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Development of blended polymeric fibers to alter the release of peptide targeting Porphyromonas gingivalis.

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DEVELOPMENT OF BLENDED POLYMERIC FIBERS TO ALTER
THE RELEASE OF PEPTIDE TARGETING *PORPHYROMONAS*
GINGIVALIS

By

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B.D.S, Rajiv Gandhi University of Health Sciences, 2009

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ABSTRACT

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THE RELEASE OF PEPTIDE TARGETING *PORPHYROMONAS
GINGIVALIS*

Sonali Sapare

Cr tkr'3; , 2018

Periodontitis is a chronic inflammatory disease that infects the tissues of the periodontium. It is estimated that 47.2% or 67.2 million American adults suffer from mild, moderate and severe periodontitis. Globally, 30-50% of the adult population is afflicted with periodontal disease, making it one of the most prevalent infectious diseases in the world. Therapeutics targeting *P. gingivalis* may be effective to alter periodontitis progression. However, the current treatment modalities that target critical pathogens to maintain host-biofilm homeostasis are limited, urging the development of specifically targeted therapeutics to limit *P. gingivalis* recolonization of the oral cavity after periodontal treatment and healing. We previously identified a peptide (BAR) that inhibits the formation of *P. gingivalis-S. gordonii* biofilms; however, formulations that effectively deliver the peptide within the oral cavity are lacking. Polymeric electrospun fibers (EFs) offer a new platform to deliver high localized concentrations of the peptide (BAR) for prolonged periods, to disrupt established biofilms and enhance BAR effectiveness. The

objective of this study was to determine if electrospun fibers (EFs) that encapsulate the BAR peptide, function as a sustained-release drug delivery vehicle for application in the oral cavity. A variety of polymer formulations were electrospun using a uniaxial electrospinning approach. Polymers including poly(lactic-co-glycolic acid) (PLGA) or methoxy-poly(ethylene glycol) (mPEG-PLGA), polycaprolactone (PCL), and poly(L-lactic acid) PLLA, were synthesized alone or blended in a 40:60, 20:80 and 10:90 w/w ratio with a hydrophilic polymer, polyethylene oxide (PEO), to increase BAR release over 24 hr. To determine the total loading of BAR in EFs, the fibers were dissolved in DMSO, and the amount of BAR encapsulated was compared to a known standard of fluorescently-labeled BAR (F-BAR). The sustained-release of F-BAR from fibers was determined by comparing the supernatant from a variety of release time points to a known standard of F-BAR. PLGA, mPEG-PLGA, PCL and PLLA EFs demonstrated encapsulation efficiencies of 68%, 94% 60% and 46% respectively, while exhibiting minimal release of BAR (9.5%, 7%, 1.4% and 1.5%) within 24 hr. Blended polymeric fibers comprised of PLGA:PEO, PCL:PEO, and PLLA:PEO with 40:60, 20:80 and 10:90 w/w ratios were fabricated to enhance release. All polymer blends incorporated high concentrations of BAR peptide, and increasing ratios of PEO significantly enhanced BAR release within 24 hr. The most promising 10:90 PLGA:PEO, PCL:PEO, and PLLA:PEO formulations provided 95%, 50% and 75% BAR release at 4 hr.

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CHAPTER 1

INTRODUCTION

Periodontal diseases are highly prevalent and can affect up to 90% of the worldwide population. Gingivitis, the mildest form of periodontal disease, is caused by the bacterial biofilm (dental plaque) that accumulates on teeth adjacent to the gingiva (gums) (Pihlstrom, Michalowicz, & Johnson, 2005). The colonization of bacteria in the supragingival area initiates an inflammatory response (Brogden & Guthmiller, 2002; Dickinson et al., 2011) and gingivitis, if left untreated, can lead to a more severe form of periodontal disease characterized by clinical attachment loss, termed periodontitis (Heijl et al., 1976). Periodontitis is a chronic, irreversible inflammatory disease, during which the chronic infiltrate of immune cells induces destruction of connective tissue, vascular proliferation and alveolar bone destruction (Pihlstrom, Michalowicz & Johnson, 2005). Periodontitis is second only to dental caries as a cause of tooth loss among adults in developed countries (Gautam et al., 2011). 30-50% of the global adult population suffers from periodontal disease making it one of the most prevalent infectious diseases in the world. It is estimated that 47.2% or 67.2

million American adults suffer from mild, moderate and severe periodontitis (P.I Eke *et al*., 2012). Severe disease (subgingival pocket depths > 6mm) occurs in 9% of U.S. adults (Adeyemi et al, 2015) and 11.2% of adults worldwide (Eke, P., et al. 2012, Kassebaum et al, 2014). This correlates to annual expenditures for the treatment and prevention of periodontal disease in excess of 14 billion dollars (Eke, P., et al. 2012). Other studies have also demonstrated that mild forms of periodontitis affect 75% of adults in the United States and more severe forms affect 20 to 30% of adults (Dhadse et al, 2010). Periodontal diseases are also proven to be risk factors for various systemic diseases such as bacteremia, infective endocarditis, cardiovascular disease, prosthetic device infection, diabetes mellitus, respiratory diseases, rheumatoid arthritis and adverse pregnancy outcomes (Kim et al, 2006, Scannapieco et al, 2013, Kaur et al 2014).

<u>Dental plaque biofilm formation</u>

A biofilm is an organized aggregate of microorganisms living within an extracellular polymeric matrix that they produce and irreversibly attach to fetish or living surface which will not remove unless rinse quickly (Hurlow et al., 2015). The National Institutes of Health (NIH) revealed that among all microbial and chronic infections, 65% and 80%, respectively, are associated with biofilm formation (Jamal et al., 2018). Microbial biofilms are known to cause a number of infectious diseases in humans, a few of which include

tonsillitis, dental disease, urinary tract infections and endocarditis. The oral cavity is a dynamic environment and from a biofilm-formation standpoint, the oral cavity is a propitious environment that allows for the growth of a diverse array of microorganisms (Gibbons, 1989). In periodontal disease, the accumulation of biofilm around the gingival margins provokes an inflammatory response by the host. This involves an increased flow of Gingival Crevicular Fluid (GCF) which not only introduces components of the host response but also many molecules that can act as potential nutrients for some of the minor components of the normal resident subgingival microbiota (Wade, 2013). A small sample of dental plaque contains, on average, between 12 and 27 species (Aas, Paster, Stokes, Olsen, & Dewhirst, 2005) hence dental plaque is a classic example of multispecies biofilm. The formation of dental plaque involves several phases within seconds of tooth eruption or after tooth cleaning (Diaz et al., 2006). Hence, understanding the growth, progression and arrangement of plaque alongside the etiology will aid in the development of novel therapies to treat and cure periodontitis (Marsh, 1994).

Development of Dental plaque
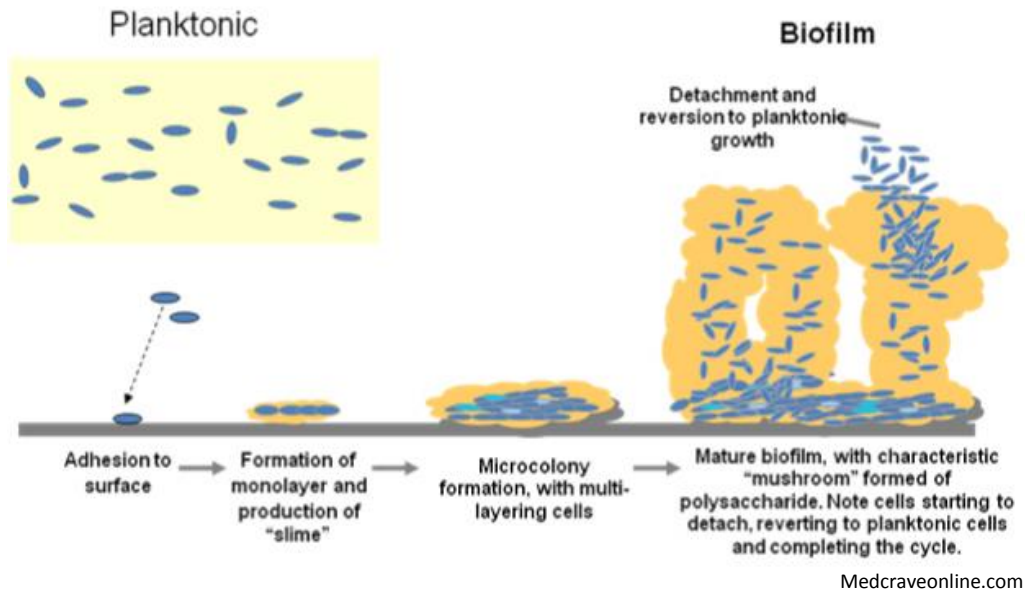
Figure 1. Biofilm development

The development of oral biofilms depends on interactions between bacterial cell-surface adhesins and host receptors. Interactions between different species of bacteria, and between bacteria and the host, are central to the development of oral biofilm (Jakubovics & Kolenbrander, 2010). The key stages in the development of a biofilm as shown in figure 1 are: first, pellicle formation; second, adhesion of a microorganism to a surface and individual colonization and organization of cells; third, secretion of extra cellular polymeric substances (EPSs) and maturation into a three-dimensional structure; and finally, dissemination of progeny biofilm cells (Seneviratne, Zhang, & Samaranayake, 2011).

