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Community Oral Health services/School Site Visits, Philadelphia, PA 2009-2013

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Abstract

Title of Thesis: A Novel Method for the Treatment of Dentinal Hypersensitivity: Penetration of Magnetic Nanoparticles into Dentinal Tubules

Se Jong Kim, Master of Science, 2016

Thesis Directed by: Dr. Radi Masri, DDS, MS, PhD, Department of EPOD

Dentinal hypersensitivity (DH) is characterized by temporary, sharp-shooting pain arising from exposed dentin in response to different types of stimuli, such as thermal, mechanical, osmotic or chemical elements This study looked the treatment of dentinal hypersensitivity (DH) by utilizing magnetic nanoparticles (MNPs). DH was simulated by creating a class V preparation on an extracted human tooth. 72 samples were divided into two groups. Three different MNPs (100nm, 300nm, 500nm) were applied to the class V preparation. A magnet was placed on the opposing side of the class V preparation for the experimental group. No magnet was used for the control group. All samples were decalcified, sectioned and mounted for visualization of MNPs through light and fluorescent microscopes. The percentage of dentinal tubule penetration of the three different MNP groups was calculated by measuring the total depth of the dentinal tubule from the inner surface of the preparation to the pulp. The second measurement was from the inner surface of the preparation to the depth that the MNPs travelled. Data were analyzed using ANOVA and Tukey's Honestly Significant Difference test. Overall, Smaller magnetic nanoparticles have a significantly higher percentage of dentinal tubule penetration than the larger magnetic nanoparticles with or without an external magnetic field ($p \leq .0005$). There was no significant difference between the percentage of dentinal tubule penetration of 300nm and 500nm in control

group. A significantly higher percentage of dentinal tubule penetration was found with application of the external magnetic field ($p \leq .0005$). In conclusion, MNPs could potentially be utilized for DH treatment.

A Novel Method for the Treatment of Dentinal Hypersensitivity: Penetration of Magnetic Nanoparticles into Dentinal Tubules

by
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Thesis submitted to the Faculty of the Graduate School
of the University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Master of Science
2016

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Introduction

Dentinal hypersensitivity (DH) is characterized by temporary, sharp-shooting pain arising from exposed dentin in response to different types of stimuli, such as thermal, mechanical, osmotic or chemical elements [1]. There have been many theories about the mode of neural transmission within the dentinal tubule but the most widely accepted mechanism is the hydrodynamic theory. This theory was first introduced by John Neill in 1838, resurfaced by Alfred Gysi in 1900 and more recently by Martin Brännström in 1963. According to Brännström [2], the disruption of dentinal fluid within patent and exposed tubules can stimulate the nerve plexus in the pulp.

The prevalence of DH has been studied extensively. Several clinical studies concluded similar prevalence of DH, around 15% [3]. Depending on the continent where DH was measured, the prevalence ranged from 3% to 84% of the adult population. [4-8]. Such a wide range of values might be explained by the assessment methods used: ranging from different types of questionnaires to clinical assessment and study locations, which could be private practice or research institution. The prevalence of DH in patients who were referred to a periodontist was 84% [4]. This may be due to the bacterial penetration into the patent dentinal tubules, which reflect a different etiology from thermal, mechanical, osmotic or chemical stimuli [9]. In another study, 77% of the teeth with dentinal hypersensitivity showed variable amounts of gingival recession [7]. Some studies conclude that DH occurs more in females than males [5, 8, 10], and four studies show cold stimulus is the most common stimulus that caused DH [4, 7, 8, 10]. Teeth that were most affected by DH were premolars and molars [5, 10]. One systematic review, however, reported that premolars and incisors were mostly like affected by DH [11]. In terms of age, many

studies conceded that DH occurs most frequently between the third and fourth decade and gradually declines thereafter [4, 5, 7].

Pathogenesis of DH

In order to understand DH, anatomical and histological aspects of the dentin-pulpal complex must be appreciated. Dentinal tubules are microscopic channels that extend the entire thickness of the dentin, radiating from the pulp to the dentinoenamel junction (DEJ) in the crown or root area. In humans, they are 2.5 to 3.5 mm long [12]. They are 1-2 um in diameter, usually taper from the pulp to the DEJ and may exhibit branching as they approach the pulp. These tubules contain cytoplasmic extensions of odontoblasts that once formed the dentin and maintain the integrity of the dentinal tubules. The cell body of the odontoblast resides in the pulp. Since odontoblast processes are extended into the pulpal end of the dentinal tubule, with its associated nerve endings, odontoblasts are primarily responsible for DH [12]. Besides odontoblastic processes, dentinal tubules contain serum-like fluid and unmineralized collagen, serving as a passage for nutrients. The dentinal tubule is, then, protected by surrounding hypermineralized tissue called peritubular dentin [12].

