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Education:

2013- Present Endodontic Resident, University of Maryland, Dental School

1999-2005 Mashhad Dental School, Mashhad, Iran. Doctor in Dental Surgery Degree (DDS)

Thesis: *Association between bone density and the bone loss of dental implants*,

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Licensure:

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Establishment of private medical and dental office in Tehran

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2006-2010 (Summer holidays) General Dental Practitioner, Voluntary practice at Rahman Care Center of Retarded Children, Chenaran, Iran

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Academic and Research Practice:

2013-Present Researcher, Biomaterial Research Center (Dr. Stansbury's lab), Denver, Colorado, United States

2008-2011 Head of International Affairs and Public Relations Division, Iranian Center for Endodontic Research, Tehran, Iran

2008-2011 Administrative director of 5-year national Clinical Trial "Vital Pulp Therapy" in five different Medical Universities

2007-2011 Researcher, Head of Article Preparing Dept, Dental Research Center, and Iranian Center for Endodontic Research Tehran, Iran

2006-Present Technical and English text editor of Iranian Endodontic Journal

2005-2011 Founder Committee member, executive director and secretary of the Iranian Section of International Association for Dental Research (IADR)

2005-2011 Head of International Affairs and Public Relations Division, Iran Center for Dental Research, Tehran, Iran

2005-2007 General Dental Practitioner and Research assistance at Shahid Beheshti University of Medical Sciences: Dental Research Center and International Affairs Department

2005-2007 Research Assistant, Basic Sciences Research Dept., Iran Center for Dental Research, Tehran, Iran

2003-2005 Dental Student, Cooperating in writing scientific articles, Mashhad School of Dentistry

Publications:

2014 Surgical management of a failed internal root resorption treatment: a histological and clinical report. Restor Dent Endod. 2014 May;39(2):137-42. doi: 10.5395/rde.2014.39.2.137.

2014 Extraoral Retrograde Root Canal Filling of an Orthodontic-induced External Root Resorption Using CEM Cement.: Iran Endod J. 2014 Spring;9(2):149-52. Epub 2014 Mar 8.

2013 Twenty years of research on Mineral Trioxide Aggregate: A scientometric report. Iran Endod J. 2013; 8(1):1-5.

2012 High-level evidence in Endodontics, Scientometrics; 2012 April, DOI: 10.1007/s11192-012-0724-5.

2012 Push-out bond strength of two root-end filling materials in root-end cavities prepared by Er,Cr:YSGG laser or ultrasonic technique. Aust Endod J. 2012 Dec.; 38(3):113-7. doi: 10.1111/j.1747-4477.2010.00264.

2011 Particle size of two endodontic materials and Portland cement. J Biointerface Res 2011; (2): 83-9.

2009 Orthodontic treatment need in 14–16-year-old Tehran high school students. Aust Orthod J. 2009 May; 25(1):8-11.

2009 Comparison of mineral trioxide aggregate's composition with Portland cements and a new endodontic cement. J Endod. 2009 Feb; 35(2):243-50.

2009 Microleakage of CEM cement in two different media. Iranian End J 2009; 4(3):91-5.

2009 Particle size of a new endodontic cement compared to Root MTA and calcium hydroxide. Iranian End J 2009; 4(3): 112-6.

2009 Particle size of a new endodontic cement compared to MTA and Portland cement. J Dent Res, special issue, Code: 20090224.

2008 The properties of a new endodontic material. J Endod 2008 Aug; 34(8):990-3.

2007 Association between bone density and bone loss of dental implants. J Dent Res, spec iss: 0006.

2007 Correlation between IGE and Histamine and Pulp Polyp. J Dent Res, spec iss: 0005.

2007 Bone reaction to different bone grafting materials: A histopathologic animal study. J Dent Res, spec iss: 0026.

2007 Sealing ability of two root-end fillings and a new endodontic material. J Dent Res, spec iss: 0038.

2007 Histologic and Histo-morphometric Analysis of Socket Preservation with three different Materials. J Dental Res, spec iss: 0025.

2007 Comparison of the pulp immunoglobulin between deciduous and permanent teeth with irreversible symptomatic pulpitis. J Dent Res, spec iss: 0007.

2007 Different storage solutions' effect on mineral trioxide aggregate and a new endodontic material as root-end fillings. Journal of Dental Research, spec iss: 0048.

Poster and Oral Presentations:

2015 Medication-related osteonecrosis of the jaws: An Update and case report, AAE annual meeting, Seattle, United States

2013 Reactive nanogels as additives in dental resin and composite materials. IADR annual conference, Seattle, United States

2013 Characterization of flow of composite restorative pastes. Annual Research Day, University of Colorado, Denver, United States

2013 Nanogel-modified dental resins and dental composites. Annual Research Day, University of Colorado, Denver, United States

2009 Particle size of a new endodontic cement compared to MTA and Portland cement. IADR annual conference, Iranian Division;

2007 Association between bone density and bone loss of dental implants. IADR annual conference, Iranian Division

2007 Correlation between IGE and Histamine and Pulp Polyp. IADR annual conference, Iranian Division

2007 Bone reaction to different bone grafting materials: A histopathologic animal study. IADR annual conference, Iranian Division

2007 Sealing ability of two root-end fillings and a new endodontic material. IADR annual conference, Iranian Division

2007 Histologic and Histo-morphometric Analysis of Socket Preservation with three different Materials. IADR annual conference, Iranian Division

2007 Comparison of the pulp immunoglobulin between deciduous and permanent teeth with irreversible symptomatic pulpitis. IADR annual conference, Iranian Division

2007 Different storage solutions' effect on mineral trioxide aggregate and a new endodontic material as root-end fillings. IADR annual conference, Iranian Division

Awards

2015 The Sherril Ann Siegel Endodontic Research Fellowship award, for demonstrating exceptional ability in the field of endodontic research. (*For the research project: "Nanogel-based scaffold in endodontics")*