When the bacterial and host products come in contact with a clean tooth surface, the negatively charged hydroxyapatite tooth surface absorbs it and forms a thin layer of conditioning film called the acquired pellicle (Armstrong et al, 1968). In the supragingival areas, this layer is covered by positively-charged molecules such as salivary glycoproteins, statherin, histatin, proline rich proteins, alpha-amylase, bacterial components like glucosyltransferases (GTFs), glucan, and by-products from gingival crevicular fluid in the subgingival areas (M. Hannig & Joiner, 2006). Acidic phosphoproteins and proline-rich proteins that aid in colonization of bacteria on to the tooth surfaces mediate the initial interaction between the pellicle and the bacteria. In addition, other environmental cues can influence biofilm formation including low pH, changes in osmolarity, and oxygen. The early plaque forming bacteria or the initial colonizers are generally Gram-positive cocci, which primarily comprise streptococcal species. (Marsh, 1994, 2006).

Adhesion of bacteria to the salivary pellicle represents the second step in the colonization of enamel surfaces in the mouth. Oral streptococci such as *Streptococcus sanguinis, Streptococcus oralis,* and *Streptococcus mitis* have been shown to be the major primary colonizers, constituting 60–80% of dental plaque within 4 to 8 hr (Diaz et al., 2006). They initially make non-specific, reversible bonds like hydrogen bonds, hydrophobic interactions, calcium bridges, van der Waals forces, acid-base interactions and

electrostatic interactions with the molecules in the acquired pellicle (C. Hannig & Hannig, 2009; Marsh, 2005). The main physical surface attachment structures of bacteria are fimbriae and fibrils (Donlan, 2002). The pellicle provides a sticky base to support further assemblage of microorganisms (Lindh et al, 2014).

Dental plaque biofilm will continue to grow and expand by the multiplication of the primary colonizers and co-aggregation and co-adhesion of secondary colonizers. Primary colonizers are mostly aerobic or facultative aerobes which reduce oxygen, allowing the anaerobic bacteria such as *Actinomyces* species*, Fusobacterium nucleatum, Prevotella intermedia,* and *Capnocytophagia* species to enter the biofilm community (Seneviratne et al., 2011). Co-aggregation is driven by specific receptor-ligand interactions that allow new bacterial colonizers to adhere to the previously attached cells (Grenier, 1992). The fundamental mechanism of aggregation is polysaccharide recognition between bacteria. The polysaccharide recognition sites vary from one paired bacterial recognition to another paired bacterial recognition, because one bacterial cell has several different receptors which are complementary to different adhesions belonging to other bacterial species (R. Huang, Li, & Gregory, 2011)

As multiplication of bacteria occurs, discrete colonies of microorganisms are formed. These microcolonies secrete extracellular polymeric substances (EPS), and get embedded in it, thus providing a physical scaffold for the biofilm. A mature biofilm community is formed as the microcolonies embedded in EPS become linked together in an organized manner (Seneviratne et al., 2011). The mature biofilm is also comprised of fluid filled channels interspersed in the microcolonies, which provide nutrients and oxygen required for bacterial growth. The final stage of biofilm development is the detachment of cells from the biofilm colony and their dispersal into the environment, which can be active or passive.

Active dispersal refers to mechanisms that are initiated by the bacteria themselves, which includes modes like seeding dispersal, referring to the rapid release of a large number of single cells or small clusters of cells from hollow cavities that form inside the biofilm colony (Boles, Thoendel, & Singh, 2005). Passive dispersal refers to biofilm cell detachment that is mediated by external forces such as fluid shear or abrasion (Lawrence, Scharf, Packroff, & Neu, 2002). Modes like erosion, which refers to the continuous release of single cells or small clusters of cells from a biofilm, and sloughing, referring to the sudden detachment of large portions of the biofilm, can be either active or passive processes (Lappin-Scott & Bass, 2001; Marshall, 1988).

Etiology of Periodontitis

The complex multifactorial etiology of Periodontitis includes genetic predisposition along with state of systemic health of the host and environmental factors. Several other risk factors including diet, stress, and smoking can be involved. At the microbial level, *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* are considered as 'periodontopathogens', and have been classified as 'red' complex oral bacteria that have are present in the pathogenic dental plaque and have strong association with periodontitis (Darveau, Hajishengallis, & Curtis, 2012; Griffen et al., 2012; Socransky, Haffajee, Cugini, Smith, & Kent, 1998).

The role of plaque bacteria in diseased individuals can be explained by two main hypotheses. The "non-specific plaque" hypothesis proposed that plaque is a conglomerate of multiple microorganisms and that no specific bacteria is responsible for the progression of periodontitis (Rosier, De Jager, Zaura, & Krom, 2014). In view of this, mechanical therapy for plaque removal was considered the best way to curb the disease. Contrary to this, the "specific plaque hypothesis" proposed by Loesche purports that out of a diverse microbial community in the oral cavity, only specific microorganisms belonging to the 'red' complex are involved in the etiology of periodontitis (Loesche, 1992). While, *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola* have strongly been identified with periodontitis, it

does not sufficiently elucidate the presence of pathogenic bacteria in healthy hosts or the absence of it in diseased individuals (G. Hajishengallis & Lamont, 2012). A more recent "keystone pathogen hypothesis" shows *P. gingivalis*, even in low levels, can play a significant role in changing the microflora from a symbiotic microbiota to a dysbiotic state, leading to disruption of the host-microbe homeostasis (George Hajishengallis, Darveau, & Curtis, 2012). Moreover, the recently described Polymicrobial Symbiosis and Dysbiosis (PSD) model highlights the importance of other microorganisms outside the 'red' complex capable in causing dysbiosis (G. Hajishengallis & Lamont, 2014). Altogether, a better understanding of the complex interactions between the microbes, host and its immunity can help discern the etiology.

P. gingivalis **and its interaction with** *Streptococcus gordonii*

As a Gram-negative anaerobic microorganism, *P. gingivalis* plays a pivotal role in periodontitis (Socransky et al., 1998). *P. gingivalis* has been extensively studied, with studies by *Hajishengalis* et al,2012 recognizing it as a keystone pathogen in mice and its relative ease of cultivation and genetic modification compared to the other species (Darveau et al., 2012). Prior to its colonization in the anaerobic environment of the subgingival pocket, it interacts with the initial colonizer *Streptococcus gordonii*, a Gram-positive commensal (Marsh, 1994). Development of biofilm occurs subsequent to the

initial adherence of *P. gingivalis* and *S. gordonii* deposited on the salivary pellicle (Kolenbrander & London, 1993). The ability of *P. gingivalis* to adhere, grow and retain in different areas is through adhesins including fimbriae, hemagglutinins and proteinases (Lamont & Jenkinson, 2000). The commensal species *S. gordonii* also provides an attachment substrate for biofilm establishment by *P. gingivalis* (Park, Y. et al., 2005).

To limit and inhibit biofilm formation, these distinct adhesion mechanisms can be targeted by therapeutic agents to curtail periodontal disease (Carlo Amorin Daep, DeAnna M. James, Richard J. Lamont, & Donald R. Demuth, 2006). *S. gordonii* expresses the antigen I/II proteins, which are multifunctional adhesins, and contribute to the initiation and development of the oral biofilm (Demuth & Irvine, 2002). The SspA and SspB polypeptides, members of the antigen I/II, adhere to the minor fimbrial antigen (Mfa) of *P. gingivalis.* A previous study by (Brooks, Demuth, Gil, & Lamont, 1997) showed the region defined by amino acid residues 1167 to 1250 of the SspB polypeptide sequence is essential for adherence to *P. gingivalis*. Further studies demonstrated that a protein determinant comprised of amino acids 1167 to 1193 designated as BAR (SspB Adherence Region) was sufficient to interact with *P. gingivalis* (Demuth, Irvine, Costerton, Cook, & Lamont, 2001). A synthetic peptide comprised of this sequence potently inhibited the protein-to-protein interactions between *P. gingivalis* and *S. gordonii*. But

asacchrolytic bacteria like *P. gingivalis* utilizes amino acids as their energy sources making it highly proteolytic in nature. This makes the BAR peptide susceptible to degradation. Study by Daep, et al. showed the inclusion of the BAR's receptor Mfa protein into *P. gingivalis* cell extracts can prevent the BAR peptide's degradation. This suggests the affinity of BAR for Mfa1 is greater than for the proteases secreted by *P. gingivalis*. This overcomes the problem of BAR's susceptibility to degradation and hence can be utilized as a potential therapeutic agent as it inhibits *P. gingivalis* adherence to streptococci in the presence of proteolytic enzymes (Daep, Novak, Lamont, & Demuth, 2010). *In vitro* experiments confirmed BAR inhibition of the resultant formation of biofilms (IC50 = 1.3μM) (C. A. Daep, D. M. James, R. J. Lamont, & D. R. Demuth, 2006). BAR inhibited *P. gingivalis* virulence in mice when administered simultaneously with *P. gingivalis* infection, *in vivo* (Daep, Novak, Lamont, & Demuth, 2011). Though BAR showed great ability to inhibit and prevent the initial interaction between *P. gingivalis* and *S. gordonii*, *in vitro* results exhibited weaker potency against already established and complex biofilms. Moreover, BAR administration demonstrated transient effects. In this study, we developed electrospun fibers, a new delivery platform to utilize and administer BAR more effectively against oral biofilms.