According to the hydrodynamic theory, once dentinal tubules are exposed to the oral environment, the rate of dentinal fluid will be influenced by thermal, tactile, osmotic, chemical or evaporative stimuli [13]. Those stimuli excite the baroreceptors of nerve endings in the dentinal tubules, causing DH. Cold stimulus causes quicker and stronger nerve response than other stimuli by drawing the dentinal fluid away from the pulp [14], which might explain why cold stimulus is the most common cause of DH.

There are several different characteristics that distinguish the dentin of teeth with DH from those without DH. A study, using a scanning electron microscope,

revealed that hypersensitive teeth have approximately eight times the number of dentinal tubules and approximately two times the diameter of non-sensitive teeth [15]. Moreover, the smear layer of dentinal tubules in sensitive teeth were found to be thinner than those in non-sensitive teeth [16]. These two studies support the idea that stimulus transmission across dentinal tubules might be mediated by hydrodynamic mechanisms. The permeability of dentinal fluid is facilitated as the greater number of patent and wider dentinal tubules is exposed to oral environment. Therefore, augmenting the hydrodynamics within the dentinal tubules with thermal, tactile, osmotic, chemical or evaporative stimuli might initiate DH. The diameter or radius of the dentinal tubules is the most important contributing factor [17]. Based on the Poiseuille-Hagen equation, the volumetric flow rate through a cylindrical pipe is proportional to the fourth-power of the radius. Thus, doubling the diameter is expected to result in 256 times the increase in the volumetric flow rate.

In normal tooth structure, dentin is covered by enamel and cementum and dentinal tubules are not exposed to the oral environment. In order for DH to develop, two phases are required. The first stage in the development of DH is the exposure of the dentinal tubules to the oral cavity. However, the dentinal tubules are not open because of the smear layer. At the second stage, with the loss of the smear layer through chemical and/or mechanical insults, DH will occur because there is communication between the oral environment and the dentinal fluid [3]. The following are predisposing factors that cause enamel loss and exposure of dentinal tubules: 1) attrition, 2) erosion, 3) abrasion, 4) abfraction 5) gingival recession and 6) periodontal surgery [3].

The critical step for treatment of DH is proper diagnosis. The clinician needs to rule out sensitivity from bleaching, defective restorations, primary or secondary

caries, postoperative sensitivity, and pulpitis because treatment of these conditions is different from that of DH. Meticulous collection of the following data should be used for diagnosis: the nature of the pain, radiographic examination, and clinical evaluation (i.e. percussion, palpation, the cold/hot test, and the pulp vitality test). For confirmation, pain can be clinically reproduced by using an air-water syringe.

Current Available Treatment Modalities for DH

Currently available treatment modalities for DH accomplish their goal in two major ways: 1) diminishing the neural transmissions and/or 2) the physical occlusion of dentinal tubules. These methods are all topical rather than systemic pharmacologic approaches because systemic treatments do not efficiently reach the pulp [18]. Some existing strategies have demonstrated their potential but their clinical efficacy is still questionable in terms of longevity and safety. Thus, there is no “gold standard” treatment for DH.

The following is a review of these modalities:

Potassium Nitrate

Potassium nitrate reduces DH by increasing the extracellular potassium ion level to depolarize the nerve. This ultimately prevents the nerve from repolarizing. This technique reduces DH by diminishing neural transmission from the nerve ending of the odontoblasts in the dentinal tubules [11]. Potassium nitrate is widely used as a dentifrice and Sensodyne Proenamel (GlaxoSmithKline, Weybridge, UK) is one example of this type of dentifrice. Achieving the optimal concentration of the extracellular potassium ion for desensitizing the nerve ending of the odontoblasts is, however, practically challenging because patients typically use toothpaste twice a day and only a minimal increase in the concentration of the potassium ion in saliva occurs

in this short period of time. A meta-analysis of six studies indicated no strong support for using a potassium containing dentifrice in managing DH [19].

Strontium Salt

Strontium salt has been known to reduce DH by utilizing its insoluble metal compound to occlude the dentinal tubules [11]. Sensodyne Original and Sensodyne Rapid Relief (GlaxoSmithKline, Weybridge, UK) are available dentifrices that contain 8% strontium acetate in a silica base. Strontium might replace calcium ions within the hydroxyapatite and therefore strengthen the dentin [20]. One recent study, however, found that artificial silica abrasive (an ingredient in these dentifrices) was actually responsible for occluding the dentinal tubules. Without further investigation of strontium, the clinical efficacy of this product remains questionable [21].