2009 2nd place for oral presentation, IADR annual conference, Iranian Division

Teaching Experience:

2009 Workshop about "Writing scientific article and most common mistakes" for members of Dental Research Center.

Membership:

2013- Present Member of American Association of Endodontics

2013- Present Member of Penick Study Club

2005-Present	Member of International Association for Dental Research, IADR (Founder committee member/Executive Director/Secretary of the Iranian Section)
2005-Present	Member of Iranian Dental Association, IDA
2005-Present	Member of Iranian Medical Council
2005-Present	Member of Iran Center for Dental Research, ICDR
2005-Present	Member of Iranian Center for Endodontic Research
2006-Present	Member of Eastern Mediterranean Editors and Translators
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Abstract

Title: Nanogel-Based Scaffold in Endodontics

Sanam Kheirieh, Master of Science 2016

Dissertation Directed By: Dr. Patricia A. Tordik, DMD

Aim: The purpose of this study was to evaluate a degradable nanogel-based scaffold with antibacterial content.

Methods: The nanogel design used in this study consisted of the cross-linker, polyethyleneglycol (PEG 4600) with 3-dimensional network. This polymer degrades over time (~30 days), delivering a controlled release of antibiotic. Amoxicillin was added to the scaffold with 25 wt% (n=26). Nanogel-scaffold only and amoxicillin only were used as controls. Agar diffusion test against E. faecalis was performed at eight time intervals (days 1, 3, 5, 7, 10, 14, 21, 30). One-Way ANOVA was used to compare the antibacterial properties of experimental groups at the eight different times.

Results: The antibacterial properties for experimental plates, at the different times, were not significantly different ($F=.624$, $p=.74$). Based on the profile, the scaffold-only group showed a smaller inhibition zone compared to the two other groups. The antibacterial profiles for the experimental group and the antibiotic-only group were similar.

Conclusion: This particular scaffold presented antibacterial properties. Findings suggest that nanogel-modified scaffolds may have potential use for drug-delivery in endodontics.

The Nanogel-Based Scaffold in Endodontics

By
Sanam Kheirieh

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
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Introduction

Success of endodontic treatment has been the subject of many investigations. Different factors are often considered as primary features that determine the rate of success. Failure of endodontic treatment is predominantly defined as the presence of apical periodontitis. (1, 2). One such investigation determined that, within 4-6 years of follow up, there are multiple influencing elements in the success rate of endodontic treatment, which include preoperative AP, treatment technique, gender, number of roots, and root-filling length; however, it concluded that AP and highlighted treatment technique are the main predictors of outcome in initial treatment. This study presented a heal rate of 81% for the endodontic treatments investigated (2). The probability of a tooth treated with apical surgery to heal completely is between 37 to 85 percent, with the average being 70 percent. Non-surgical treatment outcomes have been shown to be more consistent than the outcomes of apical surgery. As such, the probability of a tooth without apical periodontitis to remain free of disease after initial treatment is between 92 to 98 percent. Also, the probability of a tooth with apical periodontitis to completely heal after initial treatment, or retreatment, is between 74 to 86 percent (3). A retrospective study, by Hoskinson, determined that the presence of a periapical lesion, along with the pulpal status of the tooth, were the primary factors of success or failure in treatment outcomes (4).

Information regarding the success rate of surgical and non-surgical retreatments determines the ranges to be between 45 to 90 percent. A randomized clinical trial, conducted in 1999, evaluated the success rate of non-surgical compared to surgical retreatments. Upon following up at 12 months, the success rate of surgical and non-surgical retreatment was significantly different. However, there was no such difference found when a follow up examination was conducted at 48

months (5). In a prospective study by Ng *et al.*, periapical healing following endodontic treatment and retreatment was investigated, as well as factors which influence the rate of healing after performing such treatments. Evaluation was conducted of 1170 roots for endodontic treatment, as well as 1314 roots for retreatment, over the course of 2 to 4 years. Study results showed that periapical healing following root canal treatment was similar to periapical healing following retreatment. The authors also listed different factors which may work to improve the periapical healing process including the absence of a periapical lesion preoperatively, or when a periapical lesion is present, a better treatment prognosis is indicated when it is smaller in size; the absence of a preoperative sinus tract; canal terminus patency; the cleaning of the canal extending as close to the apical terminus as possible; a wash using ethylene-diaminetetra-acetic acid (EDTA) solution followed by a NaOCl solution rinse when performing retreatment; using NaOCl solution instead of 2% chlorhexidine as an irrigant; completing treatment without perforating the tooth or root; absence of pain/swelling between appointments; the prevention of root-filling extrusion; and the building of a quality coronal restoration (6).

In another prospective study, a large number of endodontic treatments were followed, which were performed by a single operator, and periodically checked over a period of 5 years. This study included, during that time period, all consecutive cases. At the conclusion of the 5 years, 470 patients, with 816 treated teeth, and 1,369 treated root canals, were viable candidates for evaluation. The study presented an 88.6 percent rate of success among the 816 teeth. Regarding the factors which influenced the success rate of root canal treatment, it was concluded that more severe disease conditions resulted in outcomes that were negative (7). Ricucci, while identifying an optimal working length, determined that an excess of root canal filling material would lead to

a decrease in the success rate. It was also indicated that an infected pulp space should optimally be treated using an intracanal dressing. It was also indicated that coronal restoration quality, as well as intracanal post retention placement, has no effect on the outcome of treatment (8). The preoperative pulp diagnosis, the periapical diagnosis, the size of the preoperative periapical radiolucency, as well as the patient's sex, were determined by Chugal *et al.* as being significant factors which may influence the outcome of endodontic treatments. An epidemiology study of 1,462,936 teeth, from 1,126,288 patients, with regard to initial endodontic treatment, was assessed over an 8-year time period. Following initial non-surgical endodontic treatment, over the course of the 8-year period, the study determined there was a tooth retention rate of 97 percent (9).

