OPTIMIZATION OF HYDROGEL SYSTEM FOR COMPLEX WOUND HEALING

by

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Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE IN BIOMEDICAL ENGINEERING

THE UNIVERSITY OF TEXAS AT ARLINGTON

AUGUST 2011

ACKNOWLEDGEMENTS

Research is difficult without opportunity and support. For that, I am very grateful to Dr. Kytai T. Nguyen both for providing me her support and permitting me to work in her laboratory independently. Her valued guidance was of great importance to accomplish my research goals. She always encouraged me in the times of my failures in research and helped me overcome them. Her faith in me had motivated me to implement new ideas in my research work. She also nourished my overall personality by giving me various responsibilities in the laboratory.

I am thankful to Dr. Jian Yang for collaborating with us on this project and allowing me to work in his laboratory. I, especially, like to thank Dr. Liping Tang for guiding me through initial struggling days of my masters program and serving on my thesis committee. Moreover, I would like to thank Dr. Young-tae Kim for serving on my thesis committee and reviewing my research work. I am very much thankful to Aniket Wadajkar for his constant help, guidance, and support. I would like to extend my gratitude to members in Dr. Yang's laboratory, in particular, Dipendra Gyawali, Michael Palmer, and Richard Tran for their help and guidance in polymer synthesis. I am very happy to express my gratitude to Tejaswi Kadapure for being a source of emotional support. I would also like to acknowledge my laboratory members including Mandy Su, Homa Homayoni, Sonia Santimano, and Roshni Iyer, who provided me with healthy research environment.

Rather than just acknowledging, I would like to say, "I love my mom and dad. Because of their unconditional love and support, I am what I am!" Lastly, special thanks to my brother and all garden club 205 members for their love and support.

July 15, 2011

ABSTRACT

OPTIMIZATION OF HYDROGEL SYSTEM FOR COMPLEX WOUND HEALING

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Various conventional therapies and preventive measures have been developed and used for treating complex wounds; however, patients with complex wounds still develop wound infection and consist of delayed wound healing. These limitations necessitate the development of a novel therapeutic system, which is cost effective, easily apply onto the complex wounds, and consists of an efficient therapeutic nature.

An *in situ* forming hydrogel system, made up of a novel biodegradable elastomer poly (ethylene glycol) maleate citrate (PEGMC), was designed and optimized for complex wound healing. Biocompatibility of this hydrogel system was studied by determining the toxicity of both the formation factors, including PEGMC monomers and APS initiator, and the effluents of the system on human dermal fibroblasts (HDFs). Cell viability was above 80% up to 5mg/ml for PEGMC, 50 µg/ml for APS, and 2.22mg/ml for the system effluents. Effects of the system formation factors on its properties, such as curing time, Young's modulus, swelling ratio, and degradation, were also investigated. Initiator APS and crosslinker PEGDA 700 concentration were the most affecting factors on the curing time in a negative manner. On other hand, crosslinker PEGDA 700 concentration positively affected the Young’s modulus and negatively

affected the swelling ratio of the system. The degradation of the system was observed to have an effect of different formation factors at different time points.

To enhance the system performance for complex wound therapies, the optimized hydrogel system was also designed to have a dual layer for sustained drug release by controlling degradation properties of each layer and to consist of bactericidal property by incorporating antibacterial peptides (CHRG01, Abu-CHRG01). Results from this research project demonstrated that the developed novel hydrogel system could be cost effective and easy to apply onto wounds of any shape and size as well as useful for providing a sustained release of a therapeutic reagent in controlled fashion, and could fight with the bacterial infection.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

Table Page

CHAPTER 1

INTRODUCTION

1.1 Background of complex wound healing

Wound healing mainly depends on factors at the wound site, cytokines, chemokines, enzymes, type of injury, and physiological conditions [1]. Complex wounds are those wounds which either take longer time (more than 3 weeks) to heal or do not heal at all. Abnormal cellular and/or molecular signaling is the main cause. Usually, a wound can heal acutely on its own, but many factors, including diabetes, bacterial infection, renal disease, ischemia, local hypoxia, malnutrition, immunodeficiency and aging, affect the normal wound healing process. According to literature, 5 million Americans are facing the problem of abnormal wound healing, making a financial burden of 20 billion dollars per year [2, 3]. Complex wounds has brought many attention in research because of their direct effect on the patient quality of life and complexity of their nature that is challenging the treatment method [4, 5]. As 'complexity of nature' is concerned, in order to develop an ideal treatment for complex wound, we should first understand the normal wound healing procedure.