Nanotechnology in therapeutics

The presence of a diverse and complex microflora in the oral cavity can make periodontitis a difficult disease to treat. Various mechanical therapies like scaling and root planning fail to eradicate the subgingival pathogens and halt the inflammatory cascade(Herrera, Matesanz, Bascones-Martinez, & Sanz, 2012). Systemic administration of antibiotics can result in side effects of inadequate concentration of the drug reaching the periodontal pockets and developing microbial resistance. Hence, local delivery systems have the advantage of direct access to disease site bypassing the systemic route and enhancing the efficacy of the drug.(Garg, Singh, Arora, & Murthy, 2012). Therapeutic approaches that can target the periodontal pathogens specifically are lacking.

Using the premise of nanotechnology, non-toxic electrospun fibers can be fabricated to administer BAR peptide locally as they offer several advantages. The small fiber size can help the drug be delivered to the appropriate site (Ferrari, 2004). They have open and interconnected pores, allowing for optimal interaction with bioactive molecules and have sufficient binding affinity to allow release of the encapsulated drug.(Morie, Garg, Goyal, & Rath, 2016). This project focuses on the synthesis of BAR-encapsulated fibers formulated via the electrospinning process.

<u>Electrospinning</u>

Electrospinning is a relatively simple, user-friendly and inexpensive process used to fabricate fibers. It is an established technique used to produce small diameter fibers in the range of several nanometers to micrometers. Since the advent of using electrostatic forces to produce fibers in the 1930s (Formhals A., 1934) it has gained popularity. Specifically, during the last 20 years it has been employed to fabricate products for use in medical applications, including fiber drug delivery systems and tissue engineering scaffolds. Reasons such as being easy to handle, minimum consumption of solution, enabling controllable fiber diameter, processing convenience, providing a cost effective method, in parallel with process reproducibility make electrospinning the method of choice for large scale preparation of fibers (Thenmozhi, Dharmaraj, Kadirvelu, & Kim, 2017). The electrospinning process converts polymeric solution into solid fibers by application of electrical force. The many advantages of electrospinning include high loading capacity, high encapsulation efficiency, simultaneous delivery of diverse active agents, ease of operation, and cost-effectiveness (Wang, Wang, Yin, & Yu, 2010). Electrospun fibers have been successfully used to achieve different drug release profiles, such as immediate, smooth, pulsatile, delayed, and biphasic releases (Prabaharan, Jayakumar, & Nair, 2012). Moreover, drugs ranging from antibiotics and anticancer agents to proteins, aptamer, DNA, and RNA have been incorporated into electrospun fibers

(Prabaharan et al., 2012). As shown in figure 2, the apparatus consists of a high voltage power supply, syringe pump, syringe needle and a stainless steel rotating mandrel as a metal collector. The main governing forces are electromechanical and hydrodynamic forces, and its working principle is based on when very high voltage supply applied on a polymer solution, which induces charge within the polymer and needle. When the charge repulsion force exceeds the surface tension, a jet is splayed from the needle tip (referred to as a "Taylor cone") creating droplets with very high surface area (Goyal, Macri, Kaplan, & Kohn, 2016; Thakkar & Misra, 2017). As the strand of solution travels to the collector, the solvent evaporates to leave fibers.
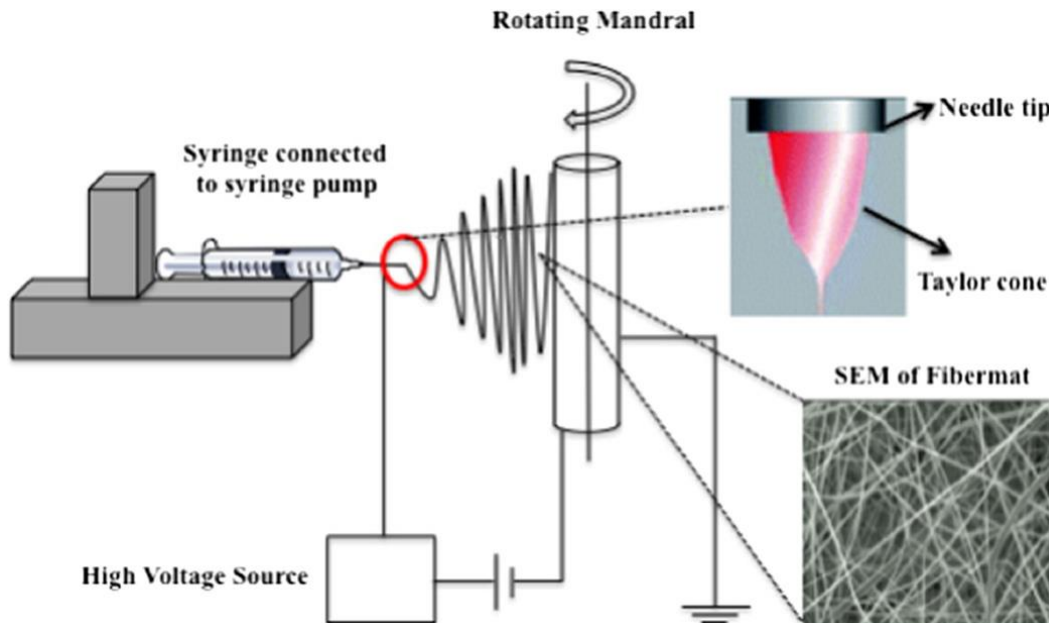
Figure 2. Overall setup of standard horizontal electrospinning setup including a syringe pump, polymer-drug/peptide solution, high voltage supply, a collecting mandrel.

In this study, we describe an approach to fabricate fibers encapsulated with BAR peptide to provide short-term release of the peptide via uniaxial/blend electrospinning. In uniaxial/blend electrospinning, a single nozzle syringe is used to electrospin polymer-solvent or polymer-solution combinations into solid porous fibers. Electrospinning is a simple approach in which water-soluble bioactive agents such as proteins, peptides, nucleic acids and also hydrophilic/hydrophobic drugs are combined into polymer solutions containing aqueous or organic solvents prior to electrospinning.
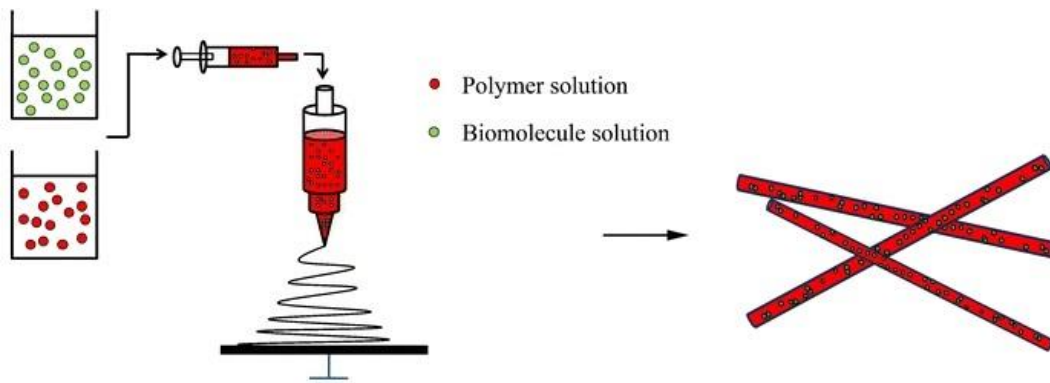
Figure 3. A schematic representation of blend electrospinning strategy to formulate electrospun fibers and the expected distribution of the bioactive molecules in the fibers.

The electrospinning process localizes biomolecules within the fibers of the scaffolds rather than simply adsorbing them to the scaffold surface. As such, this approach has the capability to improve sustained-release relative to physical adsorption (Wei Ji et al., 2011). Also compared to co-axial

electrospinning, uniaxial spinning is relatively a simpler method with no additional setup required to fabricate fibers. In this work, we adopt this straightforward approach by combining the BAR peptide solubilized in an aqueous solution (Tris-EDTA, TE) buffer and with an organic solvent blend (dimethyl sulfoxide, DMSO/chloroform) that contains polymer solution.

Electrospun fibers

Electrospinning is a versatile method to fabricate fibers that have diameters ranging from several nanometers to micrometers. Molecules such as proteins, peptide, antibodies, and small molecule drugs, can be loaded within or on the surface of fibers according to their properties. Using electrospun fibers as drug delivery systems provides many advantages including a) high drug loading (up to 60%) and encapsulation efficiency (up to 100%) so the drug is released continuously for longer duration upon insertion in the body (Ball & Woodrow, 2014), b) polymer diversity to accommodate compatibility with physico-chemically distinct agents (Ball, Krogstad, Chaowanachan, & Woodrow, 2012), c) easy modulation of drug release profile depending upon the properties of polymer/polymeric blends/other materials used (Sundararaj, Thomas, Peyyala, Dziubla, & Puleo, 2013), and d) process simplicity and cost-effectiveness (Ball, C., & Woodrow, K. A., 2014). These fibers possess high surface to volume ratio which would accelerate the solubility of the drug in the aqueous solution and enhance the efficiency of the drug (Meng et al.,

2011). With a high degree of structural perfection and resultant superior mechanical properties (Liao, Zhang, Gao, Zhu, & Fong, 2008) electrospun fibers have open and interconnected pores which allow for optimal interaction with bioactive molecules, have excellent ability to deliver the encapsulated substances to the target site and have sufficient binding affinity to allow release of the encapsulated substance for longer duration (Morie et al., 2016). Many studies have shown that fibers comprised of polymer blends have a great potential for tuning drug miscibility and the resulting drug-polymer interactions could lead to different release profiles (Chou & Woodrow, 2017).