It is important to understand the biological factors which relate to the failure of an endodontically treated tooth in order to maintain quality management of PA lesions in an endodontically treated tooth. As such, there are five primary factors that may result in the persistence of periapical radiolucency in an endodontically treated tooth. These factors include intra-radicular infection, extraradicular infection; reaction to foreign bodies; the presence of a true cyst; and the growth of fibrous scar tissue. Among these five factors, the orthograde retreatment may result in less persistence of microorganisms within the root canal. Periapical surgery is the only way to manage, foreign bodies, true cysts, and lesions associated with extraradicular bacteria. However, no treatment is required for periapical lesions that heal by the formation of fibrous scar tissue. The primary cause of failure in endodontic treatment has been found to be persisting microorganisms. Other studies have reported that in endodontically treated teeth with apical radiolucencies, bacterial growth can be seen. The microflora in teeth that have not been endodontically treated is different than that of the microflora found in root canal treated teeth. Facultative anaerobes, along

with gram-positive microorganisms are predominantly reported. *Enterococcus faecalis* is the bacterial species most often found in teeth needing endodontic treatment, with *streptococci* also being common among microflora. *Lactobacilli* is another species found in high numbers, along with *peptostreptococci* and *actinomyces* (10). Identification of bacteria found within endodontic infections, using PCR-based methods targeting bacterial 16S rRNA genes showed that *streptococcus* species were associated with preoperative symptoms (11). It has been shown that periapical lesions include the presence of bacteria (12). Sundqvist *et al.* found that with persistent periapical lesions *Enterococcus faecalis* is the most common bacteria. It was stated in this study that microflora related to persistent periapical lesions is different than the microflora of untreated teeth (13). However, it is still debatable whether micro-organisms can live within periapical endodontic lesions. In 2003 Sunde, using Fluorescence in situ hybridization (FISH), was able to directly visualize bacteria in periapical lesions of root-filled teeth. It was shown that cocci, spirochaetes, and rods were observed as microcolonies within periapical lesions. Colonies of *Prevotella intermedia, Tannerella forsythensis, Porphyromonas gingivalis,* and *treponemes* were also detected. In some lesions, *Streptococcus spp.* was observed as well (14).

In order to decrease or eliminate the bacterial population, antibiotics have been used in endodontic infections (15, 16). Multiple combinations of antibiotics have been used when assessing the susceptibility of endodontic bacteria to their effects. Gary *et al.*, in 1997, was able to isolate and identify bacteria from periapical lesions in order to determine their sensitivity to antibiotics. The periapical lesions of 28 refractory endodontic cases, which required surgical intervention, were used to perform this study. *Propionibacterium acnes, Wolinella recta, Streptococcus intermedius, Staphylococcus epidermidis, Clostridium* species, and *Fusobacterium* species were the most

common of the isolated organisms. Bacteria was tested for susceptibility/resistance to Tetracycline, Clindamycin, Metronidazole, Cefoxitin, Benzylpenicillin, Cefotaxime, and Erythromycin. Results showed that among the species tested there was no significant antibiotic resistance among (17). In 2003, an antibiotic susceptibility test was performed on *Enterococcus faecalis* and *Peptostreptococcus* species, which were isolated from persistent periapical lesions. It was shown that each of these species were susceptible to Amoxicillin, Amoxicillin when combined with Clavulanate, and Benzylpenicillin (18). Bacterial microflora within the periapical lesions of endodontically treated teeth is comprised of gram-positive species and facultative anaerobes. The most predominant was *Enterococcus faecalis,* which was found to be susceptible to Erythromycin and Azithromycin. A group of bacteriostatic antibiotics, the tetracyclines, were found to have up to 12 weeks of antibacterial substantivity. For anti-inflammatory, anti-resorptive, and anti-bacterial properties these antibiotics were typically used in combination with corticosteroids. These combinations help in the reduction of inflammatory reactions, including resorption mediated by clastic-cells. Tetracycline, when used as part of irrigating solutions, has only 4 weeks of antibacterial substantivity. Clindamycin, along with a combination of Metronidazole, Ciprofloxacin, and Minocycline, has also been reported as being effective in the reduction of bacterial numbers in infected teeth (19). A more recent, and rational, alternative to root apexification that has been studied is regenerative endodontics (20, 21). This procedure involves the initial disinfection of the root canal system with irrigation solutions and minimal instrumentation, followed by placement of a medication as a mixture of antibiotics. After a 4-week period, the medication is removed. Bleeding is induced by laceration of periapical tissue and it is subsequent blood clot formation (20, 22-24). It is assumed that the blood clot carries a variety of growth factors, acting as a natural, fibrin-based scaffold for the attachment,

proliferation, and differentiation of stem cells from the apical papillae (23, 24). The elimination and/or inhibition of bacteria have been shown to play a key role in the regenerative outcome (23, 24). Therefore, complete eradication of the residual infection and bacteria is critical in regenerative endodontics (25). This is typically achieved through applying calcium hydroxide. Double antibiotic paste (DAP), which contains MET and CIP, or triple antibiotic paste (TAP), which contains MET, CIP, along with minocycline, are alternatives that may be used (26, 27). Both Calcium hydroxide and antibiotic pastes have demonstrated important side effects. Calcium hydroxide is associated with the weakening of roots when used for either short (28, 29) or for long periods of time (29, 30). The use of TAP and DAP pastes at recommended dosages has resulted in unfavorable effects on pulp cells (24, 31), and fibroblasts of periodontal ligaments (32). It is noteworthy that irrigation methods currently in use fail to completely remove antibiotic paste remnants from root canals (33). Recently, it has been proposed that the reduction of drug concentration through the use of nanofibers, which contain antibiotics, may be an alternative drug delivery-based strategy (25, 29, 34-37). In fact, designing the scaffolds with antibacterial content has been recently a research topic in endodontics.

