A new clinical use of Ferumoxytol nanoparticles: an anti-biofilm treatment

NCT06110494

17-Dec-2021

Protocol Details

Basic Info

Confirmation Number:	**ceheehgd**
Protocol Number:	**828211**
Created By:	
Principal Investigator:	
Protocol Title:	**A new clinical use of Feraheme nanoparticles: an anti-biofilm treatment**
Short Title:	**Feraheme nanoparticles in Endodontics**
Protocol Description:	**The purpose of this study is to to develop a protocol for biofilms disinfection with a clinically approved and commercially available iron oxide nanoparticle formulation Feraheme/H2O2 treatments. This protocol will be testing local single topical application of Feraheme within the canal system in patients going through routine endodontic treatment, evaluate its potential as anti-biofilm treatment and compare it to other currently used antibacterial protocols.**
Submission Type:	**Biomedical Research**
Application Type:	**FULL**

Resubmission*
Yes

Study Personnel

Principal Investigator

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HS Training Completed:	**03/01/2018**
Training Expiration Date:	**CITI Protection of Human Subjects Research Training - ORA**
Name of course completed :	

Study Contacts

None

Other Investigator

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HS Training Completed:	**02/06/2020**
Training Expiration Date:	**CITI Protection of Human Subjects Research Training - ORA**
Name of course completed :	

Responsible Org (Department/School/Division):

5112 - Endodontics

Key Study Personnel

Name:	**Orthodontics**
Department/School/Division:	**Yes**
HS Training Completed:	**06/15/2018**
Training Expiration Date:	**CITI Protection of Human Subjects Research Training - ORA**
Name of course completed:	

Disclosure of Significant Financial Interests*
Does any person who is responsible for the design, conduct, or reporting of this research protocol have a **FINANCIAL INTEREST**?
No

Penn Intellectual Property*
To the best of the Principal Investigator's knowledge, does this protocol involve the testing, development or evaluation of a drug, device, product, or other type of intellectual property (IP) that is owned by or assigned to the University of Pennsylvania?
Yes

Certification
I have reviewed the *Financial Disclosure and Presumptively Prohibited Conflicts for Faculty Participating in Clinical Trials* and the *Financial Disclosure Policy for Research and Sponsored Projects* with all persons who are responsible for the design, conduct, or reporting of this research; and all required Disclosures have been attached to this application.
Yes

Biomedical Research

Investigator Initiated Trial
Is this an investigator-initiated trial?
Yes

Drugs or Devices*
Does this research study involve Drugs or Devices?
Yes: Drugs, products or devices prepared or used in a manner not in the FDA-approved labeling.

IND Exemption

Obtained

For studies that fall under an IND exemption, please provide the number below

For studies including IND or IDE's, please provide the number(s) below

IDE Review*
NOTE: For research involving investigational devices, you are required to review the guidance on Managing Research Device Inventory. Consult the Penn Manual for Clinical Research: https://somapps.med.upenn.edu/pennmanual/secure/pm/investigational-product-management Please check the box Yes if you have reviewed the guidance.
Yes

Research Device Management*
Please indicate how research device(s) will be managed.
Not Applicable (no investigational devices)

Drug, Herbal Product or Other Chemical Element Management *
Please indicate how drugs, herbal products or other chemical entities will be managed.
The drug, herbal product or other chemical entity will be received, stored and dispensed by the research team (please provide information in the protocol summary as to how this will be conducted)

Radiation Exposure*
Are research subjects receiving any radiation exposure (e.g. X-rays, CT, Fluoroscopy, DEXA, pQCT, FDG, Tc-99m, etc.) that they would not receive if they were not enrolled in this protocol?
No

Gene Transfer*
Does this research involve gene transfer (including all vectors) to human subjects?
No

Human Source Material*
Does this research include collection or use of human source material (i.e., human blood, blood products, tissues or body fluids)?
No

CACTIS and CT Studies*
Does the research involve Center for Advanced Computed Tomography Imaging Services (CACTIS) and CT studies that research subjects would not receive if they were not part of this protocol?
No

CAMRIS and MRI Studies*
Does the research involve Center for Advanced Magnetic Resonance Imaging and Spectroscopy (CAMRIS) and MRI studies that research subjects would not receive if they were not part of this protocol?
No

Investigational Agent or Device within the Operating Room*
Does the research project involve the use of an investigational agent or device within the Operating

Room?
No

Cancer Related research not being conducted by an NCI cooperative group*
Does this protocol involve cancer-related studies in any of the following categories?
No

Processing of Materials*
Will the research involve processing (such as over encapsulating, or compounding)?
No

In-House Manufacturing of Materials*
Will the research involve processing (such as over encapsulating, or compounding)?
No

Medical Information Disclosure*
Does the research proposal involve the use and disclosure of research subject's medical information for research purposes?
Yes

If the answer is YES, indicate which items is is provided with this submission:

Modified research informed consent document that incorporates HIPAA requirements

CTRC Resources*
Does the research involve CTRC resources?
No

Pathology and Laboratory Medicine Resources*
Will samples be collected by hospital phlebotomy and/or processed or analyzed by any of the clinical laboratories of the University of Pennsylvania Health System?
No

Research Involves Apheresis, Cell Collection, and/or Blood Product Collection*
Does this research involve collection of blood products in the Penn Donor Center and/or the use of apheresis for treatment or collection of cells or other blood components?
No

Research involving blood transfusion or drug infusions*
Will your research involve blood transfusion or infusion of study drug in 3 Ravdin Apheresis Unit for research purposes?
No

Trial in Radiation Oncology
Is this research a prospective trial being done in Radiation Oncology, and if so, has this protocol been approved by the Radiation Oncology Protocol committee?
N/A

Study in Radiation Oncology
Is this research a retrospective study being done in Radiation Oncology, and if so, has this project been reviewed by the Radiation Oncology Clinical Research Group?
N/A

Use of UPHS services*
Does your study require the use of University of Pennsylvania Health System (UPHS) services, tests or procedures*, whether considered routine care or strictly for research purposes?
No

Primary Focus*
Clinical Trial (prospectively assigning subjects to health-related interventions to evaluate outcomes)

Protocol Interventions

	Sociobehavioral (i.e. cognitive or behavioral therapy)
x	**Drug**
	Device - therapeutic
	Device - diagnostic (assessing a device for sensitivity or specificity in disease diagnosis)
	Surgical
	Diagnostic test/procedure (research-related diagnostic test or procedure)
	Obtaining human tissue for basic research or biospecimen bank
	Survey instrument
	None of the above

The following documents are currently attached to this item:

There are no documents attached for this item.

