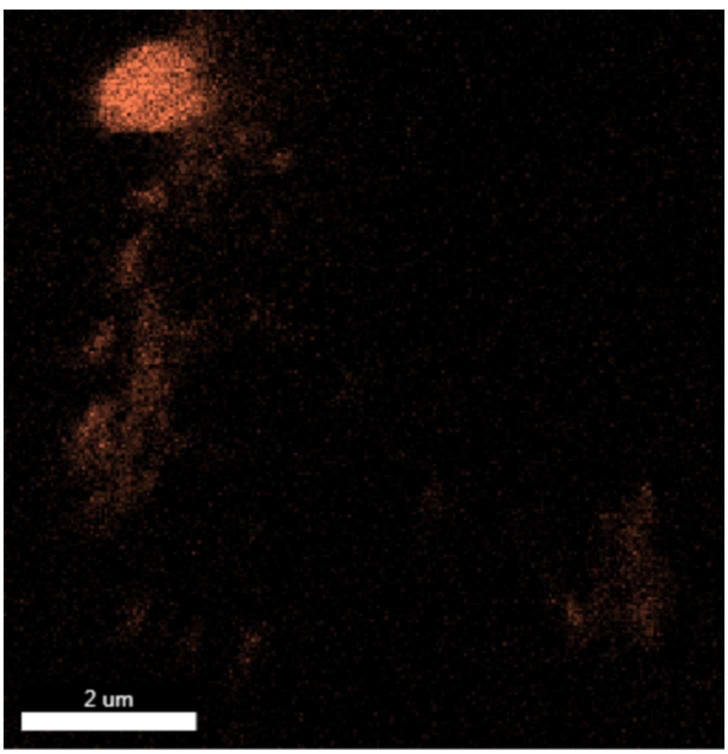

Figure 5. Representative image for Fe distribution detected by EDX mapping in paper fibers of mixing pad. Scale bar 2 μm.

DISCUSSION

The objective of this study was to determine if SPIONs could diffuse from the outer cementum to the pulp chamber using a magnetic field to help guide diffusion. Our initial ICP- OES results had a high degree of variance, and had no correlation to the time spent in the magnetic field, root wall thickness, and root diameter. Despite this, our LA-ICP-MS and SEM/EDX results provide some evidence that SPIONs can penetrate into the pulp chamber after being placed on the external surface of the tooth and subjected to an external magnetic field. Therefore, the null hypothesis was rejected. It is important to note that the techniques used could only provide qualitative information, signifying the presence or absence of SPIONs. To this date, other studies looking at the use of nanoparticles for drug delivery have not explored this route of entry into to the tooth.[4,5,6] This method of delivery could expand the possible uses of nanoparticle-based drug delivery systems in teeth and the surrounding periodontal tissues since it is a minimally-invasive drug delivery method.

CONCLUSIONS

Despite the limitations of this study, it was possible to demonstrate that superparamagnetic iron oxide nanoparticles are able to completely penetrate human teeth under the influence of a magnetic field, making them an attractive candidate for localized drug delivery, while offering a minimally invasive delivery method.

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