Nanogels, a type of highly branched polymer, have internal crosslinked polymers which are less than 100nm in dimension (38, 39). With multiple crosslinking points, a nanogel is a structure comprised of numerous connected primary chains. Free radical chain growth has been used in the polymerization of methyl methacrylate, with a diacrylate crosslinker, in order to randomly generate highly branched polymers (nanogels) in a solution. Microemulsion polymerization, applying significant amounts of surfactant, is the most commonly used technique in the generation of smaller, 50nm, domains. Both before and after microemulsion polymerization,

thermodynamically stable, and optical clear, dispersions can be obtained. However, with large amounts of surfactant/co-surfactant present, after emulsion polymerization, purifying the nanogel can be tedious. When compared to solution polymerization, during emulsion polymerization there is no significant change in viscosity and particles of polymer, with monodispersed molecular dimension and weights are provided. Effective crosslinking is emphasized with emulsion polymerization more than cyclization, as emulsion polymerization is bulk polymerization in small domains, when compared to solution polymerization. As such, when there is high crosslinking density, the potential for nanogel swelling is limited (40).

Nanogels may be inappropriate for some applications, such as those that need near-perfect polymer structural regulation, when formed from random copolymerization. As such, the need for controlling the composition, polymeric nanoparticle structure, and properties of the nanogel has increased. The ability to tune physical and chemical characteristics with processing techniques, reaction conditions, and widely available monomers has been demonstrated by block copolymers. As block copolymers are essentially building blocks, they are used for the formation of a variety of micro- and nano-domain patterns (41). These patterns include vesicles, lamellae, cylinders, spheres, and more complicated structures such as the double diamond and double gyroid. Developed decades ago, one approach for the synthesis of well-controlled nanogel structures uses block copolymer assemblies in solution (42). This technique, over more recent years, has received substantial attention (43). Composed of block segments with different hydrophilicity, block copolymers interact in different ways with solvents. For example, micelle structures can be formed by amphiphilic block copolymers in an aqueous environment. In such a case, the hydrophilic blocks are compatible in water while, to minimize free energy, the

hydrophobic segments, as allowed by their mobility, collapse together (44). Micelle dimension can be controlled by block copolymer chain length and chemical composition, placing them in the order of 10-100nm. However, many factors, such as concentration, solvents, pH, and temperature can easily disrupt the structure of micelles. For more stabilized, robust, and controlled nanogel structures, reactions between specific blocks can lead to internal crosslinking of micelles (45). It is very common to form core crosslinked micelles, through crosslinking of hydrophobic blocks, given a hydrophilic aqueous environment. Due to shell layer protection, when compared to shell crosslinked approach, intra-micelle reaction is provided through core crosslinking (46). However, as the formation of dense shell layer vesicles is suitable for drug delivery applications, investigation of crosslinking within the shell is also a priority. Drug delivery application is based on controlled degradation rate of nanogels which subsequently result in to controlled release of the drug.

Regarding the importance of scaffolds with antibacterial content in order to be used as drug delivery system in endodontics, the rationale of this study was to develop a degradable scaffold with antibiotic content, presenting a controlled rate of degradation and therefore a controlled release of antibiotics to the surrounding environment.

Purpose: The aim of this study was to evaluate the antibacterial properties of the nanogel-modified scaffold with antibiotic content at eight different time intervals.

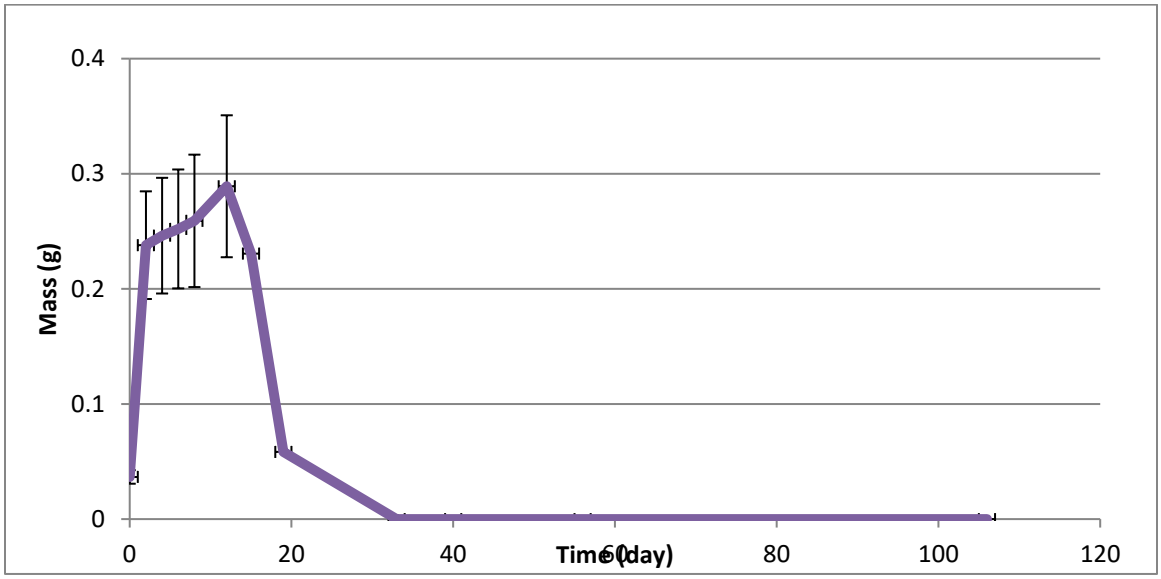
Hypothesis: The hypothesis was that there is no sig difference in the size of the inhibition zone created by the antibiotic-containing scaffold at eight different time intervals.