Sponsors

Business Administrator

none

Department budget code

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Funding Sponsors

Funding sponsors billing address
If you have selected a commercial or industry sponsor, please provide the appropriate address and contact information for the Sponsor for the purposes of billing for IRB review fees (initial review, continuing review and convened modification fees apply here). If the Sponsor is not industry/ commercial, this information is not necessary to provide with your application.

Funding sponsors gift
Is this research being funded by a philanthropic gift?

Regulatory Sponsor

IND Sponsor

none

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Industry Sponsor

None

Project Funding*
Is this project funded by or associated with a grant or contract?
No

Sponsor Funding
Is this study funded by an industry sponsor?

Status of contract

The following documents are currently attached to this item:

There are no documents attached for this item.

Multi-Site Research

Other Sites

No other sites

Management of Information for Multi-Center Research
N/A

The following documents are currently attached to this item:

There are no documents attached for this item.

Protocol

Abstract
Biofilms are aggregates of microorganisms that are attached to a surface and enmeshed in a protective extracellular polymeric matrix, causing many infectious diseases. Biofilm-associated infections are notoriously difficult to treat. Bacterial infections in the root canal lead to high rates of failure of endodontic treatments. Conventional drugs often fail to kill the microbes embedded within biofilms and are incapable of destroying the matrix. New approach using catalytic nanoparticles (CAT-NP) that are biocompatible and have potent anti-biofilm action has been proposed. Studies on CAT-NP have shown that CAT-NP rapidly catalyze low concentrations of H2O2 to generate free radicals in situ to degrade the biofilm matrix and simultaneously kill embedded bacteria with exceptional efficacy. Moreover, the treatment suppresses virulent biofilm formation without cytotoxicity in an animal biofilm model. Recently, a clinically approved and commercially available iron oxide nanoparticle formulation with similar catalytic activity and anti-biofilm properties of CAT-NP became available. The purpose of this study is to develop a protocol for biofilms disinfection with a clinically approved and commercially available iron oxide nanoparticle formulation Feraheme. This protocol will be testing a local single topical application of Feraheme within the canal system in patients going through routine endodontic treatment, will evaluate its potential as anti-biofilm treatment and compare it to other currently used antibacterial protocols

Objectives

Overall objectives
The purpose of this study is to develop a better, more effective disinfection protocol against biofilms within the root canal system, subsequently, improve the success of root canal treatment in teeth with apical periodontitis. This protocol will be testing local single topical application of a very low dose of Feraheme within the canal system in patients going through routine endodontic treatment, will evaluate its potential as a novel antibacterial, anti-biofilm treatment by intra-canal bacterial sampling and compare it to other currently used antibacterial protocols

Primary outcome variable(s)
Studies has shown that the bacteria within tooth root canal system is the primary etiology for the root- end disease process. The reduction or elimination of microorganisms from the root canal system of infected teeth has been shown to correlate with the long-term success of the endodontic treatment. The main objective of root canal treatment is to disinfect root canal systems entirely and seal to prevent further contamination. However, with current techniques, it is impossible to eradicate bacteria and biofilm completely from the complex root canal system. This study is designed to evaluate the antibacterial efficacy of Feraheme/H2O2 and to compare it to the routinely used protocol. The outcome

will be evaluated by taking bacterial samples from the root canals during routine root canal treatment after applying different disinfection protocols. Bacteria from each sample will be quantified. The primary outcome parameter will be the difference in bacterial reduction between the experimental group (Feraheme) and a comparison group (routinely used protocol).

Secondary outcome variable(s)
Complex anatomy limit the efficacy of disinfection solutions and protocols. By evaluating bacterial reduction in different canal configurations, we will have a better understanding on the efficacy of tested solutions. Furthermore, proof-of-concept experiments in endodontic treatment could open the door for Feraheme to be used in numerous clinical anti-biofilm applications.