Fluoride

Fluoride ions have been included in major dentifrices used for relieving tooth sensitivity. Fluoride causes the precipitation of calcium fluoride (CaF2) within the dentinal tubules, thereby occluding these tubules and reducing the permeability of the dentinal fluid [22]. Topical fluoride (1.23% sodium fluoride) has been shown to decrease postoperative sensitivity after bleaching in a randomized controlled clinical trial [23]. Besides sodium fluoride products, stannous fluoride is another form of fluoride dentifrice. It is presented in an aqueous solution carrier or with carboxymethyl cellulose [24]. One study demonstrated 0.454% stannous fluoride was superior to 1000 ppm fluoride (as sodium monofluorophosphate) in reducing the pain of DH at four and eight week time points [25]. The anhydrous version of stannous fluoride has demonstrated near complete occlusion of the dentinal tubules by forming a tin-rich surface deposit in vitro and in vivo but the challenging part for the clinician was maintaining the anhydrous version of stannous fluoride simultaneously in the

aqueous preparation [26]. The major disadvantage of use of a stannous fluoride dentifrice is that it stains the teeth, the tongue, [27] and ceramic materials [28], and that it takes a long time to be effective.

Oxalate

Oxalate products were first introduced in the late 1970s for treating DH. They have demonstrated a decrease in hydraulic conductance in the dentinal tubules [29]. Oxalate products are relatively insoluble in acid which render them more resistant to dissolution [30]. A systematic review showed that, other than 3% monohydrogen-monopotassium oxalate, oxalates has not been proven to be more effective in treating DH than placebos [31]. Oxalate solutions have also been shown to be inferior to sodium chloride solutions in reducing DH [32].

Lasers

Introduced in the mid-1980s, low and middle output-power lasers have been used to treat DH. Low output-power lasers (i.e., diode lasers and He-Ne lasers) are thought to depolarize the nerve. Middle output-power lasers (i.e., Nd:YAG, CO2, Er-YAG) are thought to reduce DH by sealing or narrowing dentinal tubules [33]. However, effectiveness of lasers has varied from 5-100% [34]. Three randomized clinical trials revealed that the reduction was not significantly different from using a placebo [33]. Although it has not been fully investigated, laser-induced pulp damage may also be a concern [33]. More research should be conducted to reveal the mechanism by which lasers reduce DH and the potential side effects associated with them.

Iontophoresis

The use of iontophoresis was first introduced in dentistry in 1984. Its major

mechanism in reducing DH is to utilize electrostatic repulsion to discharge fluoride ions into the dentinal tubules [35]. With electrical current, discharged fluoride ions are thought to cause the precipitation of calcium fluoride within the dentinal tubules, thereby decreasing the permeability, or the paresthesia of the sensory nerve [36]. The implication of iontophoresis for dental practice may be, however, impractical because molecules have to be charged in order to repulse them. The instrument used for iontophoresis has to be maintained and disinfected after each use. Also, there are no long term randomized clinical trials of effectiveness or the absence of side effects.

Mucogingival Plastic Surgery

Mucogingival plastic surgery aims to minimize the area of exposed root surface by means of a soft-tissue pedicle graft and a free soft tissue graft [37]. In addition to reducing DH by covering exposed dentinal tubules, it improves esthetics in the area where there is gingival recession. [38, 39]. The application of mucogingival plastic surgery is limited because this technique can only be used on the cervical area of teeth and recession may occur again even after surgery. It is painful both at the site of grafting and the donor site and can be expensive. There are not enough randomized controlled clinical trials and long term studies to draw the conclusion that mucogingival plastic surgery can reduce DH [37].

Nanodentistry

In 1959, nanotechnology was first introduced by Nobel Prize–winning physicist Richard P. Feynman. Since then, nanoparticles have been applied routinely in dentistry. They are an integral element in composite resins, porcelains, cements, impression materials, and mineralized bone scaffolds. Beyond the scope of dental materials, other possible applications include diagnosis of oral cancer, nanoanesthesia,

bone replacement materials, major tooth repair/nanotissue engineering, and dental implants.