1.1.1 Normal wound healing

Wound healing, is well orchestrated with a combination of many cellular and molecular events (Figure 1.1), and these events can grouped in three main phases: inflammatory, proliferative, and remolding (or maturation) phases. Being the pioneer of the wound healing,

inflammatory phase initiates a signaling cascade involving cell migration, proliferation, and differentiation. Inflammatory phase actually starts with hemostatis. A coagulation cascade, ending with a fibrin plug and reflex vasoconstriction, achieves hemostatis. Different cytokines, chemokines, and growth factors play major roles in the inflammatory phase by affecting the cells involved. As inflammation forms a base for the wound healing, proliferative phase starts building the blocks required for the wound to heal. It starts with inward migration of fibroblasts from the wound margins. The phase of proliferation mainly includes granular tissue formation, neo-angiogenesis, and re-epithelialization. Granular tissue formation depends on glycosaminoglycans, proteoglycans, and collagen, produced by fibroblasts, in response to growth factors like Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-β (TGF-β), and basic Fibroblast Growth Factor (bFGF). Neo-angiogenesis, as a result of proliferating endothelial cells, and epithelial layer covering the wound are required for the growing granular tissue. After all building blocks of wound healing placed in to the position, remolding phase constructs the ceiling. In this phase, maturation of collagen type I gets dominant while replacing collagen type III. Remolding phase can lasts up to 2 years, until 4:1 ratio of collagen I: III achieved, which is same as normal skin tissue. Migration of epithelial cells to cover entire defect and transformation of fibroblasts into myofibroblasts, continue until the wound is healed completely [1, 2, 6].

1.1.2 Complex wound healing

Although all wounds are programmed to heal in orderly and timely manner, various factors might hamper the normal healing process (Figure 1.2). For instance, bacterial infection maintains high levels of pro-inflammatory cytokines and proteases causing delay in the collagen