Background
Endodontic research has established the importance of microorganisms in the pathogenesis of apical periodontitis (1-3). The reduction or elimination of microorganisms is a logical aim for successful endodontic treatment. Sjörgen et al (4) evaluated the long-term outcome after one step endodontic treatment of teeth with negative or positive cultures after instrumentation. 94% of the teeth filled after a negative culture succeeded, whereas only 68% of the canals filled after a positive culture had been detected were successful. Based on this study the optimal clinical success will be reached if a tooth is filled when the bacterial load of the canal is undetectable with culturing methods. Because culturing is as yet only effective as a research tool, the clinician should expect optimal success when treating an infected tooth with a technique or protocol that has been shown experimentally to result in consistent negative cultures. Byström et al (5-7) published a series of studies to evaluate the antibacteriological effects of the individual steps in the endodontic procedure. Only after instrumentation using endodontic instruments with NaOCl and EDTA irrigation and a dressing of calcium hydroxide it was possible to obtain a predictable negative culture (5-7). However, Histo-bacteriological studies have shown that even after meticulous chemo-mechanical preparation using endodontic instruments with NaOCl and EDTA and obturation of the root canal system, a significant amount of bacterial biofilm is often present in the anatomical complexities of the root canal system (isthmuses, apical ramification, accessory canals, and dentinal tubules) and on the walls of the main root canal that was not engaged by the root canal instruments (8-10). Endodontic biofilms may play a key role in infections persisting after primary or secondary endodontic treatment, as microorganisms growing in biofilms are better protected from adverse environmental changes and other antimicrobial agents. Ricucci et al, (11) evaluated the prevalence of bacterial biofilms in untreated root canals of teeth showing apical periodontitis in 80% of the cases. Nevertheless, the persistence of endodontic infections is also attributed to the presence of the aforementioned anatomical complexities, which can harbor biofilm and protect it from the effects of the current disinfection solutions (12). Based on these facts, advanced disinfection strategies with the ability to disrupt biofilm are needed to achieve the goal of endodontic treatment and to overcome the persistence of endodontic infections, if possible using less harsh chemicals as currently used (e.g. NaOCl). Hydrogen peroxide (H_2O_2) is commonly used as a safe green chemical (13) for disinfection purposes or as tooth-whitening agent (at concentrations as high as 10%) because it generates free radicals that exhibit antibacterial activity or stain removal (through degradation of polymeric substances). However, the process of free radical generation is slow and H_2O_2 by itself has a modest anti-biofilm or endodontic disinfection effects when used alone. It has been shown that catalytic iron oxide nanoparticles (CAT-NP) have enhanced peroxidase-like activity that catalyzes hydrogen peroxide (H_2O_2) to create a potent anti-biofilm action in vivo (14). Very low concentrations of CAT-NP (at 0.5 mg/ml) rapidly catalyze H_2O_2 to generate free radicals in situ that simultaneously degrade the biofilm matrix and kill embedded bacteria with exceptional efficacy within 5 minutes (5,000-fold more effective than H_2O_2 alone), without cytotoxic effects on oral tissues in vivo (14). Recently, in a preliminary in- vitro study we tested the antibacterial activity of CAT-NP/ H_2O_2 in comparison to 3% H_2O_2, 3% NaOCl, and 2% CHX, the latter two solutions are current disinfectants used in endodontic treatment. The solutions were topically placed on dentine blocks that were infected with E. faecalis, a commonly isolated microorganism from root canal treated teeth with persistent apical periodontitis. Our results show that the antibacterial activity of CAT-NP/H_2O_2 was significantly better than the conventional irrigants. CAT-NP/H_2O_2 was up to 30-fold more effective in bacterial killing compared to NaOCl and CHX. These results reveal the potential to exploit CAT-NP/H_2O_2 as a potent alternative approach for treatment of endodontic infections. Recently, an FDA-approved iron oxide nanoparticle formulation known as Feraheme became available commercially. Our in-vitro studies have shown that the peroxidase-like activity of Feraheme is similar to CAT-NP and this nanoparticle with H_2O_2 exhibited similar catalytic activity and potent anti-biofilm effects against endodontic biofilms. Feraheme is approved as an intravenous treatment for iron deficiency with repeated dosing at 510 mg. For

endodontic applications, only a single topical treatment of up to 6 mg (which is 85 times less than the FDA-approved dose) would be used for a transient exposure (for less than 10 minutes) of infected tooth canal in the oral cavity, supporting the potential clinical translation of a Feraheme/H2O2 anti- biofilm treatment. Proof-of-concept experiments in endodontic treatment could open the door for Feraheme to be used in numerous clinical anti-biofilm applications. The assessment of bacterial reduction during endodontic therapy by bacterial sampling and culturing is an established technique and has been used to evaluate treatment protocols or variations thereof (15-18). There is a current need for a better disinfecting agent that is capable of overcoming the limitations of the currently used irrigation solutions, and based on our preliminary findings, CAT-NP/H2O2 has a potential as a novel anti-biofilm clinical therapy with its potent antibacterial and anti-biofilm activities. The purpose of this study is to develop an irrigation disinfection protocol for eradicating biofilms with a clinically approved and commercially available iron oxide nanoparticle formulation Feraheme. This protocol will be testing local application of Feraheme within the canal system in patients going through routine endodontic treatment, will evaluate its potential as a novel anti-biofilm disinfection solution and compare it to other currently used antibacterial protocols.

References: 1. Kakehashi S, Stanley H, Fitzgerald R. The effect of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965; 20:340-9. 2. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. Odontol Revy 1974; 25:347-58. 3. Kerekes K, Tronstad L. Long-term results of endodontic treatment performed with a standardized technique. J Endodon 1990; 16:498-504. 4. Sjörgen U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. International Endodontic Journal 1997; 30:297-306. 5. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981;89:321-8. 6. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol. 1983;55:307-12. 7. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J. 1985;18:35-40. 8. Nair, P.N., et al., Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2005. 99(2): p. 231-52. 9. Ricucci, D., et al., Histologic investigation of root canal-treated teeth with apical periodontitis: a retrospective study from twenty-four patients. J Endod, 2009. 35(4): p. 493-502. 10. Vera, J., et al., One- versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. J Endod, 2012. 38(8): p. 1040-52. 11. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod. 2010;36:1277-88. 12. Kishen, A., Advanced therapeutic options for endodontic biofilms. Endodontic topics, 2010. 22: p. 99-123. 13. Andrea, G. F., C., Hydrogen peroxide in green oxidation reactions: recent catalytic processes. In Green Chemical Reactions, Tundo, P.; Esposito, V., Eds. Springer: The Netherlands, 2008; pp 191-212. 14. Gao, L.; Liu, Y.; Kim, D.; Li, Y.; Hwang, G.; Naha, P. C.; Cormode, D. P.; Koo, H., Nanocatalysts promote Streptococcus mutans biofilm matrix degradation and enhance bacterial killing to suppress dental caries in vivo. Biomaterials 2016, 101, 272-284. 15. Dalton BC, Orstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. J Endod. 1998;24:763-7.

16. Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod. 2000;26:751-5. 17. McGurkin-Smith R, Trope M, Caplan D, Sigurdsson A. Reduction of intracanal bacteria using GT rotary instrumentation, 5.25% NaOCl, EDTA, and Ca(OH)2. J Endod. 2005;31:359-63. 18. Wang CS, Arnold RR, Trope M, Teixeira FB. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. J Endod. 2007 ;33:1283-9.