Materials and Methods

Nanogel synthesis:

The nanogel used in this study was synthesized and supplied by the Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO and the Biomaterials Research Center, School of Dental Medicine, University of Colorado, Aurora, CO, USA. Monomer and nanogel synthetic procedures were modified from previously described protocols (47). The degradable monovinyl monomer was synthesized using a 1:1 molar ratio of 2-hydroxyethyl methacrylate (HEMA) and 3,6-dimethyl-1,4-dioxane-2,5-dione (lactide). The mixture was heated to 140°C under a nitrogen purge to melt the lactide. One drop of catalyst dibutyltin dilaurate was added and the reaction was carried out for four hours. After the reaction was completed, excess lactide was removed by heating the mixture to 180°C under high vacuum for 30 min. 1H-NMR was used to calculate the molecular weight of the HEMA-poly(lactic acid) (HEMA-PLA). A free-radical solution polymerization was used to synthesize the degradable nanogel. The degradation rate of the nanogel was with a complete release of degradation products in 20-30 days (Figure 1) (47).

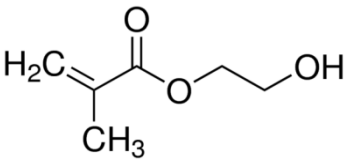
Figure 1. Degradation profile of nanogel showing complete degradation at 30 days.



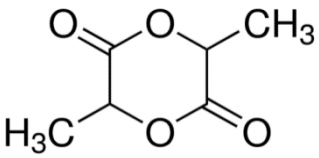
Polyethylene glycol 4600 dimethacrylate (PEG4600DMA) was combined with HEMA-PLA at a 1:1 molar ratio. Mercaptoethanol was used as a chain transfer agent (10 mol% relative to monomer content) and methyl ethyl ketone was used at a 4-fold excess relative to the monomer mass. 1 wt% azobisisobutyronitrile (AIBN) was added as a thermal initiator. The reaction was carried out at 80°C for approximately two hours until a degree of conversion of approximately 80% was achieved, as confirmed by mid-IR spectroscopy (C=C absorption at 1637 cm-1).

The nanogel was then isolated by precipitation from a 10-fold excess of hexanes and additional methacrylate functionality was added to the nanogel via isocyanatoethyl methacrylate attachment to PLA-based OH groups. Methacrylate functionalization provided the capability for inter-particle nanogel polymerizations and hydrolysis of the PLA segments allowed for polymer network degradation over time. Formation of crosslinked nanogel networks involved dispersing the nanogels in water (P/W 2:1) and adding ammonium persulfate (APS) (2wt%) plus tetramethylethylenediamine (TEMED) (2wt%) as a co-initiating system (Figures 2, 3) (47,48).

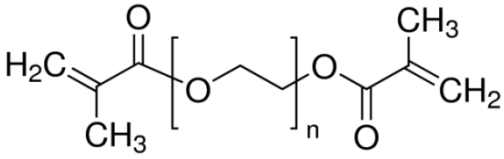
Figure 2. Chemical structures of materials to synthesize nanogel.



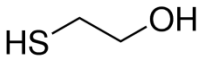
Hydroxyethyl methacrylate



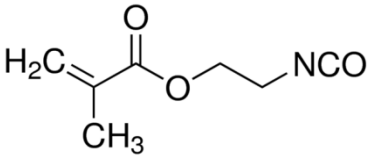
3,6-dimethyl-1,4-dioxane-2,5-dione (lactide)



Poly(ethylene glycol) dimethacrylate (PEG4600DMA)

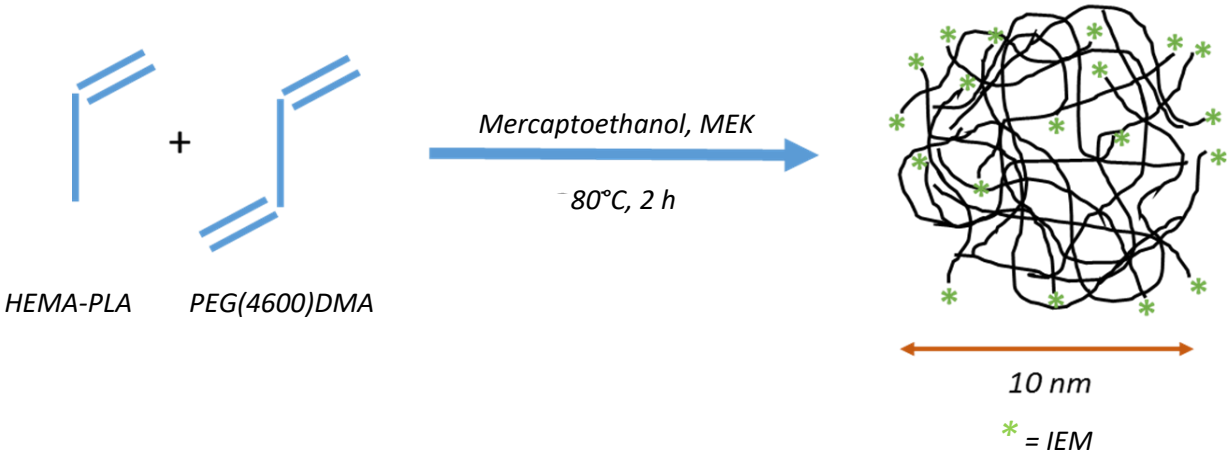


Mercaptoethanol



Isocyanatoethyl methacrylate (IEM)

Figure 3. Schematic of crosslinking reaction to form nanogels.



HEMA-PLA *PEG(4600)DMA*

Bacteria

Use of Entrecoccus faecalis (ATCC 29212, American Type Culture, Manassas, VA, USA, ATCC® Medium 2174: Brain heart infusion medium with vancomycin 4 mcg/ml) was approved by the University of Maryland. Enterococcus faecalis was cultured in Mueller-Hinton agar from stock culture and used for experiments.