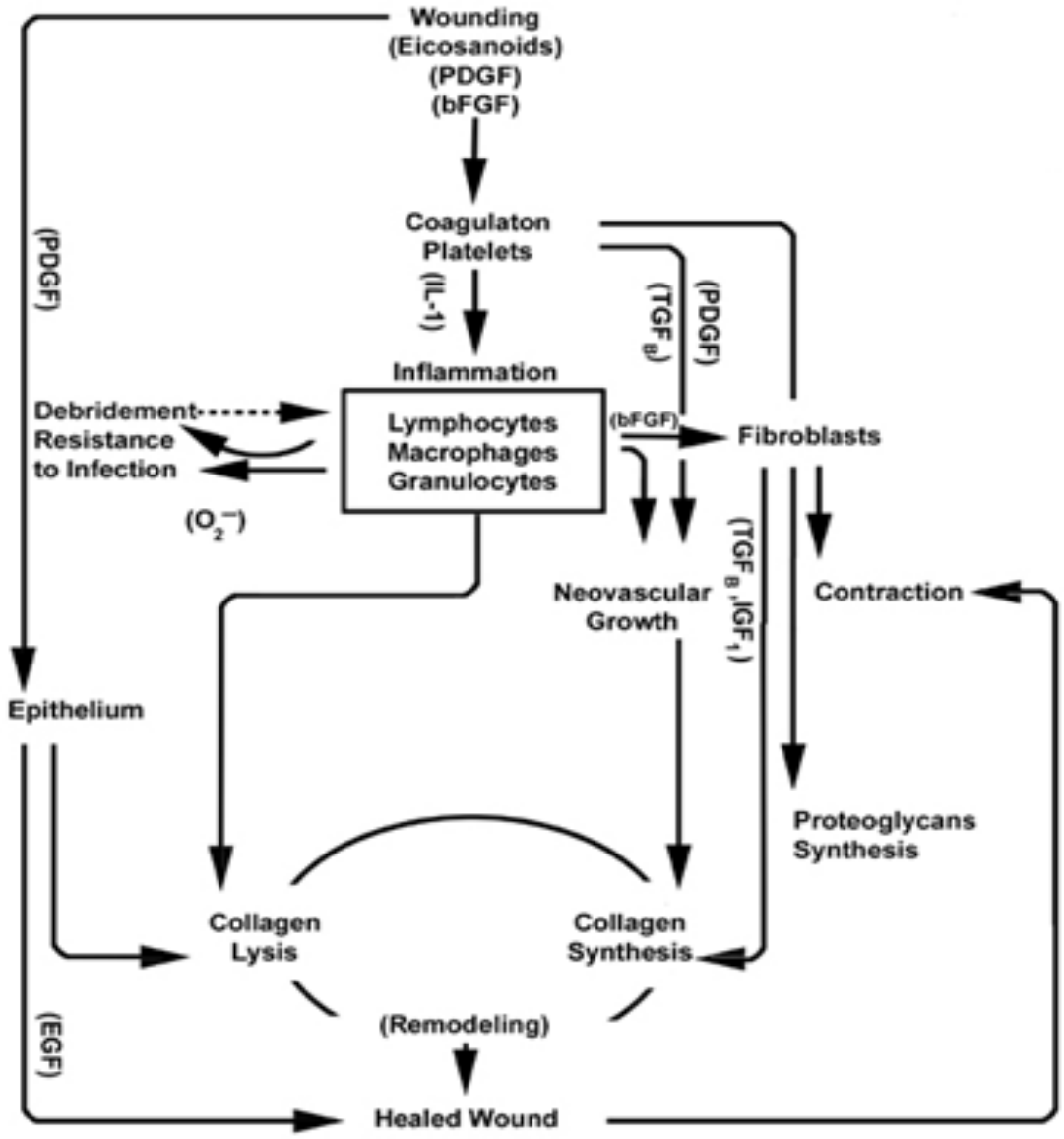

Figure 1.1 Normal wound healing cascade [2].

deposition and granular tissue degradation. In addition, low oxygen levels affect collagen fibril crosslinking and leukocyte oxydative phosphorylation, and both of them slow down the healing procedure, earlier one with poor tissue remolding and later one with bacterial infection. Diabetes mellitus also increases the risk of prolonged wound healing. Thicker and weaker blood vessel walls and poor phagocytosis by neutrophil and macrophage are some of the reasons related for

complex wounds in Diabetes. Malnutrition is another factor, hampering wound healing by poor immunity, poor neovascularization and lack of fibroblast proliferation. Obesity and aging are also interfering factors to the normal wound healing [7].

Broadly, chronic wounds can divide in to five groups: 1) Diabetic foot ulcers, which are caused mainly due to obstruction of blood vessels and neuropathy caused by microangiopathy. 2) Pressure ulcers that are most commonly seen in older people. This type of chronic disorder mainly occurs due to compression of soft tissues in between osseous prominence and hard surface. 3) Chronic venous ulcers / venous stasis ulcer is a result of insufficient blood supply in the lower extremities. Venous statis ulcer is known for its incurable nature [2, 8]. Definite treatment is not available for this chronic disorder due to incomplete understanding of its etiology and pathology. 4) Extensive necrosis due to infection, for example Fournier's gangrene, is an infection caused by aerobic and anaerobic bacteria at perineum and ganital regions. 5) Wounds in the people, who are suffering from vasculitis and immunosuppressive disorders [2, 8].

1.2 Current methods, preventive measures and advancements in treatment of complex wounds

As, the quality of life gets hampered due to complex wounds, effective treatment is an immediate need for many patients suffering from complex wounds. There are different treatments, preventive measures, and some on-going research for the treatment of complex wound [4, 8-12]. Until date, the most effective treatment is surgical treatment. This treatment