Study Design

Phase*
Not applicable

Design
Patients presenting to the Department of Endodontics, School of Dental Medicine, University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis will be asked to take part in the study if they meet the inclusion criteria specified below and volunteer to participate. Prior to patient recruitment a randomized chart was drafted that assigns teeth to either NaOCL or Feraheme/H2O2 (amended). After eligibility of the patient is confirmed, the randomly assigned protocol for the tooth to be treated will be looked up by the principal

investigator. Before treatment, patients will be thoroughly informed about the nature, potential risks and alternatives of the study as well as the root canal treatment. Patients will be presented with a written consent form regarding the above mentioned study characteristics (amended to this IRB proposal) as well as the regular consent forms for the root canal therapy, including the consent form for endodontic treatement, acknowledgement of privacy practices and a patient understanding and informed consent form. Patients will be eligible to participate in the study for NaOCL or Feraheme/H2O2 if the following inclusion criteria are met: 1. Patients to welling to participate in the study. 2. Patients are 18 years or above. 3. Non-contributory medical history (Patient can be seen for regular dental appointment in PDM; ASA classes I and II). 4. Tooth requiring root canal treatment with radiographic presence of periapical radiolucency and responding to thermal sensitivity testing negatively (difluordichlormethane at 50 °C) (Endo-Ice, Coltène/Whaledent Inc., Cuyahoga Falls, Ohio) and Negatively to EPT testing. 5. Tooth with adequate remaining tooth structure for proper isolation with rubber dam. 6. No history of previous endodontic treatment on the tooth. 7. Teeth with single canal and single and roots with single canals in multirooted teeth. Patients will not be eligible to participate in the study if any of the following exclusion criteria applies: 1. Self-reported Pregnancy. 2. Patients requiring antibiotic premedication prior to dental treatment. 3. Patients with multiple drug allergies. 4. Patients with known hypersensitivity to Ferumoxytol nanoparticles or any iron products. 5. Patients who are scheduled for MRI for the head region within three months after Fer nanoparticles application. 6. Periodontal changes (pockets 3 mm, mobility Grade I or gingival edema). 7. Radiographic presence of resorptive processes. 8. Cracked and fractured teeth. 9. if one of the inclusion criteria is not met.The treatment and sampling procedures will largely follow the protocol described by Shuping et al. (19). Briefly, Patients presenting to the Department of Endodontics for evaluation and treatment of infected, necrotic teeth with chronic apical periodontitis will be asked if they want to participate in the study. Informed consent will be obtained by the principal investigator. The patient will be anesthetized, and the tooth will be isolated with rubber dam and opal dam will be placed around the tooth. The tooth, the clamp, and the rubber dam will be cleansed using 30% hydrogen peroxide and disinfected with 3% NaOCl. After access preparation with sterile burs and sterile saline irrigation, thermoplastic gutta percha will be placed to temporarily block the orifice. The field, including the pulp chamber, will be cleaned and disinfected as described previously. NaOCl will be neutralized with 10% sodium thiosulfate. After removal of the gutta percha from the root canal orifices and working length determination, Liquid Dental Transport Media (LDT) will be placed in the canal with a sterile 31g needle, and a #15 Hedstrom file will be used in a filing motion to scrape the canal walls and agitate the canal content. (S1) sample will be taken by placing a sterile paper point in the canal, allow it to saturate for 30 seconds, and then transfer to a tube containing LDT. This step will be once more with a second paper point. The treatment solution (saline, NaOCl, or Fer/H2O2) 2 mL will be introduced into the canal. Canals will be instrumented with 15/0.04, 20/0.04, and 25/0.04 using 2 mL of treatment solution after each file with a total of 8 mL and a total contact time of 10 minutes. Canal contents will be deactivated with sodium thiosulphate for NaOCl, and saline wash will be used for Fer/H2O2 and saline treatments. A wash step with 1 mL saline will be done to wash the deactivating solution, and paper points will be used to dry the canals. A second bacterial sample will be taken (S2) by placing LDT inside the canal, agitating it with 25/0.02 Hedstrom hand file, followed by absorbing the content with 2 paper points placed in the canal for 30 seconds each. The paper points will be placed inside a tube containing LDT. An additional step was included for all the test groups to evaluate if further irrigation after (S2) will lead to more reduction of bacterial counts inside the root canal system. This could inform future experiments evaluating the possibility of synergistic antimicrobial effects for the sequential Fer/H2O2 and NaOCl treatment. All canals in all groups were irrigated with 2 mL of 3% NaOCl (1 min), followed by ultrasonic irrigant activation for 30 seconds. This step was repeated once again making the total activation time 1 min and the total contact time of the irrigant 3 min. Upon completion of irrigation, a third bacterial sample (S3) was taken following the deactivation, washing, drying, and sampling steps as described above.The remaining treatment sequences of the routine root canal therapy will be carried out after these procedures including further root-end enlargement and final routine irrigation protocol. The root canals will be dried with paper points, a medication (calcium hydroxide) will be placed and the teeth sealed with a temporary restoration. Patients will return after one to four weeks for completion of the root filling. For the any of the groups, the treatment procedures carried out during this investigation do not differ from the standard root canal treatment protocol with the exception of additional irrigation step with the experimental solution and the bacteriologic sampling procedures. The paper points used to take the bacteriological sampling will be transferred to the microbiology laboratory using a vial containing 1 ml of reduced transport fluid (LDT). The laboratory procedures will be performed at the University of Pennsylvanias Leon Levy Oral Health Sciences Building of the School of Dental Medicine in the Microbiology Laboratory (Dr. M. Koo's laboratory). Vial labels will contain information on tooth number, sample number (S0-S1-S2-S3) and the experimental group. Patient information will not be used in any case. LDT vials with samples will be vortexed before preparing aliquots. Samples with dilutions of 10, 100, and 1000-fold will be prepared

under anaerobic conditions using sterile glassware. Cell culture dishes with anaerobic sheep blood agar will be inoculated with 0.25 ml of undiluted sample, as well as each of the three dilutions. The culture plates will be incubated at 37°C in an anaerobic glove box containing 5% hydrogen, 10% nitrogen, and 85% CO2 for 7 days. After incubation the number of colony forming units will be determined by using a stereoscope. ANOVA and Students t-test will be used for statistical analysis.