Specimen Fabrication

For the Nanogel-only group, a powder:water ratio of 2:1 (w/w) was used in order to reach a clinically useful consistency. The powder consisted of nanogel powder (PGE 4600), two initiators were added including Ammonium persulfate (APS, Sigma-Aldrich, St. Louis MO) and tetramethylethylenediamine (TEMED, Sigma-Aldrich, St. Louis MO). PGE 4600 was used as 96 wt%. Two initiators were used as 2 wt% each.

For the Antibiotic-only group, Amoxicillin was used as 25 wt% mixed with distilled water. For the experimental group, the mix of powder and water was made with the powder:water ratio of 2:1. This mix included 25wt% of Amoxicillin. Therefore, 75wt% of the mix contained PGE 4600, APS, TEMED, and water. The remaining was 25wt% Amoxicillin added to the mixture. The consistency of final mixtures in different groups reached a paste texture.

Each paste as described for different groups was placed into a circular well (10 mm diameter, 2 mm thick) at the center of Mueller-Hinton agar plates. A total of 26 plates were used in the experimental group. The Nanogel-only group and the Amoxicillin-only group included 5 plates each. The specimens were then incubated at 37 °C for 24 h.

Measurement of Inhibitory Effect Against Bacterial Growth by a Disk-Diffusion Method

Agar disk diffusion test (ADT) was used to examine the antibacterial effect of antibiotic-loaded nanogels. Sterilized nanogel specimens were placed onto Mueller-Hinton agar plates inoculated with 350 µL of 1 x 10^8 CFU/mL of *E. faecalis* suspension and the plate was incubated at 37ºC for 24 h. After incubation, elution of the antibacterial component was estimated from the production of inhibition zones around the specimens (49). The distance between the disk and the closest bacterial colony was measured with digital calipers. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) or those included in the US Food and Drug Administration (FDA) approved product inserts for the disks.

Statistical analysis

Using power analysis, a One-Way ANOVA, with a large effect size, n=21 plates checked at eight different time intervals and p=.05, power was equal to.80.

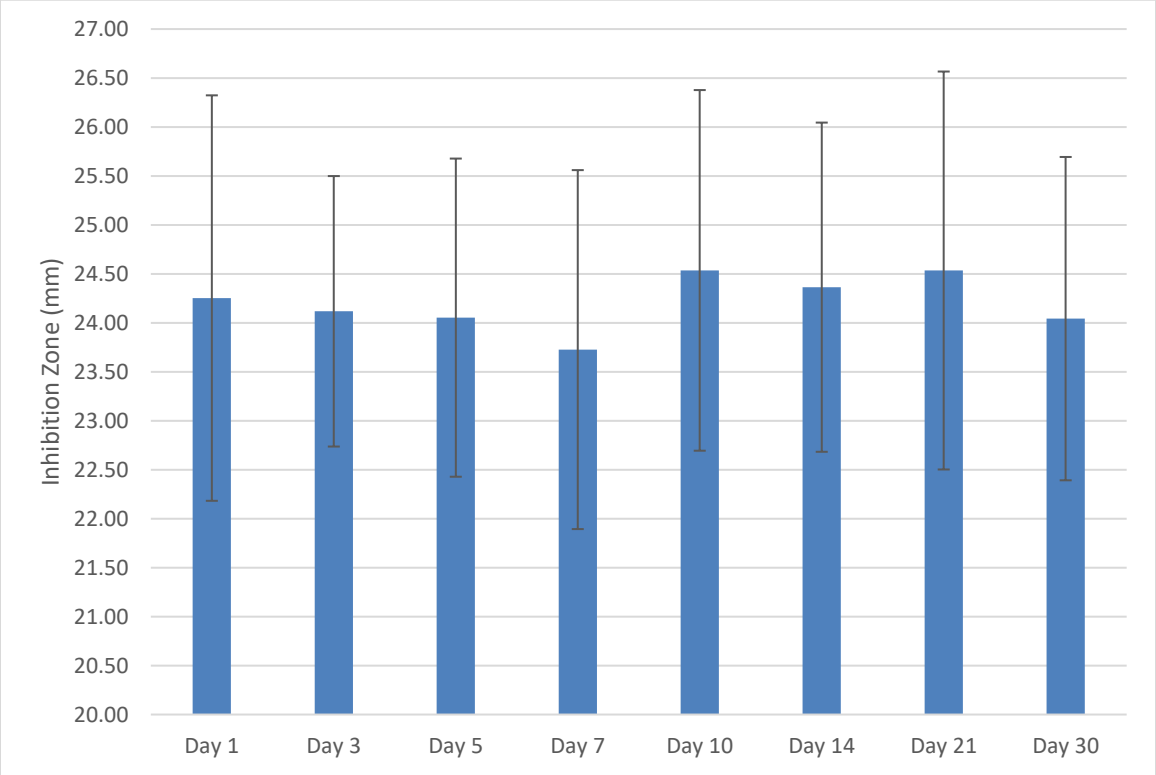
One-way ANOVA was used to analyze the difference between antibacterial properties, (as indicated by the size of the inhibition zone) at the different time intervals. Since a significant difference was not found, Tukey's Honestly Significant Difference Test was not needed. A $p \leq .05$ was considered significant.

Results:

The aim of this study was to evaluate the antibacterial properties of a nanogel-modified scaffold with antibiotic content at eight different time intervals (days 1, 3, 5, 7, 10, 14, 21, 30).

The results of the present study showed that the inhibition zone measurements for each of the experimental plates (n=26), at the different times (Day 1 up to Day 30), were not significantly different (F=.624, p=.74). The antibacterial properties of the nanogel-modified scaffold, with the incorporated antibiotic content, did not indicate significant differences between any of the eight time intervals (Figure 4).