Managing DH with nanoparticles is a new field of investigation [40]. A number of studies have investigated the application of nanoparticles to manage DH. During a randomized clinical trial, a new dentifrice containing zinc-carbonate hydroxyapatite (CHA) nanocrystal was shown to reduce DH significantly after four and eight weeks when compared to a commercially available potassium nitrate/fluoride dentifrice [41]. In another study, a mixture of nano-sized calcium oxide particles containing a mesoporous silica biomaterial (NCMS) and 30% phosphoric acid was applied in vitro on dentin. NCMS showed a significant reduction in dentin permeability under simulated pulpal pressure [42]. Application of nano-hydroxyapatite paste after tooth bleaching was shown to reduce the number of days of sensitivity in half compared with a placebo group [43]. Nano-carbonate apatite dentifrice demonstrated its effectiveness in occluding dentinal tubules when compared to its control and strontium chloride [44]. An in vitro study demonstrated that the occlusion effect of nano-carbonate apatite was 87% compared with its control group. This was 33% higher than the Er:YAG laser irradiation group as well [45]. There are not enough randomized controlled clinical trials and long-term studies to prove the effectiveness of nanoparticles to manage DH.

Magnetic Nanoparticles in Dentistry

For the present study, metallic magnetic-fluorescent nanoparticles were utilized as a possible DH treatment modality. Magnetic nanoparticles consist of three elements: magnetic iron cores which can be magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3), lipophilic fluorescence dye as an inner layer, and a polysaccharide or chitosan matrix as an outer layer that can be used to carry therapeutic agents if desired. Other types of

polymer matrix are available as a substitute for the polysaccharide or chitosan matrix. This allows magnetic nanoparticles to be used for other purposes. If clinical studies are as promising as prior animal studies have been, magnetic nanoparticles will benefit humans in many different ways. Several possible applications include target imaging with MRI and drug targeting [46].

Magnetic nanoparticles have been utilized frequently in biomedical and biotechnology studies due to their large surface-to-volume ratio, their quantum size effect as well as their magnetic elements. Possible applications for magnetic nanoparticles include targeted drug delivery with help of an external magnetic field, rapid separation in environmental biology, tumor magnetic hyperthermia therapy, contrast enhancement of magnetic resonance imaging, biosensors, and concentration tracing of specific targets. Unlike cobalt or nickel, magnetite or maghemite is less susceptible to oxidation which makes both of them less toxic to humans. Higher biodegradability is another advantage of these iron cores. They degrade into iron which is an essential component of our body. In fact, these particles have been tested to be biocompatible and non-toxic in preclinical models [47, 48] as well as in human clinical trials [49, 50].

Magnetite or maghemite are superparamagnetic and ferromagnetic respectively. Superparamagnetic particles have no macroscopic magnetic moment but develop a mean magnetic moment when affected by an external magnetic field. However, ferromagnetic particles have a permanent mean magnetic moment and they can be separated easily in viscous media. Agglomeration of magnetic nanoparticles are prevented by the outer layer of the magnetic nanoparticles (polysaccharide or chitosan matrix).

Purpose

In this project, a novel technique was studied as a possible treatment modality for DH. The occlusion of the dentinal tubules was achieved by pulling starch-coated magnetic nanoparticles (MNPs) using an external magnetic force (EMF). The EMF allowed the MNPs to penetrate the dentinal tubules more efficiently. The aggregation of these nanoparticles is expected to partially or completely occlude the tubules and therefore reduce the dentin permeability. Dentinal tubules vary in size (0.3-2um) and they branch and taper as they reach the pulp. The size of the MNP is expected to affect the degree of mobility of these nanoparticles within the dentinal tubules. The advantage of using larger MNPs is that they can be pulled by the EMF more efficiently. The disadvantage is that they are more likely to aggregate, impeding their ability to move within the tubules. The reverse is true of smaller MNPs.

Hypotheses

Null Hypothesis 1: There is no significant difference in the percentage of dentinal tubule penetration between the following sizes of magnetic nanoparticles (MNPs): 100nm, 300nm, or 500nm.

Specific Research Hypothesis 1: Smaller MNPs have a significantly higher percentage of dentinal tubule penetration than the larger MNPs.

Null Hypothesis 2: There is no significant difference in the percentage of dentinal tubule penetration between the experimental and the control group.

Specific Research Hypothesis 2: The experimental group has a significantly higher percentage of dentinal tubule penetration than the control group.