Study duration

The projected time frame for this research project is from May 2017 to May 2018. The recruitment and

treatment of patients with 60 teeth matching the inclusion criteria mentioned above can be reasonably achieved within these 12 months. Included patients will not participate in this proposed study longer than the time necessary for the routine root canal therapy.

Resources necessary for human research protection

Describe research staff and justify that the staff are adequate in number and qualifications to conduct the research. Describe how you will ensure that all staff assisting with the research are adequately informed about the protocol and their research related duties. Please allow adequate time for the researchers to conduct and complete the research. Please confirm that there are adequate facilities for the research.

Patients will be treated in the Endodontic Clinic of the University of Pennsylvania's Department of Endodontics of the School of Dental Medicine (PDM). The Endodontic Clinic exclusively handles all root canal procedures carried out at the PDM with the exception of Dental Care Network facilities. By postgraduate residents alone, more than 1500 root canal procedures are done routinely per year. Patients are PDM patients and come from the Restorative Department of the PDM, emergency admissions or referred by outside dental clinics directly to the clinic. The recruitment and treatment of patients with 60 teeth matching the inclusion criteria mentioned above can be achieved within a reasonable time. 16 residents are enrolled at any time for postgraduate studies at the Endodontic Department. The instructions regarding the technical aspects and the human research protection issues will be given to the residents during specific research meetings that are held regularly in the Department for updates on progress and conduct of research activities within the Department. The primary investigator will inform all residents seeing patients about all aspects concerning the protection of human research participants and will review the research protocol and all associated consent forms to ensure calibration, proper conduct and participants protection at all times. Before treatment of an individual patient potentially suitable for treatment under the conditions of this investigation, the research consent form, the general root canal treatment consent form, OSHA, HIPPA, privacy notice etc. will be reviewed with the patient. Besides the principal investigator (BK), who is a full-time member of the Endodontic Department, and the co-investigator (AB), who is one of the endodontic residents, endodontic residents are under supervision of full-time and/or part-time faculty members during patient treatments at any time. The principal investigator and co-investigator received training, conducted research in cell culture techniques and analysis. Needs for medical or psychological services are not a possible consequence of the bacterial sampling procedures to be undertaken during the routine root canal therapy for this research project. Any medical needs that should arise during the routine endodontic therapy are covered by the faculty and staff of the PDM or the University of Pennsylvania’s medical emergency services (511) as for any other dental procedure routinely carried out at the PDM. The laboratory part of the investigation will be performed in the Microbiology Laboratory (Dr. M. Koo's laboratory) of the Leon Levy Oral Health Sciences Building of the School of Dental Medicine. This laboratory is well equipped for cell culture and the handling of anaerobic bacteria, including an anaerobic glove box as well as all standard equipment for laboratory cell culture.

Characteristics of the Study Population

Target population

Patients presenting to the Department of Endodontics, School of Dental Medicine, University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis.

Subjects enrolled by Penn Researchers

60

Subjects enrolled by Collaborating Researchers

0

Accrual

Patients will be treated in the Endodontic Clinic of the University of Pennsylvania's Department of Endodontics of the School of Dental Medicine (PDM). The Endodontic Clinic exclusively handles all root canal procedures carried out at the PDM, with the exception of Dental Care Network facilities. By

postgraduate residents alone, more than 1500 root canal procedures are done routinely per year. Patients are PDM patients coming from the Restorative Department of the PDM, emergency admissions or referred from outside private clinics directly to the clinic. The recruitment and treatment of patients with 60 teeth matching the inclusion criteria mentioned above can be achieved within a reasonable time. The clinical significance of this investigation will not be evaluated by statistical analysis based on overall sample size. A target negative culture threshold is well established in the literature for an acceptable success rate (refer to Background, reference 4) and the experimental and comparison groups are evaluated as successful (or unsuccessful) for their ability to reach this threshold (or not). Comparable studies investigating the reduction of intra-canal bacteria after changes in treatment protocol have been conducted with similar sample sizes (refer to Background, references 15-18).

Key inclusion criteria

Gender is not an inclusion or exclusion criteria. Patients will be eligible to participate in the study if the following inclusion criteria are met: 1. Patients to welling to participate in the study. 2. Patients are 18 years or above. 3. Non-contributory medical history (Patient can be seen for regular dental appointment in PDM; ASA classes I and II). 4. Tooth requiring root canal treatment with radiographic presence of periapical radiolucency and responding to thermal sensitivity testing negatively (difluordichlormethane at 50 °C) (Endo-Ice, Coltène/Whaledent Inc., Cuyahoga Falls, Ohio) and Negatively to EPT testing. 5. Tooth with adequate remaining tooth structure for proper isolation with rubber dam. 6. No history of previous endodontic treatment on the tooth. 7. Teeth with single canal and single and roots with single canals in multirooted teeth.

Key exclusion criteria

Economic status, gender, race or ethnicity are not of concern for the proposed investigation and therefore for study exclusion. The cut-off age of 18 years was only chosen so as to limit the need for parental consent. Patients will not be eligible to participate in the study if any of the following exclusion criteria applies: 1. Self-reported Pregnancy. 2. Patients requiring antibiotic premedication prior to dental treatment. 3. Patients with multiple drug allergies. 4. Patients with known hypersensitivity to Ferumoxytol nanoparticles or any iron products. 5. Patients who are scheduled for MRI for the head region within three months after Fer nanoparticles application. 6. Periodontal changes (pockets 3 mm, mobility Grade I or gingival edema). 7. Radiographic presence of resorptive processes. 8. Cracked and fractured teeth. 9. if one of the inclusion criteria is not met.

Vulnerable Populations

	Children Form
	Pregnant women (if the study procedures may affect the condition of the pregnant woman or fetus) Form
	Fetuses and/or Neonates Form
	Prisoners Form
	Other
x	**None of the above populations are included in the research study**

The following documents are currently attached to this item:

There are no documents attached for this item.