 A number of natural, synthetic, semi synthetic and biological polymers are used. Since biocompatible, biodegradable, and Food and Drug Administration (FDA) approved polymers including PLGA (W. J. Li, Laurencin, Caterson, Tuan, & Ko, 2002), PCL (59),PLLA (Jun, Hou, Schaper, H. Wendorff, & Greiner, 2003) and PEO (Son, Youk, Lee, & Park, 2004) have been successfully electrospun into fibers, all the mentioned polymers are used in this study.

CHAPTER 2

HYPOTHESIS AND SPECIFIC AIMS

Research Hypothesis

We hypothesize that BAR-encapsulated EFs, will provide short-term release of therapeutically relevant concentrations of BAR. Moreover, we hypothesize that BAR release from EFs may be modulated by changing hydrophilic:hydrophobic polymeric fiber blend ratios. Long-term, we believe EFs will offer a new dosage form in which to encapsulate BAR, and will function as an effective drug delivery vehicle within the oral cavity.

Specific Aims

1. Synthesize electrospun fibers (EFs) in different formulations that encapsulate BAR peptide.

2. Characterize the electrospun fibers (EFs) to provide release of the peptide for up to 24 hr.

CHAPTER 3

MATERIALS AND METHODS

Peptide Synthesis

The peptide used in this study (shown in Table 1) was synthesized by BioSynthesis, Inc. (Lewisville, TX). It was obtained with a purity greater than 85% and comprised residues 1167 to 1193 of the SspB (Antigen I/II) protein sequence of *S. gordonii.*

Table 1 Sequence of BAR peptide.

Peptide	Peptide Sequence
BAR	NH2-LEAAPKKVQDLLKKANITVKGAFQLFS-OH
F-BAR	NH2-LEAAPK-Kflc-VQDLLKKANITVKGAFQLFSOH

A fluorescent BAR peptide (F-BAR), synthesized by covalently attaching 6-carboxyfluorescein (Flc) to the epsilon amine of the lysine residue (underlined in Table 1), was used to determine the amount of BAR peptide encapsulated in the electrospun fibers.

Materials

Hydrophobic polymers including poly (lactic-co-glycolic acid) (PLGA, 50:50 lactic:glycolic acid, MW 30,000-60,000), methoxy-poly(ethylene glycol) (mPEG-PLGA, MW 5,000:55,000), polycaprolactone (PCL, MW 80,000), and poly(L-lactic acid) (PLLA, MW 50,000) and the hydrophilic polymer, polyethylene oxide (PEO, MW 100,000) were purchased from Sigma-Aldrich (St.Louis, MO, USA). Tris-EDTA (TE) buffer (pH 8.0), phosphate buffered saline (PBS) and the organic solvents chloroform, dimethyl sulfoxide (DMSO) and hexafluoroisopropanol (HFIP) were also purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used directly without further purification. One milliliter plastic syringes, petri dishes, and 20 ml scintillation vials were obtained from VWR. One ml glass syringes were purchased from Fisher Scientific. The electrospinner was provided courtesy of Dr. Stuart Williams at the Cardiovascular Innovative Institute, University of Louisville.

Preparation of Polymer solutions

To prepare the hydrophobic polymer batches, PLGA, mPEG-PLGA, and PLLA were dissolved in HFIP at a concentration of 15% (w/w). This solution was aspirated into a 7 ml glass scintillation vial, and sealed using parafilm to prevent evaporation of the organic solvent. Keeping the vial at eye level, the level of the polymer solution was marked to ensure a constant volume of

organic solvent was maintained post-incubation. The vial was placed in a shaker at 150 rpm and incubated at 37°C overnight to solubilize the polymer. The final volume of the polymer solution was 1 ml. The following day, BAR peptide was dissolved in 200 µl TE buffer in a 1 ml eppendorf tube and vortexed for 5 minutes. The BAR solution was mixed with the polymer solvent at a concentration of 1% (w/w), followed by vortexing the polymer/BAR solution for another 5 minutes.

To prepare blended polymers, the hydrophobic polymers PLGA, PCL and PLLA were mixed with PEO at different ratios (40:60, 20:80, 10:90) in (w/w) to form PLGA/PEO, PCL/PEO, and PLLA/PEO blends in chloroform at a concentration of 15% (w/v). The blended solutions were aspirated into a 20 ml glass scintillation vial, and sealed using parafilm to prevent evaporation of the organic solvent. Keeping the vial at eye level, the solvent meniscus was marked to ensure a constant volume of organic solvent was maintained post-incubation. If any solvent evaporated during incubation, the marking was used to add fresh solvent to maintain the original solvent volume. The vial was placed in a shaker at 150 rpm and incubated at 37°C overnight to solubilize the polymer. The final volume of the polymer solution was 1ml. The following day, BAR peptide was dissolved in 60 µl DMSO in a 1 ml eppendorf tube and vortexed for 5 minutes. The BAR solution was mixed

with the polymer solvent at a concentration of 1% (w/w), followed by vortexing the polymer/BAR solution for another 5 minutes.

Electrospinning

The experimental set-up used for conducting electrospinning included a high voltage power supply, syringe pump, 1ml plastic and glass syringe needle and a 4 mm metal mandrel.

On the day of synthesis, the plexiglass case of the electrospinner was opened to adjust the position of the collector. A 4 mm diameter stainless steel mandrel was cleaned using sand paper to remove residue and was secured to the collector. For the non-blended polymer solution with HFIP as the solvent once the BAR peptide solution was mixed, 1 ml of this solution was loaded into a 1ml plastic syringe with an 18-gauge needle tip. The syringe was held upright for a few minutes and pushed gently to let the air bubbles out. The syringe was then mounted on the syringe pump, setting the holder plate on top of syringe to keep it in place. The inner diameter of the syringe pump program was set to the internal diameter of the BD plastic syringe (4.78 mm). The needle tip was pushed into the circular hole until the tip was roughly 1 inch into plexiglass case, and centered. The collector was adjusted such that there was at least 10 cm distance maintained from the needle tip. The alligator clip from the voltage source was attached to the needle tip 1 inch away from syringe but still outside the plexiglass case. The syringe pump

motor controls were adjusted by setting the "slide" control to 4.5 and the "rotor" to 8. The voltage supply was set at 20 kV, and the syringe pump flow rate was set to 0.8 ml per hour. The polymer solution was electrospun at room temperature, under atmospheric conditions, for 1 hr 15 min, and the resulting fine mist of solution was collected on the mandrel and allowed to dry for 15 minutes. The mandrel was removed from the collector and using a razor blade, the fiber was cut and gently peeled off the mandrel. The fiber was placed in a labeled petri dish and kept in desiccator for 24 hr before any characterization (weighing) occurs, preventing residual solvent from contributing to the fiber weight. The desiccated fibers were stored in 4°C until use.

For the blended polymer solution with BAR peptide, 1 ml of the solution was aspirated into a 1 ml glass syringe with a 22-guage metal blunt needle and mounted on the automated syringe pump. The internal diameter of the Hamilton Gastight syringe was set to 4.61 mm. A distance of 15 cm was kept between the needle tip and the collector. The "slide" control was set to 4.5 and the "rotor" was set to 8. A voltage of 20-25 kV was applied, at a flow rate of 0.3 ml per hr. The electrospinning processes were employed under ambient conditions for 3 hr 20 min. The stretched and solidified polymeric fibers were collected on a 4mm diameter stainless steel mandrel and allowed to dry for 15 minutes. The fiber was peeled off the mandrel and placed in

petri dish and kept in desiccator for 24 hours. After desiccation the fibers were stored in 4°C.
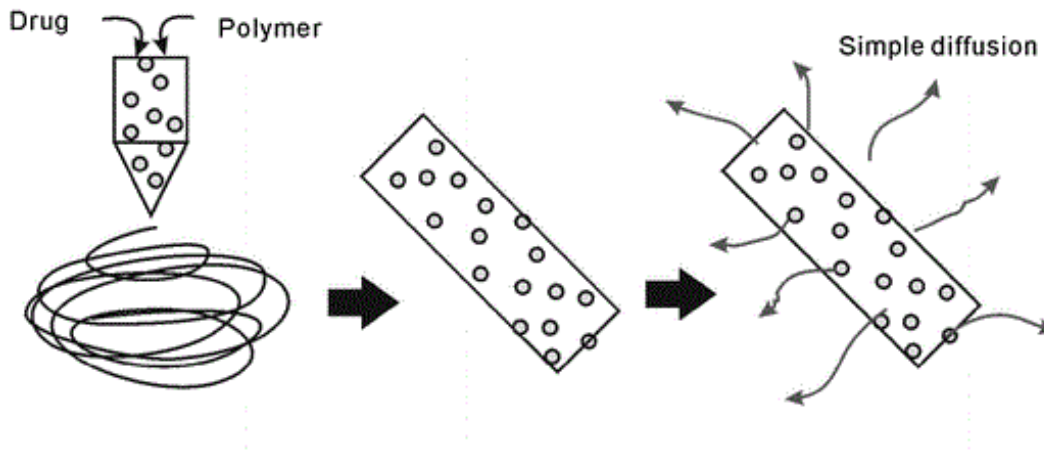
Figure 4. Schematic of drug/peptide loading method within the electrospun fiber and its proposed release kinetics.

Characterization of electrospun fibers

Functional Characterization

Encapsulation efficiency of BAR peptide in fibers.