Materials and Methods

Any type of extracted teeth from the prosthodontics, periodontics, oral maxillofacial surgery and orthodontics clinics at University of Maryland, Baltimore were collected, mechanically cleaned with a toothbrush and stored in diluted (1:10) 5.25% sodium hypochlorite solution. A class V cavity preparation on the buccal surface was done with the following dimensions: one millimeter above and below the cervico-enamel junction (CEJ) corono-cervically by five millimeters mesio-distally. The smear layer was removed by etching with 38% phosphoric acid for one minute. The samples were ultra-sonicated in distilled water for 30 minutes as described in Lee et al. [44].

Prepared samples were randomly divided into three experimental and three control groups (n=12 per group). The three experimental groups were exposed to ten uL of three different size MNPs (100 nm, 300 nm, and 500 nm, Chemicell, Berlin, Germany). MNPs were applied, using calibrated pipettes, to the class V preparations. An external magnetic force (EMF, a neodymium magnet) was placed on the lingual surface of the sample, ten millimeters away from the class V preparation. This was achieved by placing soft wax between the samples and the magnet. This distance was verified by a ruler. Three control groups were exposed to the same MNP conditions without the application of EMF.

Exposure time was set to 30 minutes as this was enough time to deliver MNPs into the dentinal tubules as determined in a similar experiment performed in the laboratory (Masri et al, under review). A thirty-minute time frame is equivalent to the time needed by a dentist to perform a restoration on a tooth, so this was considered a “clinically acceptable time frame” according to Masri et al. Magnetite nanoparticles were utilized as opposed to maghemite because the superparamagnetic

properties of magnetite provide a mean magnetic moment only in EMF. This allowed control of the movement of the magnetite nanoparticles [51]. After 30 minutes, the excess material was washed away using isotonic saline and suction.

Samples were decalicified in 12% EDTA for four weeks, Formical-4 for four weeks, and 8% HCl for two weeks. They were then fixed, cryoprotected with 8% sucrose solution and frozen before being sectioned, horizontally, along the long axis of the tooth, into 25 µm sections. Sectioning was done using a Leica CM3050 Research Cryostat (Leica Biosystems Inc., Buffalo Grove, IL, USA). Six sections from each sample were mounted on slides and examined under a light microscope only, or under both light and fluorescent microscopes.

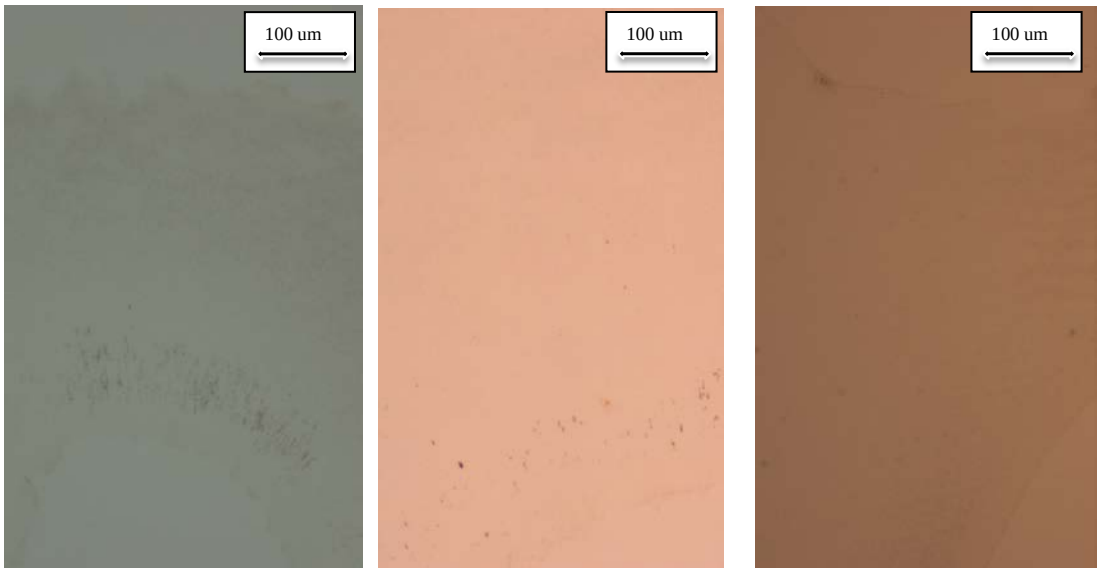
The following two microscopes were used to detect MNPs in the mounted sections: 1) a fluorescent stereomicroscope (MVX 10; Olympus Co., Tokyo, Japan) equipped with a sensitive black/white charged-coupled device camera and a long-pass filter (600 nm; CVI, Seoul, Korea) and 2) a light microscope (BX53M, Olympus Co., Tokyo, Japan). An image from each section was captured and analyzed [52] using IMAGEJ software (Wayne Rasband, National Institute of Health, v1.43u). The percentage of dentinal tubule penetration was calculated using the following formula:

$$Percentage\ of\ dentinal\ tubule\ penetration = \frac{total\ depth\ of\ dentinal\ tubule}{depth\ that\ MNP\ travelled}$$