Populations vulnerable to undue influence or coercion

Given the nature of the suggested study and the non-invasive procedures (irrigation within the closed environment of root canal, procedure of taking bacterial samples from a root canal) none of the potential participants appear to be likely to be vulnerable to coercion or undue influence or influence. Potential participants will receive the elected root canal treatment if they are participating in the study or if they are not participating in the study. In the event that Penn students or employees present for screening they will be informed that their decision regarding whether or not to participate will in no way impact their standing at the university. Given the number of patients seen on a regular basis in the Department of Endodontics and the common nature of the diseases pulp necrosis with apical periodontitis undue influence or coercion is not likely.

Subject recruitment
Patients presenting to the Department of Endodontics, School of Dental Medicine (PDM), University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis will be asked to take part in the study if they meet the inclusion criteria specified above. Patients will be asked if they were interested to participate in this investigation after a clinical diagnosis matching is confirmed and the patient/ tooth meets the inclusion criteria specified above. Initial clinical diagnosis will be made by a postgraduate resident and verified by the supervising faculty. The PI, a full-time faculty, and the co-investigator, endodontic resident, will be informed about a potential study participant, verify the diagnosis and ask for participation (including consent and alternatives). No additional recruitment materials, such as radio/video scripts, flyers, internet postings, etc. will be used. No non-registered PDM patients will be asked to participate in the study.

Will the recruitment plan propose to use any Penn media services (communications, marketing, etc.) for outreach via social media avenues (examples include: Facebook, Twitter, blogging, texting, etc.) or does the study team plan to directly use social media to recruit for the research?
No

The following documents are currently attached to this item:

There are no documents attached for this item.

Subject compensation*
Will subjects be financially compensated for their participation?
No

The following documents are currently attached to this item:

There are no documents attached for this item.

If there is subject compensation, provide the schedule for compensation per study visit or session and total amount for entire participation, either as text or separate document

N/A

Study Procedures

Suicidal Ideation and Behavior
Does this research qualify as a clinical investigation that will utilize a test article (ie- drug or biological) which may carry a potential for central nervous system (CNS) effect(s)?
No

Procedures
Patients presenting to the Department of Endodontics, School of Dental Medicine, University of Pennsylvania for evaluation and routine endodontic treatment of infected, necrotic teeth with chronic apical periodontitis will be asked to take part in the study if they meet the inclusion criteria specified below and volunteer to participate. Prior to patient recruitment a randomized chart was drafted that assigns teeth to either NaOCL, saline or Feraheme/H2O2 (amended). After eligibility of the patient is confirmed, the randomly assigned protocol for the tooth to be treated will be looked up by the principal investigator. Before treatment, patients will be thoroughly informed about the nature, potential risks and alternatives of the study as well as the root canal treatment. Patients will be presented with a written consent form regarding the above mentioned study characteristics (amended to this IRB proposal) as well as the regular consent forms for the root canal therapy, including the consent form for endodontic treatement, acknowledgement of privacy practices and a patient understanding and informed consent form. Patients will be eligible to participate in the study for NaOCL (positive control), saline (negative control or Feraheme/H2O2 if the following inclusion criteria are met: 1. Patients to welling to participate in the study. 2. Patients are 18 years or above. 3. Non-contributory medical history (Patient can be seen for regular dental appointment in PDM; ASA classes I and II). 4. Tooth requiring root canal treatment with radiographic presence of periapical radiolucency and responding to thermal sensitivity testing negatively

(difluordichlormethane at 50 °C) (Endo-Ice, Coltène/Whaledent Inc., Cuyahoga Falls, Ohio) and Negatively to EPT testing. 5. Tooth with adequate remaining tooth structure for proper isolation with rubber dam. 6. No history of previous endodontic treatment on the tooth. 7. Teeth with single canal and single and roots with single canals in multirooted teeth. If upon access this canal configuration can not be verified, the routine endodontic treatment will continue without bacterial sampling and the tooth not included in the investigation. Patients will not be eligible to participate in the study if any of the following exclusion criteria applies: 1. Self-reported Pregnancy. 2. Patients requiring antibiotic premedication prior to dental treatment. 3. Patients with multiple drug allergies. 4. Patients with known hypersensitivity to Ferumoxytol nanoparticles or any iron products. 5. Patients who are scheduled for MRI for the head region within three months after Fer nanoparticles application. 6. Periodontal changes (pockets 3 mm, mobility Grade I or gingival edema). 7. Radiographic presence of resorptive processes. 8. Cracked and fractured teeth. 9. if one of the inclusion criteria is not met. The treatment and sampling procedures will largely follow the protocol described by Shuping et al. (19). Briefly, Patients presenting to the Department of Endodontics for evaluation and treatment of infected, necrotic teeth with chronic apical periodontitis will be asked if they want to participate in the study. Informed consent will be obtained by the principal investigator. The patient will be anesthetized, and the tooth will be isolated with rubber dam and opal dam will be placed around the tooth. The tooth, the clamp, and the rubber dam will be cleansed using 30% hydrogen peroxide and disinfected with 3% NaOCl. After access preparation with sterile burs and sterile saline irrigation, thermoplastic gutta percha will be placed to temporarily block the orifice. The field, including the pulp chamber, will be cleaned and disinfected as described previously. NaOCl will be neutralized with 10% sodium thiosulfate. After removal of the gutta percha from the root canal orifices and working length determination, Liquid Dental Transport Media (LDT) will be placed in the canal with a sterile 31g needle, and a #15 Hedstrom file will be used in a filing motion to scrape the canal walls and agitate the canal content. (S1) sample will be taken by placing a sterile paper point in the canal, allow it to saturate for 30 seconds, and then transfer to a tube containing LDT. This step will be once more with a second paper point. The treatment solution (saline, NaOCl, or Fer/H2O2) 2 mL will be introduced into the canal. Canals will be instrumented with 15/0.04, 20/0.04, and 25/0.04 using 2 mL of treatment solution after each file with a total of 8 mL and a total contact time of 10 minutes. Canal contents will be deactivated with sodium thiosulphate for NaOCl, and saline wash will be used for Fer/H2O2 and saline treatments. A wash step with 1 mL saline will be done to wash the deactivating solution, and paper points will be used to dry the canals. A second bacterial sample will be taken (S2) by placing LDT inside the canal, agitating it with 25/0.02 Hedstrom hand file, followed by absorbing the content with 2 paper points placed in the canal for 30 seconds each. The paper points will be placed inside a tube containing LDT. An additional step was included for all the test groups to evaluate if further irrigation after (S2) will lead to more reduction of bacterial counts inside the root canal system. This could inform future experiments evaluating the possibility of synergistic antimicrobial effects for the sequential Fer/H2O2 and NaOCl treatment. All canals in all groups were irrigated with 2 mL of 3% NaOCl (1 min), followed by ultrasonic irrigant activation for 30 seconds. This step was repeated once again making the total activation time 1 min and the total contact time of the irrigant 3 min. Upon completion of irrigation, a third bacterial sample (S3) was taken following the deactivation, washing, drying, and sampling steps as described previously. The remaining treatment sequences of the routine root canal therapy will be carried out after these procedures further root-end enlargement and final routine irrigation protocol. The root canals will be dried with paper points, a medication (calcium hydroxide) will be placed and the teeth sealed with a temporary restoration. Patients will return after one to four weeks for completion of the root filling. For the any of the groups, the treatment procedures carried out during this investigation do not differ from the standard root canal treatment protocol with the exception of additional irrigation step with the experimental solution and the bacteriologic sampling procedures. The paper points used to take the bacteriological sampling will be transferred to the microbiology laboratory using a vial containing 1 ml of reduced transport fluid (LDT). The laboratory procedures will be performed at the University of Pennsylvania's Leon Levy Oral Health Sciences Building of the School of Dental Medicine in the Microbiology Laboratory (Dr. M. Koos laboratory). Vial labels will contain information on tooth number, sample number (S0-S1-S2-S3) and the experimental group. Patient information will not be used in any case. LDT vials with samples will be vortexed before preparing aliquots. Samples with dilutions of 10, 100, and 1000-fold will be prepared under anaerobic conditions using sterile glassware. Cell culture dishes with anaerobic sheep blood agar will be inoculated with 0.25 ml of undiluted sample, as well as each of the three dilutions. The culture plates will be incubated at 37°C in an anaerobic glove box containing 5% hydrogen, 10% nitrogen, and 85% CO2 for 7 days. After incubation the number of colony forming units will be determined by using a stereoscope. ANOVA and Students t- test will be used for statistical analysis.

