Drug-loaded Bio-responsive Liposomal Gels for Wound Care Applications

Kelsey Swingle

Biomedical Engineering, Concentration in Biomaterials

Mentor: Dr. Anirban Sen Gupta, PhD

Department of Biomedical Engineering

Goals and Objectives

Clinical Significance

The central goal of this project is to bioengineer and evaluate a nanoparticle-based hydrogel biomaterial matrix technology that can be used as a wound-dressing material to allow spatio-temporally regulated delivery of anti-microbial drugs, signaling molecules, and growth factors to facilitate wound healing. Every year, millions of people are affected by skin wounds that require clinical attention, leading to wound care costs bearing an annual overall cost burden of more than $20 billion in the US alone (1,2). A principal component of wound-care is the 'wound dressing' material that allows protection of the wound from the outside environment but can also interact actively with the wound to facilitate healing. In this clinically significant area of research, several matrices have been synthesized from natural (e.g. chitosan, alginate, pullulan, etc.) and synthetic (e.g. hydrocolloids, hydrogels, foams, etc.) biomaterials to treat wounds by providing a moist, biocompatible surface to aid tissue regeneration along with providing absorbent and exudate removal properties (5,7,8). Moreover, it has been shown that supplementing the wound site with growth factors such as Platelet-derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), etc. can enhance healing response by modulating macrophage function towards a pro-healing phenotype, as well as stimulating endothelial cell and fibroblast migration to promote angiogenesis, matrix degradation, and remodeling (9-12). Finally, anti-microbial drugs have been used to treat wounds prone to infection in order to provide a wound environment that is conducive to both repair and regeneration (6-8, 13). An ideal wound dressing should (i) maintain a moist environment, (ii) allow oxygen permeability and gas exchange, (iii) provide protection against microbial infection, (iv) enable removal of excess wound exudates, and (v) enable spatio-temporally controlled release of pro-healing bioactive factors (3,4). Based upon these design criteria, my project will focus on developing a nanoparticle-based hydrogel platform that allows spatio-temporally regulated release of anti-infective and pro-healing factors. This will be achieved via a unique approach where biodegradable liposomal nanoparticles will be designed to allow gelation via transglutaminase-induced crosslinking. The nanoparticles will contain therapeutic agents (anti-microbial drugs and pro-healing factors) and their gelation will create the hydrogel platform. The nanoparticles themselves (and hence the resultant gel) will be degradable by enzymes upregulated at wound sites (e.g. thrombin, plasmin, matrix-metalloproteases, etc.) to allow enzyme-triggered biodegradation of the gel matrix for payload release. Figure 1 shows the conceptual design of this system, along with a schematic of the wound dressing application. I hypothesize that such a design will allow modular control of drug release within a biodegradable 'wound dressing' matrix for promotion of wound healing during various phases.

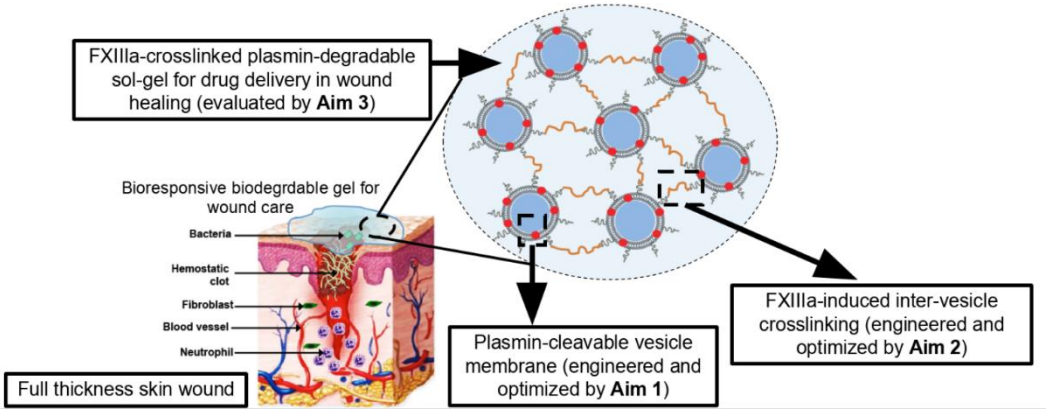

Figure 1. Components of a full thickness wound and proposed wound dressing technology with research aim components.