Statistical Analysis

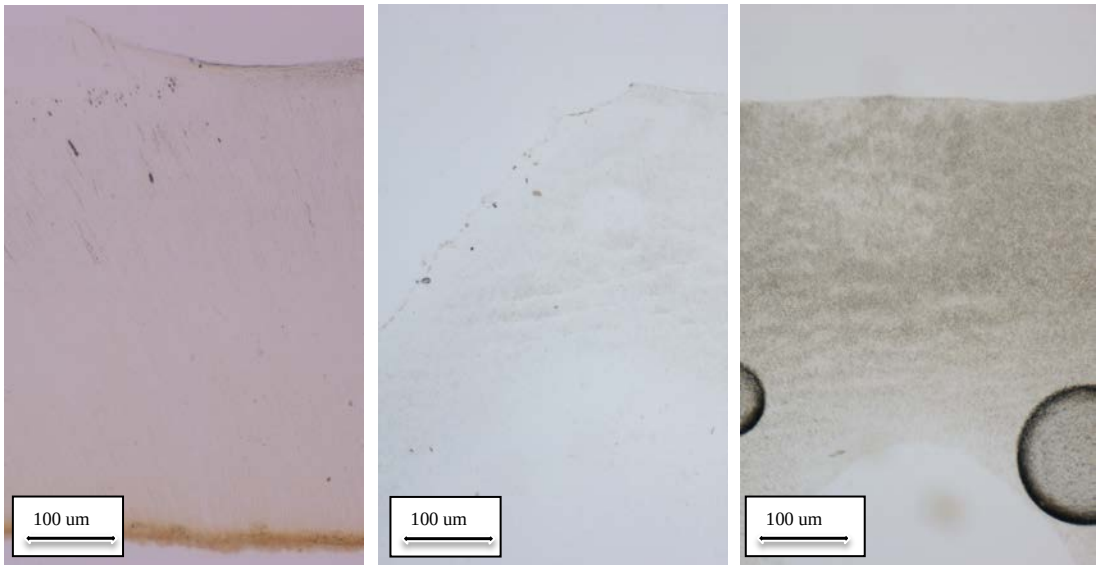
Since this research was new in focus, no data was available to use in conducting a power analysis. Masri et al. (under review), with a similar research design, used an n=12 per group. Therefore, for the present study, sample size was n=12. An analysis of variance (ANOVA) was used to test for significant differences followed by Tukey's Honestly Significant Difference (HSD) test, if needed. A p-value

of ≤.05 was considered significant.



A B C

Figure 1: Light micrographs of section of three different experimental groups (A) 100mm (B) 300nm and (C) 500nm



A B C

Figure 2: Light micrographs of section of three different control groups (A) 100mm (B) 300nm and (C) 500nm

Result

This study looked at a novel method for the treatment of dentinal hypersensitivity (DH) by utilizing magnetic nanoparticles (MNPs). DH was simulated by creating a class V preparation on an extracted human tooth. Then MNPs were applied to the class V preparation. A magnet was placed on the opposing side of the class V preparation for the experimental sample. No magnet was used for the control sample. All samples were decalcified, sectioned and mounted for visualization of MNPs through light and fluorescent microscopes (Fig. 1 and 2). The percentage of dentinal tubule penetration of the three different MNP groups (100nm, 300nm, 500nm) was calculated by measuring the total depth of the dentinal tubule from the inner surface of the preparation to the pulp (A). The second measurement was from the inner surface of the preparation to the depth that the MNPs travelled (B).

$$The\ percentage\ of\ dentinal\ tubule\ penetration = \frac{A}{B}\text{x}100$$

Although an n of 12 was the planned size for each group, in the 100nm experimental group, two samples were not included in the statistical analysis due to an improper mounting procedure. In the 300nm experimental group, one sample was not included due to loss of the sample during the freezing procedure.

Comparison Between 100, 300, and 500nm Sized NMPs in the Experimental Group

In the experimental group, there was a significant difference between the three different size MNPs ($F=180.365$, $p\leq.0005$). 100nm sized MNPs had a significantly higher percentage of dentinal tubule penetration (98.45±2.5%) than the two other sizes of MNP. 300nm sized MNPs had a significantly higher percentage of dentinal tubule penetration (18.55±20.8%) than the 500nm sized MNPs (4.46±3.5%). See Table 1.