The following documents are currently attached to this item:

There are no documents attached for this item.

Deception
Does your project use deception?
No

Analysis Plan
After incubation the number of colony forming units will be determined by using a stereoscope. ANOVA and Students t-test will be used for statistical analysis.

The following documents are currently attached to this item:

There are no documents attached for this item.

Are you conducting research outside of the United States?
No

Data confidentiality

- x **Paper-based records will be kept in a secure location and only be accessible to personnel involved in the study.**
- x **Computer-based files will only be made available to personnel involved in the study through the use of access privileges and passwords.**

  Prior to access to any study-related information, personnel will be required to sign statements agreeing to protect the security and confidentiality of identifiable information.
- x **Wherever feasible, identifiers will be removed from study-related information.**

  A Certificate of Confidentiality will be obtained, because the research could place the subject at risk of criminal or civil liability or cause damage to the subject's financial standing, employability, or liability.

  A waiver of documentation of consent is being requested, because the only link between the subject and the study would be the consent document and the primary risk is a breach of confidentiality. (This is not an option for FDA-regulated research.)

  Precautions are in place to ensure the data is secure by using passwords and encryption, because the research involves web-based surveys.

  Audio and/or video recordings will be transcribed and then destroyed to eliminate audible identification of subjects.

Subject Confidentiality
Patients will be presented with a written consent form regarding the above mentioned study characteristics (research consent form, amended to this IRB proposal) as well as the regular consent forms for the root canal therapy, including the consent form for endodontic treatement, acknowledgement of privacy practices and a patient understanding and informed consent form. Patients signed consent forms will collected and stored by the PI in a secure, restricted area. Study data will be stored on an institutionally secured and managed device or server. The information collected and used for research purposes will be the School of Dental School Medicine chart number, diagnosis and tooth number. No protected health information such as name, address, telephone number, date of birth, social security number, personal and family medical history or results from physical examinations, tests or procedures will be used or collected. The chart number will only be recorded for a possible future verification of the research data such as tooth number and diagnosis. Patients chart number is secured by a login and password only accessible to students, residents and faculty of the dental school. Diagnosis and tooth number will be used in this investigation for scientific publication or presentation at scientific meetings.

Sensitive Research Information*
Does this research involve collection of sensitive information about the subjects that should be excluded from the electronic medical record?
No

Subject Privacy
Privacy refers to the person's desire to control access of others to themselves. Privacy concerns people, whereas confidentiality concerns data. Describe the strategies to protect privacy giving consideration to the following: The degree to which privacy can be expected in the proposed research and the safeguards that will be put into place to respect those boundaries. The methods used to identify and contact

potential participants. The settings in which an individual will be interacting with an investigator. The privacy guidelines developed by relevant professions, professional associations and scholarly disciplines (e.g., psychiatry, genetic counseling, oral history, anthropology, psychology).
Patients signed consent forms will collected and stored by the PI in a secure, restricted area. The information collected and used for research purposes will be the School of Dental School Medicine chart number, diagnosis and tooth number. No protected health information such as name, address, telephone number, date of birth, social security number, personal and family medical history or results from physical examinations, tests or procedures will be used or collected. The chart number will only be recorded for a possible future verification of the research data such as tooth number and diagnosis. Patients chart number is secured by a login and password only accessible to students, residents and faculty of the dental school. Treatments will be done at the University of Pennsylvania School of Dental Medicine Endoodontic clinic. All root canal procedures within the Dental School is done in the Endodontic clinic where work stations are designed to accommodate patient's privacy. Endodontic clinic contains a separate private room in cases where research instructions or patient's concerns can be addressed.