The loading and encapsulation efficiency of the peptide in the non-blended and blended fibers were determined by dissolving F-BAR fiber scaffolds in DMSO. A clean razor blade was used to cut three samples of each fiber, each weighing ~ 2-3 mg. The fiber samples were placed in a 1.5 ml eppendorf tube and DMSO was added to create a 1mg/ml solution. The samples were vortexed one minute, sonicated for 5 min, and left to dissolve for 1 hour in the dark.

A standard curve of F-BAR was obtained by making a 0.1 mg/ml F-BAR stock solution in 1:9 DMSO:TE. The stock solution was serially diluted with 1:9 DMSO:TE to generate a concentration ranging from 0.0007 mg/ml to 0.1 mg/ml. The diluted solutions were transferred to a 96-well clear bottom microtiter plate in triplicate, consisting of 100µl in each well.

After the incubation period, the sample solutions were vortexed and sonicated again. The solutions were diluted 1:2, 1:5, 1:10 and 1:100 with 1:9 DMSO:TE solution, and transferred to a microtiter plate.

The diluted fiber samples and standard were measured for fluorescence at 488nm/520nm (excitation/emission) using a spectrophotometer. The amount of BAR peptide encapsulated in fibers was determined from the known standard curve of the BAR peptide. The loading and encapsulation efficiency of the BAR peptide in the fiber were calculated by taking the average of the values obtained in each dilution (1:2, 1:5, 1:10 and 1:100) of the fiber samples.

Determination of peptide release profiles from fibers

Three samples for each of the non-blended and blended fibers each weighing 2-3 mg, were placed in clean 1.5 ml eppendorf tube. One milliliter of 1x PBS (pH 7.4) was added to the eppendorf tube. The fiber samples were placed in an incubator shaker at 130 rpm and 37°C for 1 hr. The supernatant was subsequently removed and pipetted into cluster tubes arranged in a freezer

box. To the remaining fiber in the Eppendorf tube, fresh 1x PBS was added and incubated until the next time point. This procedure was repeated and supernatants were obtained at 1, 2, 4, 8, 12, and 24 hr time points. The freezer box was parafilmed and covered in aluminum foil and stored at -20°C.

A standard curve of the F-BAR was obtained and plotted by making a 1 mg/ml F-BAR stock solution with TE buffer. This stock solution was serially diluted with TE buffer to generate a standard curve ranging from 0.03 µg/ml to 1000 µg/ml. One hundred microliters of the standard samples were transferred to each well of a 96-well clear bottom microtiter plate.

The supernatants at every time point of each fiber sample and the standard were measured for fluorescence at 488nm/520nm (excitation/emission) using a spectrophotometer. The amount of F-BAR released at each time point was measured by comparing to a known standard of F-BAR in TE buffer.

CHAPTER 4

RESULTS

Functional Characterization

Previous studies have demonstrated that EFs have been effectively used to deliver of proteins (Casper, Yamaguchi, Kiick, & Rabolt, 2005; Chew, Wen, Yim, & Leong, 2005; Xiaoqiang Li et al., 2010; Puhl, Li, Meinel, & Germershaus, 2014; Zeng et al., 2005). In this study we tested various formulations of the electrospun fibers using different polymers to deliver the BAR peptide. Initially non-blended EFs were fabricated using hydrophobic polymers PLGA, mPEG-PLGA, PLLA with HFIP (15% w/w). PCL/HFIP solution at 15% w/w was highly viscous and was not electrospinnable, hence a 12% w/w of PCL:HFIP was used.

PLGA, PCL, PLLA were also blended in 40:60, 20:80, 10:90 w/w ratio with hydrophilic polymer PEO and dissolved in chloroform (15% w/v). All blends were theoretically loaded with 1% w/w F-BAR: polymer, such that 10 µg of F-BAR was loaded for every milligram polymer. The loading and encapsulation efficiency were determined by assessing the fluorescence of the dissolved polymers. The total fluorescence emitted by the dissolved EF solution was converted to a concentration of BAR by comparing to the

fluorescence of a known F-BAR standard (in 1:9 DMSO:TE buffer). Figure

5 shows the standard curve of free F-BAR that was used to quantify the

concentration of F-BAR in fibers.
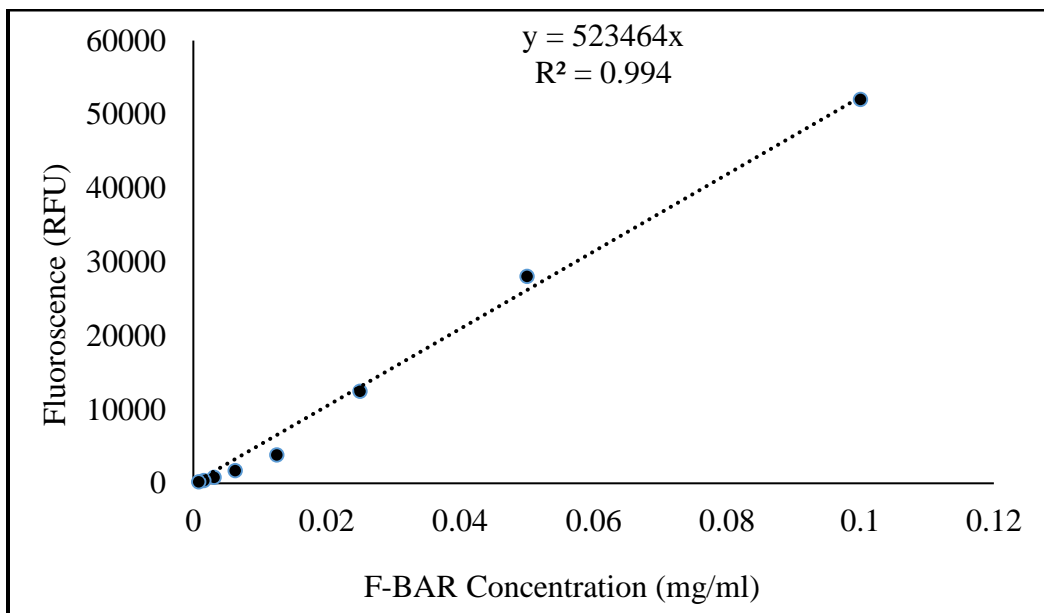
Figure 5. Standard Curve for free F-BAR showing increased fluorescence
with an increase in F-BAR concentration. This graph was used to calculate
the output concentration of F-BAR for BAR-EFs. The x-axis represents the
concentration of BAR in mg/ml and the y-axis represents the fluorescence in
Relative Fluorescence Units (RFU).

Fiber Formulation	Blend Ratio	Overall Polymer Yield (%)	Loading BAR/Fiber (μg/mg)	Encapsulation Efficiency (%)
PLGA		59.0	6.9 ± 0.1	68.6 ± 2.5
mPEG-PLGA	100:0	52.0	9.4 ± 0.4	94 ± 0.5
PCL		51.0	6.0 ± 0.4	60 ± 4.0
PLLA		42.3	4.6 ± 0.6	46 ± 5.2

PLGA:PEO		82.9	7.4 ± 0.5	74 ± 5.5
PCL:PEO	**40:60**	91.5	8.6 ± 0.2	86 ± 2.4
PLLA:PEO		82.0	9.1 ± 0.3	92 ± 3.1
PLGA:PEO		80.9	8.8 ± 0.2	88 ± 2.6
PCL:PEO	**20:80**	89.3	8.9 ± 0.4	89 ± 4.0
PLLA:PEO		85.2	8.3 ± 0.4	83 ± 4.2
PLGA:PEO		82.8	8.8 ± 0.5	88 ± 5.6
PCL:PEO		80	6.0 ± 0.4	60 ± 4.0
PLLA:PEO	**10:90**	80.9	8.5 ± 0.3	85 ± 3.5

Table 2. The amount of BAR (mg) loaded in non-blended and blended polymeric EF formulations (µg/mg). High loading capacity and encapsulation efficiency was achieved by all fiber formulations. Non-blended EFs showed comparatively lower polymer yield than the blended EFs.

As summarized in Table 2, the total payload for non-blended and blended EFs ranged from 4.6 – 9.4 µg/mg and 6.0– 9.2 µg/mg, respectively, indicating that high loading of F-BAR was achieved in all fiber formulations. The amount of polymer incorporated into the electrospun fiber scaffold, calculated as the overall polymer yield, ranged from 40-60% for the non-blended formula, while the blended fibers achieved higher polymer yields (80-90%).