Background

Wound healing occurs in humans in a complex cohort of four overlapping and inter-dependent phases: (1) hemostasis, (2) inflammation, (3) angiogenesis, and (4) remodeling (9,14-18). These four phases occur over different lengths of time and involve interplay between a variety of cellular entities, enzymes, and signaling molecules regulating various aspects of the wound-healing cascade. This proposed research aims to leverage some of these regulatory molecules found at a wound site to achieve a biodegradable gel matrix for spatio-temporal control of drug delivery at the wound site. Specifically, the components I propose to use are: (i) transglutaminase enzyme to render nanoparticle crosslinking into a gel, (ii) plasmin as an enzymatic trigger for nanoparticle (hence gel) degradation and (iii) clinically approved anti-microbial agents like Gentamicin and natively present growth factors like PDGF as payloads for release from the degrading gel. For the transglutaminase enzyme, an ideal choice is the coagulation factor FXIIIa, which is part of a family of Calcium-dependent enzymes that catalyze covalent crosslinking between glutamine and lysine residues of amino acids in a variety of physiological polypeptide reactions. Specifically, FXIIIa is responsible for crosslinking fibrin in the hemostatic clot formation phase of wound healing (8,11,18,19). Plasmin is a prominent enzymatic component in the wound healing process, contributing to the degradation of fibrin clots (fibrinolysis), in addition to the signaling mechanisms for cell recruitment, angiogenesis, formation of granulation tissue, and the re-epithelialization and degradation of extracellular matrix proteins. The abundance of plasmin at the interface of hemostasis and inflammation/angiogenesis phases during wound healing

provides the rationale for using it as the enzymatic trigger to enable wound site-specific degradation of the gel matrix. Also, plasmin-induced biodegradation of the matrix avoids the secondary injury complications that are otherwise associated with periodic removal of non-degradable wound dressings (5,6).

Project Description and Methodology

The central hypothesis of my research project is that FXIIIa-induced crosslinking of plasmin-cleavable liposomal nanoparticles will render a hydrogel matrix that enables spatio-temporally controlled delivery of antibiotics and growth factors via wound site enzyme-triggered degradation to enhance wound healing. To test this hypothesis, the research project has been divided into three aims. This project will focus on the completion of Aim 1 during the ten-week program. A brief description of Aim 1 and the corresponding methodology is found below.

Aim 1: Engineer plasmin-degradable liposomes and characterize encapsulation and plasmin-triggered release of candidate drug payloads from them.

The primary goal of this aim is to engineer plasmin-degradable liposomal nanoparticles and characterize encapsulation and plasmin-triggered release of model payloads and candidate therapeutic agents from them. For this, an amphiphilic lipid-peptide conjugate will be synthesized by reacting a plasmin-cleavable peptide (PCP) having amino acid sequence of KTFKC (21) to the lipid Stearylamine; the resultant PCP-Stearate conjugate will be characterized using MALDI-TOF mass spectrometry. To establish the plasmin-induced degradation of this conjugate, it will be incubated with plasmin at 37^{o}C and the resulting reaction volume will be analyzed using MALDI-TOF. Subsequently, PCP-Stearate will be combined with the lipid distearylphosphatidylcholine (DSPC) and cholesterol at controlled mole% to fabricate unilamellar lipid vesicles using the film rehydration and extrusion technique (22). The resultant liposomal nanoparticles will be characterized by dynamic light scattering (DLS) for size and cryo-transmission electron microscopy (cryo-TEM) for vesicle morphology. Next, the PCP-incorporated liposomal vesicles will be loaded with model payload and plasmin-triggered vesicle degradation for payload release will be characterized in vitro. I hypothesize that plasmin will cleave the PCP from the hydrophobic stearyl tail, resulting in the loss of its amphiphilicity, which in turn will destabilize the liposome membrane and cause the release of the incorporated payload. To test this hypothesis, carboxyfluorescein (CF, green fluorescence) will be encapsulated as a model payload within the liposomes. CF at a high concentration is self-quenched (low fluorescence signal) and its release results in dilution and thus increase in fluorescence signal. Therefore, CF release from the PCP-Stearate incorporated vesicles in absence versus presence of plasmin will be monitored over time by fluorescence spectrometry. Using liposomal systems with controlled mole% incorporation of KTFKC-Stearate will allow correlation of release kinetics with the liposome membrane composition. CF is representative of a small molecule payload like the antibiotic Gentamicin, and therefore these studies will allow optimization of the plasmin-cleavable vesicle system for this type of drug release. Similar studies of encapsulation and release as above will be repeated using fluorescein isothiocyanate (FITC, green fluorescence) labeled bovine serum albumin (BSA) as a model payload for larger molecules like growth factors. After optimizing the vesicle composition for plasmin-triggered release of CF and FITC-BSA release, candidate antimicrobial agent Gentamicin and candidate growth factor PDGF will be encapsulated in these liposomal systems and their plasmin-responsive release will be characterized by UV-Vis spectrometry.