Table 1: Percentage Penetration of Different Size of MNPs in Experimental Group

Size of MNP (nm)	n	Mean	Standard Deviation	Standard Error	F	p
100	10	98.45[a*]	2.5	.8	180.365	.0005
300	11	18.55[b]	20.8	6.3		
500	12	4.46[c]	3.5	1.0		

*Groups with different letters are significantly different

Comparison Between 100, 300, and 500nm Sized NMP in Control Group

In the control group, there was a significant difference between 100nm sized NMPs and the other two size NMPs (F=70.530, p≤.0005). 100nm size MNPs had a significantly higher percentage of dentinal tubule penetration (60.88±24.4%) than 300nm and 500nm size MNPs. There was no significant difference in percentage of dentinal tubule penetration between 300nm (1.88±0.9%) and 500nm size NMPs (1.46±1.3%). See Table 2.

Table 2: Percentage Penetration of Different Size of MNPs in Control Group

Size of MNP (nm)	n	Mean	Standard Deviation	Standard Error	F	p
100	12	60.88	24.4	7.0	70.530	.0005
300	12	1.88	.9	.3		
500	12	1.46	1.3	.4		

Comparison Between Experimental and Control Group for Each Size NMP

For all three size NMPs {100nm (F=23.364, p≤.0005), 300nm (F=7.747, p≤.011), 500nm (F=7.772, p≤.011)}, a significant difference was found. All experimental groups had a significantly higher percentage of dentinal tubule penetration than their controls. See Tables 3 to 5.

Table 3: Comparison Between 100nm Experimental and Control Groups

	n	Mean	Standard Deviation	Standard Error	F	p
Experimental Group	10	98.45	2.5	.8	23.364	.0005
Control Group	12	60.88	24.4	7.0		

Table 4: Comparison Between 300nm Experimental and Control Groups

	n	Mean	Standard Deviation	Standard Error	F	p
Experimental Group	11	18.55	20.8	6.3	7.747	.011
Control Group	12	1.88	.9	.3		

Table 5: Comparison Between 500nm Experimental and Control Groups

	n	Mean	Standard Deviation	Standard Error	F	p
Experimental Group	12	4.46	3.5	1.0	7.772	.011
Control Group	12	1.46	1.3	.4		

Discussion

A new treatment modality of dentinal hypersensitivity (DH) was examined in this study by utilizing magnetic nanoparticles (MNPs) with an external magnetic field (EMF). A class V preparation was made on an extracted human tooth to expose its dentinal tubules. MNP with EMF was applied for 30 minutes. After the decalcification process, samples were sectioned and mounted for visualization of the MNPs through light and fluorescent microscopes.

The results support the rejection of both null hypotheses. Smaller MNPs had a significantly higher percentage of dentinal tubule penetration than the larger MNPs. For each MNP size, the experimental group had a significantly higher percentage of dentinal tubule penetration than the control group.

Problems and Implications for Final Sample Size

In the 100nm experimental group, an immature mounting technique resulted in the loss of two samples. Of the ten final samples, four were analyzed via light microscope and six were analyzed via light as well as fluorescent microscopes. The fluorescent microscope allowed visualization of the six samples whose MNPs could not be visualized clearly via light microscope. With the light microscope, the image was captured and analyzed with the software. Sometimes, it was not possible to distinguish between debris and MNPs via the light microscope. Therefore, a fluorescent microscope was used for visualization of MNPs. Penetration was visualized to be definitely higher than 90%. However, due to mechanical problems with the image capturing hardware of the fluorescent microscope, it was not possible to obtain slide images suitable for IMAGEJ. Instead of measuring the penetration precisely, therefore, the depth of the penetration of the 100nm experimental group was estimated. It is believed that this number was fairly accurate because it was close to the estimated original penetration of 90%. This measurement error would not appear to affect the results of the study because, even with accurate measurement, the 100nm experimental group would have shown significantly higher penetration than both the 300nm and 500nm experimental groups.