BAR release from BAR encapsulated EFs

To determine the release profiles of the fiber formulations, aliquots of BAR-encapsulated EFs were incubated in PBS at 37°C. Fluorescence of the

collected supernatant was measured at 1, 2, 4, 8, 12 and 24 hr. Triplicate fluorescence readings at each time point were compared to a known standard of the F-BAR in PBS. Figure 6 shows the cumulative release of BAR from non-blended EFs at each time point over a 24 hr duration. PLGA and mPEG-PLGA illustrates a slow release of 9.5% and 7% respectively at 24 hr. PCL and PLLA fibers showed much less release of BAR for the same duration, with hydrophobic-only fibers demonstrating minimal release relative to the PEO-blended EFs. Figure 7 shows the release of F-BAR from PLGA:PEO, PCL:PEO and PLLA:PEO blended fibers with different blend ratios (40:60, 80:20, 90:10). The hydrophobic fibers comprised of PLGA, mPEG-PLGA, PCL and PLLA only, released minimal concentrations of BAR within 24 hr, relative to PEO-blended fibers. Fibers comprised of 10:90 PLGA:PEO released 8.25 μg/mg, corresponding to 93% of the encapsulated F-BAR within the first 2 hr., and the highest among all the formulations. PLLA:PEO and PCL:PEO 10:90 fibers released 65% and 45% of F-BAR, respectively, within 2 hr. For the remainder of the time points, there was significant reduction in the release of BAR. For the 20:80 blended formulations, the PLGA:PEO fibers showed maximum release of 88%, compared to PLLA:PEO and PCL:PEO at 58% and 25%, respectively, after 2 hr. Similar trends in BAR release were observed for the 40:60 formulations with PLGA:PEO exhibiting the maximum release of 78%, with PLLA:PEO at 45% and PCL:PEO at 17% after 2 hr.
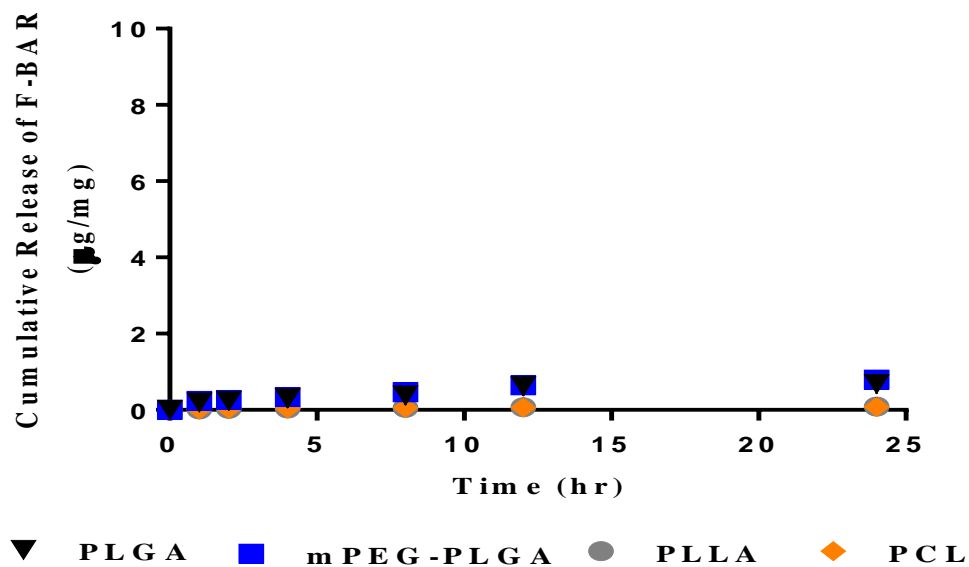
Figure 6. The cumulative release of F-BAR from 1% w/w F-BAR non-blended (100:0) PLGA, mPEG, PCL and PLLA fibers. The cumulative release is reported as (A) µg F-BAR per mg of fiber, and (B) percent of total loaded F-BAR. PLGA and mPEG-PLGA showed the greatest release of encapsulated BAR among the non-blended formulations at 24 hr.
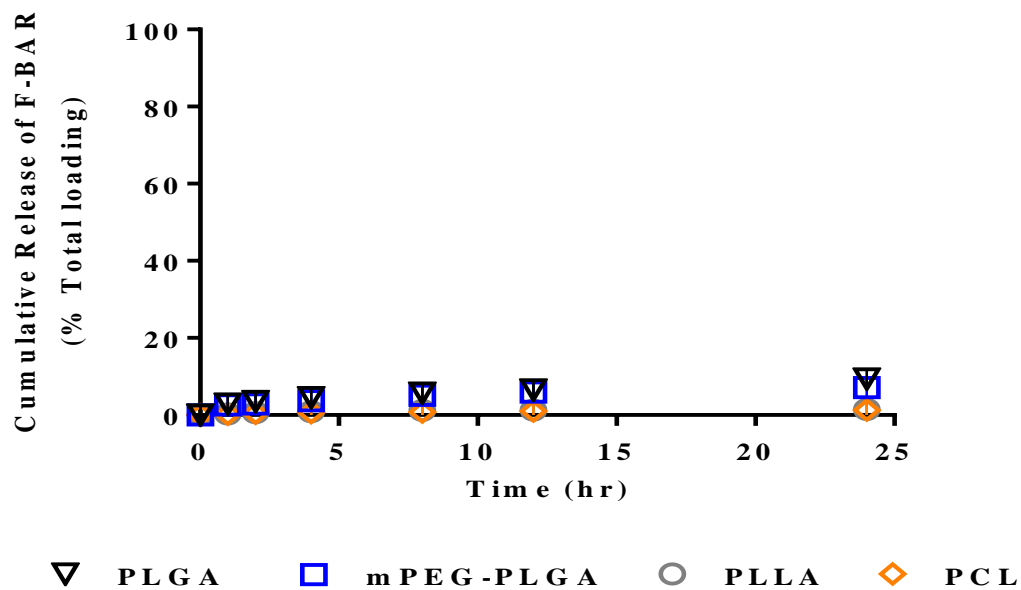
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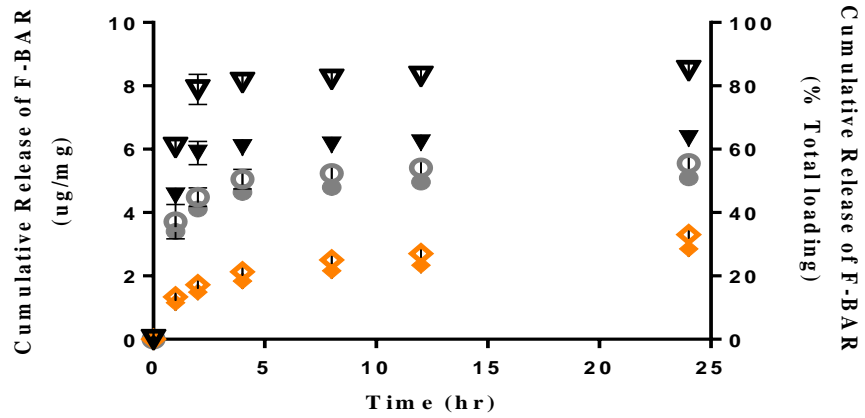
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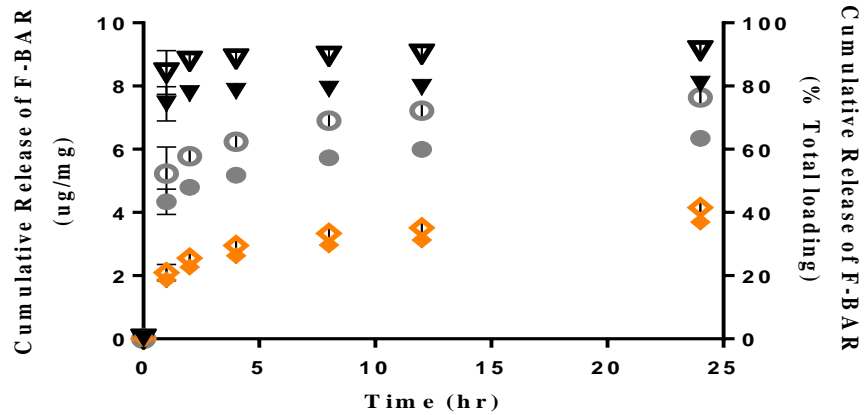
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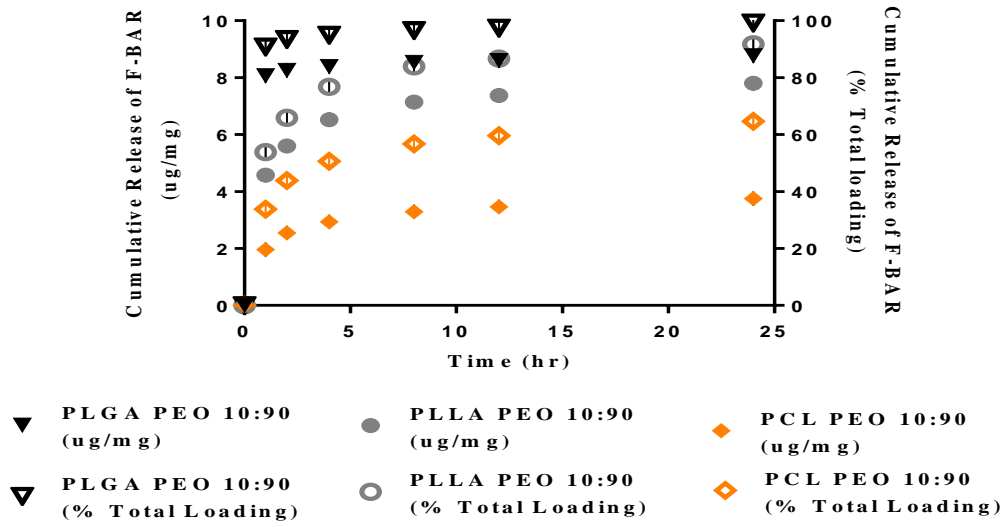
Figure 7. The cumulative release of F-BAR from 1% w/w F-BAR blended PLGA:PEO, PCL:PEO and PLLA:PEO fibers **(A)** 40:60, **(B)** 20:80, and **(C)** 10:90. The cumulative release is reported as both µg F-BAR per mg of fiber on the left axis, and as the percent of total loaded F-BAR on the right axis.
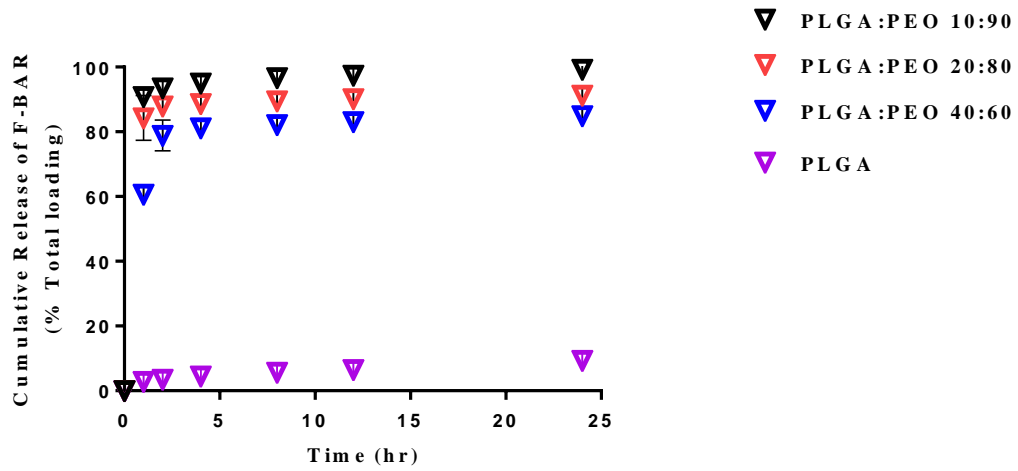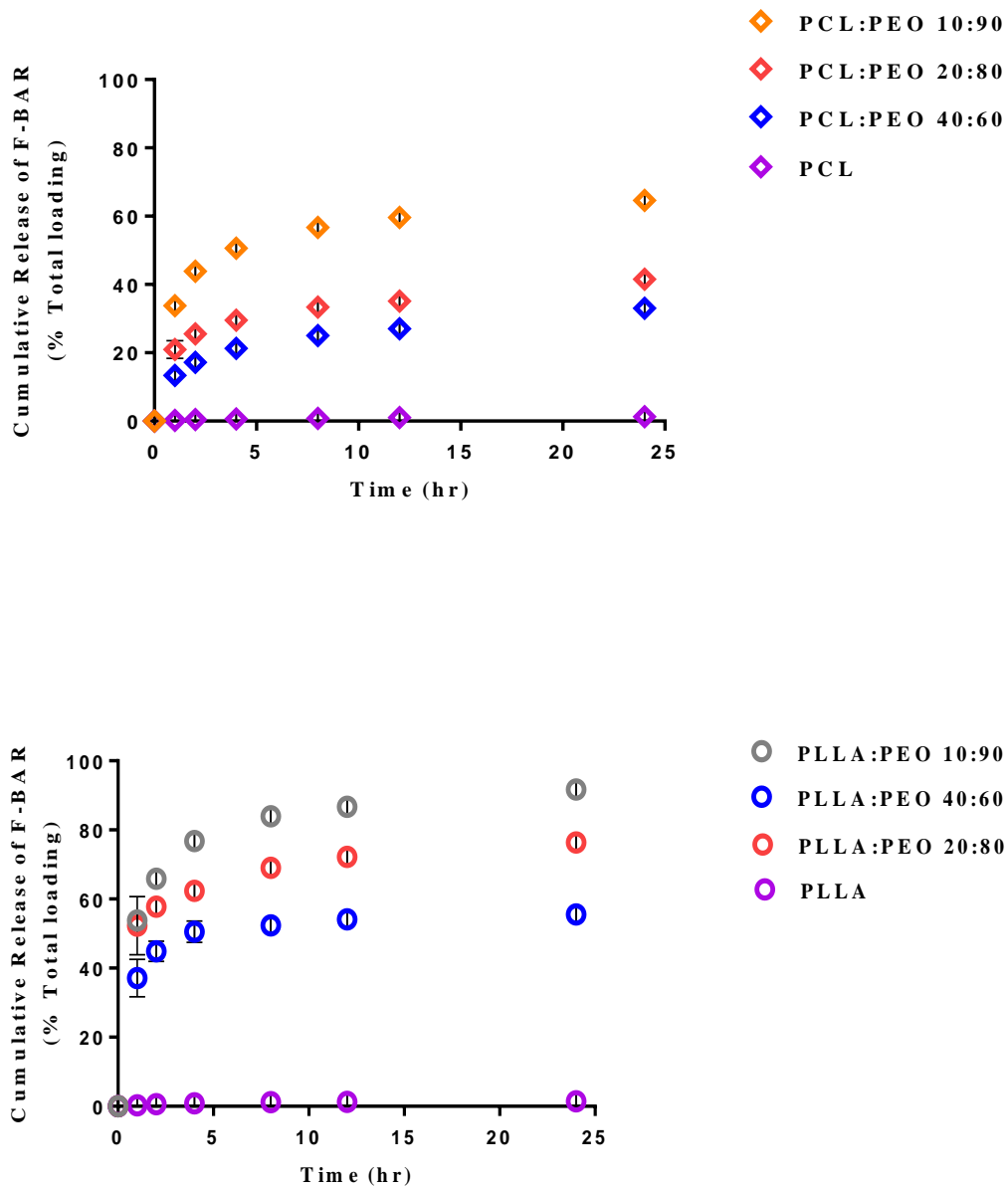
Figure 8. The cumulative release of F-BAR from the hydrophobic non-blended and PEO-blended formulations as a function of hydrophobic polymer type (PLGA, PCL, or PLLA) and PEO ratio in each blend. The release of encapsulated BAR increases with an increase in PEO fraction. PLGA and PEO blends exhibit the most significant and rapid F-BAR release, relative to PLLA and PCL blends. For all polymer types, the 10:90 blends show the greatest peptide release as compared to 20:80 and 40:60 formulations at any given time point. PLGA:PEO (10:90) fibers provide the greatest release among all formulations.