Figure 4. Inhibition zones (mean±SD) of a nanogel-modified scaffold with antibiotic at eight different time periods (F=.624, p=.74)



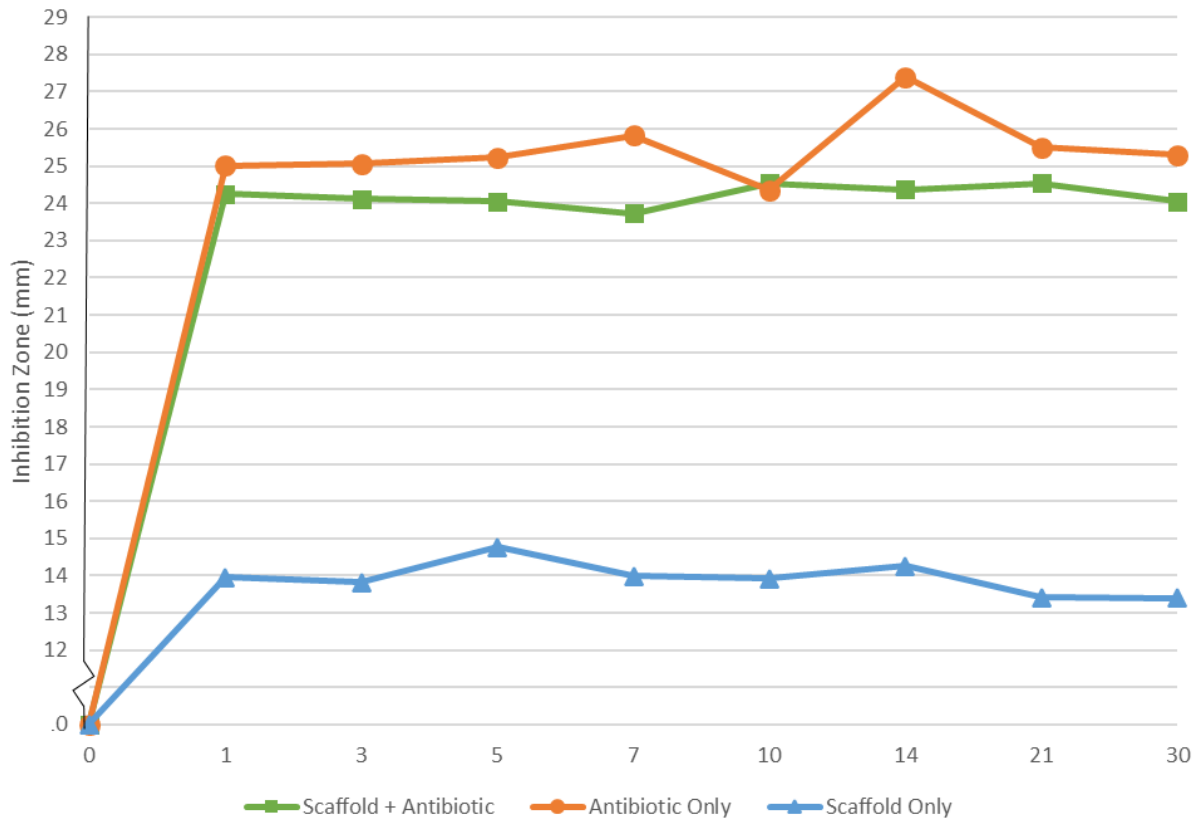
Measurements of the antibacterial properties of different groups showed that the mean ± SD of the inhibition zones, all days combined, was 24.20±1.8 for the nanogel-modified scaffold with antibiotic, 25.46±1.4 for the antibiotic only, positive control group, and 13.95±1.0 for the scaffold only, negative control group (Table 1). Statistical differences between days were not tested for the positive and negative control groups.

Table 1: Mean ± SD of Inhibition zone measurements in three groups (Scaffold+Antibiotics, Antibiotics only, Scaffold only) at different time intervals

Day	n	Scaffold + Antibiotic * (experimental group)	n	Antibiotic Only (positive control)	n	Scaffold Only (negative control)
1	26	24.25 ± 2.1	5	25.02 ± 2.24	5	13.96 ± 1.51
3	26	24.12 ± 1.4	5	25.06 ± 1.51	5	13.82 ± 1.49
5	26	24.05 ± 1.6	5	25.24 ± 2.24	5	14.77 ± 0.58
7	26	23.73 ± 1.8	5	25.83 ± 1.93	5	13.99 ± 1.32
10	26	24.54 ± 1.8	5	24.35 ± 1.14	5	13.93 ± 1.13
14	26	24.36 ± 1.7	5	27.39 ± 0.41	5	14.26 ± 0.71
21	26	24.53 ± 2.0	5	25.51 ± 0.73	5	13.42 ± 0.78
30	26	24.04 ± 1.6	5	25.30 ± 1.25	5	13.40 ± 0.54
Total	208	24.20 ± 1.7	40	25.46 ± 1.43	40	13.95 ± 1.00

Figure 5 displays the profile of the measurements of the inhibition zone for each of the different groups, including the nanogel-modified scaffold with antibiotic content, antibiotic only, and scaffold only, at different days. Based on the profile, the antibacterial properties observed for the scaffold only group had a smaller inhibition zone compared to the two other groups. The profiles for the nanogel-modified scaffold with antibiotic content group and the antibiotic only group presented similar antibacterial levels. Statistical analyses were not performed between the three groups.

Figure 5. Profile of inhibition zones of three different groups (Scaffold + Antibiotic, Antibiotic only, Scaffold only) over 30 days.



Discussion:

The purpose of this study was to develop an antibiotic delivery system for potential use in endodontic infections. Reducing the bacterial content/load of a root canal system has been shown clinically to improve the outcome of regenerative treatment with additional root development demonstrated upon radiographic assessment (50).