Time Commitment

For the summer of 2018, I will commit approximately 40 hours per week to this research project for a total of 10 weeks. I will attend all required Lunch and Learn seminars during the 10-week program. I plan to begin my research project starting in May after the spring semester ends in the event that I chose to take 1-2 weeks off during the 10-week program. I will be participating in my research project full time and will not be taking any classes in conjunction with my project.

Relevance to Educational and Career Goals

My proposed project focuses on developing therapeutic technologies for a clinically significant area of research, namely, wound care. This project will allow me to receive education and training, as well as carry out research in several aspects of the STEM fields including wet chemistry, wound biology, biomaterials engineering, and wound care technology. I joined the laboratory of Dr. Anirban Sen Gupta in Biomedical Engineering in the fall of 2017 and am currently undergoing training in techniques and methodologies such as lipid-peptide conjugation and characterization, nanoparticle fabrication, and characterization, microscopy and UV-Vis spectroscopy. The proposed project will allow me to apply this training towards exciting biomedical research with translational promise. It will also allow me to work individually on my proposed project as well as participate with other undergraduate, graduate, and post-doctoral researchers in related projects. Dr. Sen Gupta's laboratory focuses heavily on a variety of trauma treatment research and wound care is an important part of trauma care. Hence, this research will allow me to gain very valuable education and research experience in a clinically important field and sharpen my professional and social skills for pursuing a biomedical education and research career in the future through graduate

education and beyond. SOURCE funding will be a critical help towards building my biomedical research career and will also allow me to share my research findings with other students and peers in the field.

After previously collaborating on a research project at Rutgers University and currently collaborating in Dr. Sen Gupta's lab, I have realized that I want to dedicate my time to research as an undergraduate. This dedication will allow me to pursue higher education and training via a PhD program and later a post-doctoral position to build a career in academia. I have chosen this goal and career path not only because of my passion for research, but also due to my interest in the field of medicine and healthcare. Many of the women in my family have pursued higher education in medicine and healthcare and I seek to continue this legacy of intellectual women in my family and to help mentor future women in medicine and STEM. My ultimate goal involves working as a research professor in academia to lead a clinical trial for new technology that could have a tangible effect on people's health. In addition, I believe this career path will allow me to mentor future STEM students, like my family members in medicine and healthcare have done for me. A key stepping stone in achieving this goal is taking ownership of and completing a research project as an undergraduate student, something that will be made possible by SOURCE funding. As a sophomore, this summer will be crucial to achieving my goals because making significant progress on my research project will allow me to write and publish my findings in a paper. A first authorship on a published paper will demonstrate my experience and preparedness to graduate schools, the next step in my career path. Therefore, I believe that receiving SOURCE funding will be a key stepping stone towards my career goal of pursuing healthcare-related research aimed at bettering the quality of life of patients as a research professor.

Budget Summary

I am requesting the full amount of $3500 from the SOURCE office. This funding will be used to support my housing and living expenses for the summer. The Sen Gupta Lab will provide all materials and supplies required for the completion of the project.

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