Figure 8 shows the importance of the PEO ratio in the each hydrophobic fiber type, with the 10:90 formulation showing maximum release for each hydrophobic blend. The 40:60 PLGA:PEO, PLLA:PEO and PCL:PEO released the least BAR (78%, 45% and 17% respectively) within the first 2 hours. The release trends for the polymer blends of different ratios were similar, with PLGA blends achieving the highest BAR release, followed by PLLA and PCL.

CHAPTER 5

DISCUSSION

Periodontal disease is one of the most widespread oral diseases among the adult population worldwide, resulting in degradation of the supporting tissues of the teeth, thereby producing aesthetic and functional problems for the patient. A key feature of this inflammatory disease is dependent on the complex microbiome residing in the oral cavity. With a complex, polymicrobial etiology, it was shown that 'red complex', gram-negative anaerobic bacteria like *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia,* and *Filifactor alocis* were some of the species present in the pathogenic dental plaque and strongly associated with disease (Griffen et al., 2012; Socransky et al., 1998). Formation of deep periodontal pockets, and the persistence of subgingival plaque caused by adhesion and colonization of bacteria via contact dependent signaling (Donlan, 2002) stimulate a series of inflammatory reactions (Bao et al., 2014).

Porphyromonas gingivalis, being a prominent component of the oral microbiome and a successful colonizer of the oral epithelium (Yilmaz, 2008), has been suggested to function as a keystone pathogen, as it facilitates a change in both the amount and composition of the normal oral microbiota and creates dysbiosis between the host and dental plaque (Darveau et al., 2012). Before it resides in the subgingival pockets as an obligate anaerobe, its initial colonization occurs with oral commensals in the aerobic supragingival environment. The initial species-specific supragingival interaction between *P. gingivalis* and *S. gordonii* is considered to initiate the biofilm formation process (Kolenbrander & London, 1993). It is mediated by Mfa1 of *P. gingivalis* and SspB polypeptide of *S. gordonii* (Carlo Amorin Daep et al., 2006). Hence *P. gingivalis* is being targeted as a significant organism to inhibit biofilm formation.

A synthetic analog of the SspB polypeptide designated, SspB Adhering Region (BAR), was identified (C. A. Daep et al., 2006), providing a species-specific target that was successful in limiting *P. gingivalis* colonization both *in vitro* and *in vivo*. While BAR was successful in limiting *P. gingivalis* colonization both *in vitro* and *in vivo*, it was shown to be less effective against well-established and complex biofilms. Previous work has revealed that BAR potently inhibits the formation of two-species biofilms, but it is less effective in disrupting established or more complex biofilms, requiring

higher concentrations and prolonged exposure to be effective. The objective of this project was to synthesize and characterize EFs as a new dosage from alternative to deliver the bioactive molecule, BAR. Fibers as a delivery platform to the oral cavity may protect and sustain the delivery of BAR for oral administration, and provide a mechanism to improve the therapeutic outcomes by increasing the localized concentration of BAR. In this work, we hypothesized that BAR-encapsulated EFs, will provide short-term release of therapeutically-relevant concentrations of BAR and that BAR release from EFs may be modulated by changing polymeric fiber blend ratios.