Data Disclosure
Will the data be disclosed to anyone who is not listed under Personnel?
The data will not be disclosed to anybody who is not listed under Personnel.

Data Protection*

	Name
	Street address, city, county, precinct, zip code, and equivalent geocodes
	All elements of dates (except year) for dates directly related to an individual and all ages over 89
	Telephone and fax number
	Electronic mail addresses
	Social security numbers
	Medical record numbers
	Health plan ID numbers
	Account numbers
	Certificate/license numbers
	Vehicle identifiers and serial numbers, including license plate numbers
	Device identifiers/serial numbers
	Web addresses (URLs)
	Internet IP addresses
	Biometric identifiers, incl. finger and voice prints
	Full face photographic images and any comparable images
x	**Any other unique identifying number, characteristic, or code**
	None

Does your research request both a waiver of HIPAA authorization for collection of patient information and involve providing Protected Health Information ("PHI") that is classified as a "limited data set" (city/town/state/zip code, dates except year, ages less than 90 or aggregate report for over 90) to a recipient outside of the University of Pennsylvania covered entity?
No

Tissue Specimens Obtained as Part of Research*
Are Tissue Specimens being obtained for research?
No

Tissue Specimens - Collected during regular care*
Will tissue specimens be collected during regulator clinical care (for treatment or diagnosis)?
No

Tissue Specimens - otherwise discarded*
Would specimens otherwise be discarded?
No

Tissue Specimens - publicly available*
Will tissue specimens be publicly available?
No

Tissue Specimens - Collected as part of research protocol*
Will tissue specimens be collected as part of the research protocol?
No

Tissue Specimens - Banking of blood, tissue etc. for future use*
Does research involve banking of blood, tissue, etc. for future use?
No

Genetic testing
If genetic testing is involved, describe the nature of the tests, including if the testing is predicative or exploratory in nature. If predictive, please describe plan for disclosing results to subjects and provision of genetic counseling. Describe how subject confidentiality will be protected Note: If no genetic testing is to be obtained, write: "Not applicable."
Not applicable

Consent

1. Consent Process

Overview
Patients routinely sign a consent form before being seen in the endodontic clinic. This form reviews the routine root canal procedure and any risks associated with it. We review routinely the consent form and health history with the patient and answer any questions that they may have on root canal procedures. The language used in the consent form is meant for lay-people and is not scientific in nature. If a patient chooses to participate in our study, they will sign an additional consent form for participation prior to participating. They will be made aware that if they do not wish to participate, they will not receive any treatment that is subpar as opposed to if they did agree to participate. The examiners will review the consent form with the patient and answer any questions they may have. If at any point, a patient decides they no longer want to participate, they will be excused from the study and will continue treatment as needed.

Children and Adolescents
N/A

Adult Subjects Not Competent to Give Consent
N/A

2. Waiver of Consent

Waiver or Alteration of Informed Consent*
No Waiver Requested

Minimal Risk*

Impact on Subject Rights and Welfare*

Waiver Essential to Research*

Additional Information to Subjects

Written Statement of Research*
No

If no written statement will be provided, please provide justificiation

The following documents are currently attached to this item:

There are no documents attached for this item.

Risk / Benefit

Potential Study Risks
Only the risks or discomforts that can arise during or after any routine root canal treatment procedure apply. Bacterial sampling will not have any adverse effect on the treatment or on treatment outcome. There are no potential risks expected from the noninvasive bacteriological sampling, including any potential physical, psychological, social, economic, monetary, legal or other risks. Identifying the antibacterial benefits of Feraheme/H2O2 irrigation is the goal of our experiment. Feraheme with much higher concentrations has been used in medicine as IV to improve and heal patients iron deficiency. Feraheme used in this experiment is limited within the root canal system and applied on dentine surfaces only. It will not be used systemically. Solution will be kept within the root canal system for a couple of minutes proposed in this protocol and will be evacuated by sterile saline, dental suction and paper points. Experiment was designed that the final root canal disinfection is not relying on experimental solutions antibacterial effect only. After bacterial sampling, antibacterial medication (CaOH2) will placed as done routinely to treat cases with pulp necrosis and apical periodontitis. Besides tooth number, diagnosis and the PDM chart number (for potential verification of study correctness) no patient data will be collected by the PI. All treatment procedures at the PDM, including the routine root canal treatment of the participants, during which the samples will be taken, are subject to strict HIPPA (privacy) and OSHA (hygiene) regulations.

Potential Study Benefits
The participant is not expected to get any personal benefit from taking part in this proposed research study. Microbial elimination is a key to the successful treatment of infected, necrotic teeth. Finding a better protocol to reduce intracanal infection will increase the successful outcome of endodontically treated necrotic, infected teeth and will result in an increased retention of natural teeth for the general population. The proposed study will verify by the means of microbial sampling and culturing, whether Feraheme/H2O2 irrigation protocol can provide better, effective disinfection on root canal surfaces and a significant reduction of intra-canal infection in-vivo. If this can be demonstrated by the proposed investigation it will have clinical significance as an improvement of root canal therapy.

Alternatives to Participation (optional)
If patient does not want to participate in the study, elected root canal treatment will be completed.

Data and Safety Monitoring
Investigators listed on the proposal will monitor the progress of the experiment. Routine meetings will be conducted to assess the number of samples collected, results, patient comments and complains, investigators' comments and findings.

The following documents are currently attached to this item:

There are no documents attached for this item.

Risk / Benefit Assessment
The risks or discomforts can arise during or after any routine root canal treatment procedure. There are no potential risks expected in this experiment. Our consent forms (for routine root canal procedures and for the experiment) contain clinic and investigator contact information. However, if occurs, patients'

concerns or complaints will be reviewed routinely.

General Attachments

The following documents are currently attached to this item:

HIPAA Authorization or Waiver (babeer.feahemeinendodontics.hipaa.consent.doc)

Additional forms (feraheme-fda.pdf)

Informed consent form (babeer.feahemeinendodontics.hipaa.consent.doc)