Possible Explanations of High Standard Deviation found in Some Groups

In the 300nm experimental group, one sample was discarded because it was fractured during the sectioning process and was unable to be mounted. In addition, the high standard deviation in relation to the mean (18.55±20.8%) in this group might be due to the non-homogeneous size of the dentinal tubules within the group. This could have occurred for several reasons: age and specific health conditions of the patient and the time elapsed from extraction. Also, the 300nm size could have been small

enough to go through the dentinal tubules but large enough to create friction against the dentinal tubules or other MNPs. On the other hand, the 100nm size travelled through the dentinal tubule without any friction. The 500nm size was large enough that it could not even enter the dentinal tubule. Therefore, the 100nm and 500nm size had low standard deviations in relation to the mean (98.45±2.5% and 4.46±3.5%, respectively).

Although all three control groups had a lower percentage of dentinal tubule penetration than the experimental groups, clinically important penetration might have occurred only in the 100nm control group (60.88±24.4%). 90% of penetration indicates that the MNPs could be either close to the pulp or in the pulp. Therefore, this could make clinically important result. 60% of penetration might be enough penetration so that penetrated MNPs would stay and seal the patent dentinal tubules longer. EMF might not be the only force that permits MNPs to penetrate through the dentinal tubules. Other possible forces occurring in the control group might include osmotic pressure or other forces. The different sized dentinal tubules might have created different osmotic pressures in varying sized samples, therefore producing high standard deviation (24.4%). This standard deviation was one third of the mean of 100nm control group, whereas that of 300nm experimental group was much higher than the mean. Therefore, the data for 100nm control group was relatively more acceptable.

Comparing 100nm, 300nm and 500nm Experimental Groups

The 100nm experimental group showed a higher percentage of dentinal tubule penetration than the 300nm and 500nm groups. The 100nm size appeared to be an effective size to receive EMF from the magnet without causing any friction among the nanoparticles, allowing them to penetrate deeper into the dentinal tubule. The

500nm experimental group had virtually no penetration. This was because the average size of the dentinal tubules in this study was smaller than 500nm. This result could be due to the inadvertent desiccation of samples after the extraction and the collapse of the dentinal tubules. An in vivo study might eliminate the above variable.

Comparing 100nm, 300nm and 500nm Control Groups

Similar to the 100nm experimental group, the 100nm control group showed a higher percentage of dentinal tubule penetration (60.88±24.4%) than the 300nm and 500nm groups. Penetration was clinically important and this was probably due to osmotic pressure and other possible forces. The 300nm and 500nm control groups had virtually no penetration thus were clinically unimportant (1.88±.9% and 1.46±1.3%, respectively)

Comparing Experimental and Control Groups at the Three Different Sizes

Overall, the experimental group had a significantly higher percentage of dentinal tubule penetration than the control group in all three size MNPs. The difference between the two was large in all three size MNPs. This indicates that EMF appears to be an effective force in achieving a significant difference in penetration between the experimental and control groups.

Limitations to the Research

Given the small sample size of each group, it was impossible to control all the variables that might have affected the dependent variable, penetration. Following variables might have affected the size and shape of the dentinal tubule: 1) age and specific health conditions of the patient, 2) existing restoration on the tooth, 3) the time elapsed from extraction and 4) the different types of teeth. Direction of the dentinal tubule might also have affected the results. Even if clinically important

penetration was found, MNPs might not have been detected because the plane of the section might not coincide with the direction of the dentinal tubule. Because of all these variables, in vitro study may not reflect to in vivo study.

Suggestions for Further Research

A study with fewer uncontrolled variables should be conducted to provide a better understanding of the effect of MNPs in the closing patent dentinal tubules. variables could be minimized by collecting the same type of human tooth from the similar age of patients. An in vivo study could also be done to eliminate the chance of samples being desiccated. This would prevent the dentinal tubules from collapsing. Once the effect of MNPs for sealing patent dentinal tubules is shown, further research should be focused on the relationship between dentinal hypersensitivity (DH) and sealed dentinal tubules. Furthermore, an active pharmacological ingredient could be coupled to the MNPs. This agent could be delivered into pulp and thus chemically desensitize the nerve endings. In this way, DH could be more effectively treated.

Conclusions

Within the limitations of this in vitro study, the following conclusions were drawn:

1. Smaller magnetic nanoparticles have a significantly higher percentage of dentinal tubule penetration than the larger magnetic nanoparticles with or without an external magnetic field.
2. A significantly higher percentage of dentinal tubule penetration was found with application of the external magnetic field.
3. Application of the external magnetic field made a clinically important difference in the percentage of dentinal tubule penetration for the 100nm and

300nm groups.

This new technology could allow the clinician to seal the patent dentinal tubule for longer periods of time and desensitize the nerve endings. Therefore, dentinal hypersensitivity can be managed in a better fashion.

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