In this study *E. faecalis* was utilized because it is a common bacteria found in endodontic infections. This bacterium plays a role in the initiation and progression of endodontic infections

in the form of a biofilm (51, 52). Also, *E. faecalis* has been shown as the common microorganism associated with refractory infection (53, 54).

Various in vitro antimicrobial assays have demonstrated that polymer-based scaffolds containing antibiotics, such as vancomycin, rifampicin, metronidazole, and CIP, hold major potential in terms of the prevention of growth and the elimination of bacteria. This potential is important in that it also may be clinically relevant in the field of endodontics, specifically with root canal disinfection (25, 29, 34, 36, 37). The nature of the few antibiotic-containing scaffolds that have been studied is diverse, as each has demonstrated varying characteristics regarding antibacterial properties. Current knowledge indicates that this study is the first to investigate the antibacterial properties of a nanogel-modified scaffold in endodontics. This scaffold is unique in that it degrades over 20-30 days.

In this study, Mueller-Hinton plates were cultured with *E. faecalis*. Plates treated with Amoxicillin only were used as a positive control group in order to analyze the zone of inhibition created by this antibiotic against *E. faecalis*. The results confirmed the antibacterial properties of the antibiotic used as it developed a inhibition zone within the first 24 hours. The antibiotic maintained this zone at a relatively constant level (25.46±1.43) during the eight time periods of this 30-day study.

As a negative control group, plates were treated with the nanogel-modified scaffold only. This group was a negative control. It was expected to have no antibacterial properties. It was notable that the nanogel demonstrated a degree of antibacterial activity against *E. faecalis*, with an

average inhibition zone of about half the size of the zones exhibited by the positive control and experimental groups. This antibacterial property also maintained an almost constant zone of inhibition (13.95±1.00) through the 30-day study. This is the first study known to demonstrate antibiotic properties of a nanogel.

Additionally, the nanogel scaffold by itself showed a smaller, but still present, inhibition zone. It might be expected that the observed antibacterial effect of the nanogel alone would contribute to the inhibition zone profile of the antibiotic+nanogel. However, in this case, that is not observed. It is theorized that the concentration of antibiotic used in the study is large enough to exert maximum antibiotic effect and the inhibition zone are as large as they can be under the given conditions. Because of this, any antibacterial effect of the scaffold alone will be overwhelmed by the antibiotic.

The possible reason for the antibacterial properties of the nanogel itself may be that the nanogel scaffold, as a polymer, was not being fully polymerized and therefore had some non-polymerized monomers remaining. The maximum level of the polymerization in nanogels is approximately 70% to 80%. However, this is less possible because none reacted monomers are eliminated after polymerization using nano-filters. Another possibility for the sustained antibacterial properties might be related to the products of the nanogel-modified scaffold, created through the course of its degradation. These degradation products may themselves present antibacterial properties. This may also account for the initial burst of antibiotic inhibition within the first 24 hours, as also exhibited by the positive control and experimental groups.

In previous studies investigating the antibacterial properties of scaffolds containing antibiotics, antibiotic release was measured in order to determine the effectiveness of the scaffold in sustained drug delivery (21). In the present study, the maintenance of antibacterial properties was assessed through the regular measurement of inhibition zones created against *E. faecalis.* The antibacterial measurements in this study were performed over the course of 30 days, at eight different times, which provided information over a longer period of time when compared to previous studies with a measurement period of 14 days (21, 36). Therefore, this study provides evidence of bacterial elimination for a longer duration of time.

The experimental data reported in this study indicated that after an initial burst release of antibiotic within the first 24 hours (day 1), the measured inhibition zones remained relatively constant (24.20±1.76) for all time-points observed. The inhibition zone measurements for each of the experimental plates (n=26) at the different time periods (day 1 through day 30) were not significantly different. Findings suggest that a sustained level of antibiotic, through steady release from a nanogel-modified scaffold, may be effective in the inhibition/eradication of an existing infection. Therefore, possible applications of this scaffold may be considered for primary endodontic infections, secondary endodontic infections, as well as regenerative treatments in endodontics.

The primary characteristic considered through the course of this research was that this nanogel-modified scaffold degrades within 30 days. This property makes this scaffold unique when compared to other reported scaffolds with similar applications. These remain stable and are non-degradable. They remain in the environment after the antibacterial agent is released. The property

of this scaffold to provide a controlled release of antibiotic into the environment for 30 days while itself degrading makes it ideal for use in endodontics. The ability to maintain the antibiotic in the scaffold with a slow release creates the possibility of using a lower concentration of antibiotics through a typical course of treatment. This possibility is due to the maintained concentration of antibiotic, as there would be a lower degree of antibiotic being washed out from the infected area. It is clear however that the in-vitro situation in this study cannot be considered as an accurate representative of the clinical situation and that further study is needed. Additionally, the degradation of the nanogel is beneficial since it does not interfere with regeneration of pulpal tissue. It is clear however that the in-vitro situation in this study cannot be considered as an accurate representative of the clinical situation and that further study is needed.

Future studies may include further investigation into the antibacterial properties of this scaffold alone. Researchers should also examine different concentrations (wt %) of antibiotic in order to determine an optimal concentration. Also, further research, ideally, would include investigating the different types of antibiotics most commonly used in endodontic research and their effectiveness when incorporated into the nanogel-modified scaffold.

In addition, the characteristics of these antibiotic agents against different bacteria species or in the form of a biofilm should be studied as well.

Conclusions:

Throughout the course of this study, there were no significant differences in the effectiveness of the antibiotic alone compared to the antibiotic when incorporated within the nanogel-modified scaffold. This particular scaffold presented antibacterial properties, which is an unexpected new

finding with regard to nanogel-modified polymers. These findings suggest that nanogel-modified scaffolds may have potential use with drug-delivery in endodontics.

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