Local drug delivery carriers in the form of films (Shifrovitch, Binderman, Bahar, Berdicevsky, & Zilberman, 2009), strips (Friesen, Williams, Krause, & Killoy, 2002; Leung, Jin, Yau, Sun, & Corbet, 2005), and wafers (Bromberg et al., 2000) have been applied for periodontal disease, where the subgingival pockets act as a natural reservoir for these drug loaded devices. However, the methods used in the fabrication of these dosage forms include solvent casting, melt spinning and direct milling methods. These procedures are labor intensive, time consuming, and expensive. In contrast, the electrospinning method provides a simple to use, time and cost efficient process. The EFs also offer several advantages compared with other dosage forms including the large surface-to-volume ratio for better contact of the encapsulated bioactive molecule to the surrounding medium, small diameter fibers for efficient drug release, ability to tailor the drug release profile,

mechanical stability, and ease of fabrication for similar application (Su, Li, Tan, Chen, & Xiumei, 2009).

Electrospun fibers have been used as a delivery vehicle in several biomedical applications. Some examples of their applications include utilization as wound dressing materials, due to their unique composition and morphology that mimics the extracellular matrix (R. Chen J.A. Hunt, 2007). For tissue regeneration purposes − where many polymers have been electrospun with PCL and PLGA into fibrous membranes for Guided Tissue Regeneration in periodontics (GTR) (Inanc, Arslan, Seker, Elcin, & Elcin, 2009; Yang, Both, Yang, Walboomers, & Jansen, 2009) and as drug delivery vehicles for bioactive molecules, antimicrobial agents, anti-inflammatory drugs, and anesthetics. Studies have used human periodontal ligament (hPDL) cells seeded on PLGA EFs (Inanc et al., 2009), in combination with PCL and PLA to deliver doxycycline (Chaturvedi, Srivastava, Srivastava, Gupta, & Verma, 2013) and metronidazole (Reise et al., 2012), respectively. Similarly, we hypothesized that fibers that incorporated BAR peptide may be used to facilitate delivery and enhance the local concentration of BAR in the oral cavity.

Moreover, research by Kim et al, indicated that blending hydrophobic with more hydrophilic polymers increased the release of lysozyme, while maintaining lysozyme activity. Many studies also have shown the addition

of PEO into the protein solution can be beneficial for improving protein stability (Casper et al., 2005; C. Li, Vepari, Jin, Kim, & Kaplan, 2006; Y. Li, Jiang, & Zhu, 2008). Given the favorable properties of the polymers mentioned, we successfully synthesized the electrospun fibers encapsulating the BAR using uniaxial-blend electrospinning technique for both the non-blended and blended formulations. To demonstrate the functionality of BAR-incorporated fibers, we initially formulated a 1% w/w of BAR:polymer, resulting in a theoretical loading of 10 μg BAR per mg of polymer. To achieve continuous release and the release of defined quantities of the peptide, the polymer yield and BAR encapsulation was assessed. The initial polymer mass, used to electrospin fibers, is required to adjust the calculation for F-BAR incorporation in absolute amount (ug BAR/mg polymer) and % efficiency. The unblended fibers, with HFIP as their solvent showed a much lower polymer yield ranging between 40-60% compared to blended fibers, suggesting that HFIP may not be the best solvent in which to synthesize BAR-encapsulated fibers. Chloroform-based blended fibers exhibited higher overall polymer yield in comparison, indicating chloroform as the preferred solvent to formulate BAR encapsulated fibers.

With respect to BAR encapsulation, all the resulting EFs displayed high BAR payload, ranging from 4.7 to 9.4 μg/mg and high encapsulation efficiency ranging from 60-90%. To further evaluate effective fiber formulations for

BAR, release kinetics of the non-blended and blended fibers were assessed. The cumulative release of BAR from the fibers was expressed as µg/mg and % total, and calculated as the concentration of the BAR in the release media relative to the actual concentration of BAR in the fibers. The release data of the unblended fibers revealed minimal release of 9.5%, 7%, 1.5% and 1.4% from PLGA, mPEG-PLGA, PLLA and PCL fibers respectively over a duration of 24 hr. The high hydrophobicity of the non-blended fibers of PLGA, PCL and PLLA likely enables release eluent to penetrate only the outermost layer of fibers, resulting in the rapid release of BAR only near the fiber surface.

By modulating the hydrophobicity of the fibers with the addition of hydrophilic PEO in ratios (40:60, 20:80 and 10:90), the release was significantly improved. The PLGA:PEO (10:90) fibers exhibited 90% release of BAR, the highest among all the blended and non-blended formulations within the first 2 hr, with PLLA:PEO (10:90) exhibiting 65% release, and PCL:PEO (10:90) releasing 45% in the same time frame. Negligible quanities of BAR were released after 24 hr.

As the concentration of PEO increased, BAR release accelerated with 10:90 ratio of PLGA/PCL/PLLA: PEO illustrating higher cumulative BAR peptide release percent than 20:80 and 40:60 blend fibers. Studies have shown that by introducing hydrophilic PEO into fiber formulations, the physical and

mechanical properties of the fiber change, in addition to providing the ability to tune encapsulant (here, BAR) release (Evrova et al., 2016). While hydrophobic polymers provide structural integrity to the scaffold, the PEO makes it more porous, caused by the dissolution of PEO in solution, enabling the release of the hydrophilic BAR peptide. In addition, hydrophilic molecules have been shown to have more affinity and compatibility with PEO, explaining the initial burst release presented by the blended fibers.

Among the hydrophobic polymers utilized, PLGA formulations demonstrated the highest release at early time points. PLLA formulations with different concentrations of PEO showed much better release profiles than PCL fibers over the desired duration of 24 hr. We propose that PLGA fibers demonstrate the highest release due to its amorphous and less hydrophobic properties, relative to PLLA and PCL. In contrast, we propose that PCL:PEO fibers demonstrated the least burst release due to its crystalline and slightly more hydrophobic features.

Among the formulations, though mPEG-PLGA and PLLA:PEO (40:60) reveals the highest encapsulation efficiency of 94% and 92%, a low release of 7% and 55% of the encapsulated BAR at the end of 24 hrs may not be effective formulations. Taking both encapsulation efficiency and release properties into consideration, PLGA:PEO (10:90) is considered the most effective formulation from this study.

In addition to materials properties, the electrospinning process itself can affect encapsulant location within the fibers, prompting different release kinetics. During electrospinning, the electrical force may drive BAR, to aggregate close to the fiber surface, due to charge repulsion (Szentivanyi, Chakradeo, Zernetsch, & Glasmacher, 2011). This localization may also contribute to burst release.

The fibers fabricated in this study were formulated with 1% w/w BAR:polymer. As such, they demonstrated high encapsulation efficiency spanning 60-90%, with burst release in the first 2 hr and minimal release thereafter. To achieve the IC_{50} of BAR (4 µg/ml) at every time point over the duration of 24 hr, loading capacity may be increased. However, previous work has shown that using a theoretical loading higher than 1 % w/w (Kim et al., 2004) via uniaxial blended spinning process may still result in significant initial burst release. To overcome burst release, optimize the release kinetics, and maintain peptide stability, techniques like emulsion electrospinning and co-axial electrospinning may be adopted (Sebe, Szabo, Kallai-Szabo, & Zelko, 2015) (X. Li et al., 2010).

Co-axial electrospinning utilizes two different capillary channels concentrically arranged to keep the protein solution and polymer solution separate creating a core and sheath morphology. The drug is trapped within the core, which is surrounded by a polymer shell. Several studies have shown

sustained-release of bioactive molecules using this methodology. Moreover, the bioactivity biological agents is also maintained since it is not incorporated into the polymer/solvent solution prior to electrospinning (W. Ji et al., 2010).

Emulsion electrospinning adopts a similar principle, where the peptide is dissolved in an aqueous solution, which is then added to an immiscible polymer-solvent solution creating a water-in-oil type of emulsion. This emulsion helps to encapsulate the aqueous agents within the core, to provide sustained and incremental release of the encapsulant. We speculate that if the blended polymeric fibers are synthesized using this approach, more prolonged administration of BAR may be achieved via sustained release

In addition to the overall electrospinning technique, particular processing parameters have been shown to impact fiber morphology. If the distance between the syringe tip and collector is too close, there may not be enough time for the solvent to evaporate, promoting EF adhesion to the mandrel (Z.-M. Huang, Zhang, Kotaki, & Ramakrishna, 2003). We found the distance of 15 cm between the needle and the collector to be ideal, when a voltage of 20-25 kV was applied. In addition, inadequate voltages and flow rates can promote bead formation on the electrospun fibers, resulting in unstable and irreproducible fiber morphology (Sill & von Recum, 2008).

Future studies include plans to assess the functionality of BAR encapsulated EFs against biofilms. Given that the more hydrophilic blended fibers released

higher amounts of BAR over 24 hr, relative to the non-blended fibers, we suggest testing the PLGA:PEO (10:90) fibers against *in vitro* biofilms. Previous work showed free soluble BAR potently inhibits *P. gingivalis-S. gordonii* biofilm formation at $IC_{50} = 1.3 \mu M$. However, with the transient activity observed using free BAR, the high loading and encapsulation efficiency of the fibers as shown in the results, Table 2, in parallel with the ability to tailor the release kinetics may enable greater efficacy against biofilm formation and disruption.

In this work, we demonstrated the feasibility, versatility and straightforward approach of blend electrospinning to prepare BAR encapsulated EFs that release therapeutically-relevant concentrations of BAR. Following studies will be focused on optimizing the release kinetics of BAR EFs and testing their efficacy against established and complex biofilms. This will be helpful in formulating long-term therapeutics for periodontitis as an intra-pocket delivery system, where the fibers can be immobilized in the subgingival pocket for a longer duration of time since it can provide a durable scaffold.

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