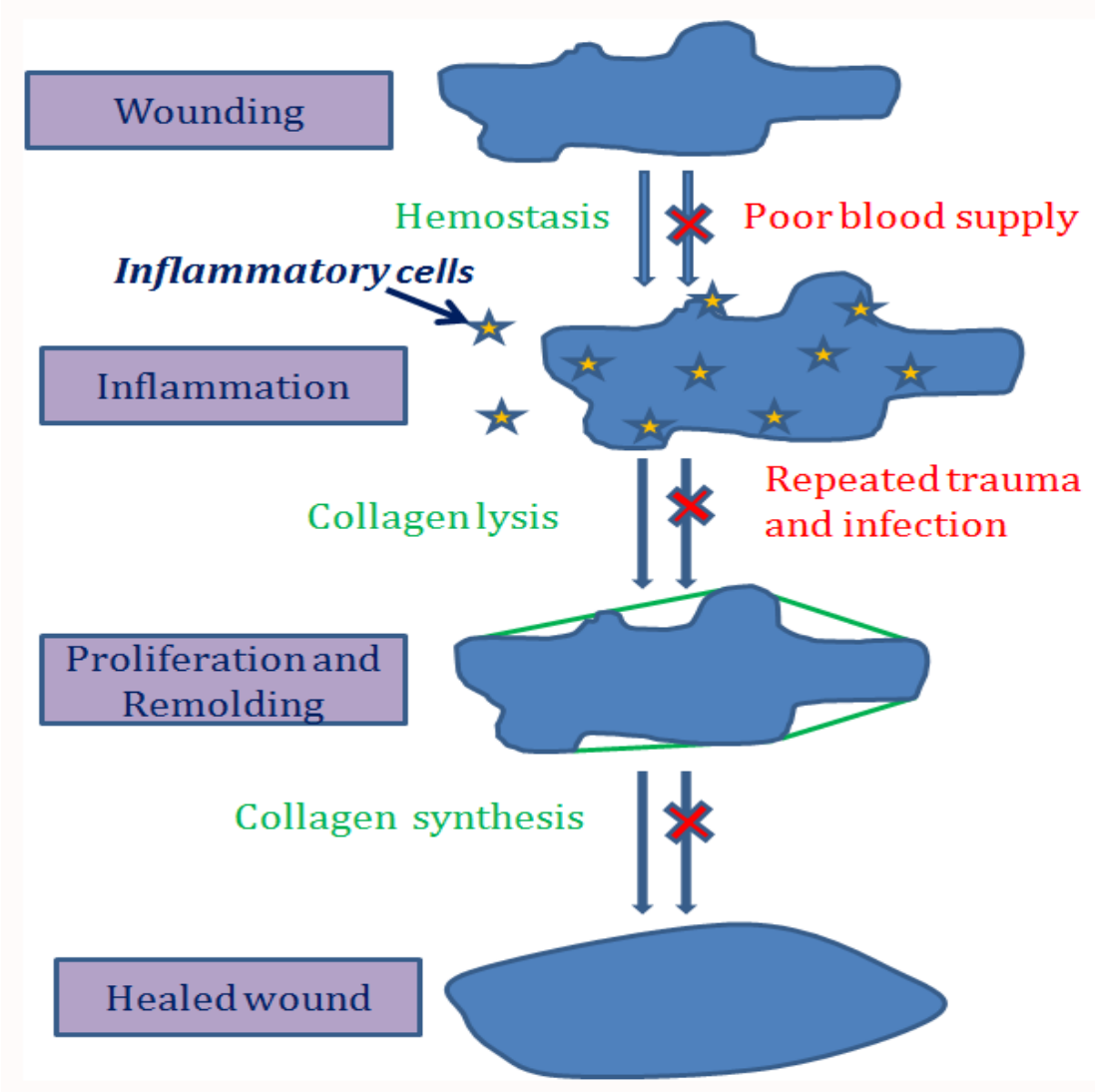

Figure 1.2 Local factors affecting normal wound healing.

includes a cascade of procedures such as removing of necrotic debris, controlling infection, and relieving the pressure on the wound. Though it is effective, it takes 10-12 weeks to heal the wound. To reduce the healing time, graft/ flap treatments are done after surgery. But this imposes the problem of wrinkling and fluting with uncomfortable nature of the graft/flap application. Another standard treatment includes aggressive pressure offloading or total contact casting, which again needs a period of 10 weeks to heal with an intensive care [9]. Routine

debridement with infection control, revascularization, and wound bed preparation; can be a good option if performed systematically and skillfully. These treatments will fail against poor revascularization and recurrent infection [9].

Some commercially available or in phase III study products play a vital role in therapies for complex wounds. Apligraf is the only tissue-engineered graft approved by FDA. It mimics the skin tissue except sweat glands and hair follicles. Despite of FDA approval, short half-life and cost ($1000/piece) are two main issues hindering the large scale application of the Apligraf. Dermagraft® is another example of tissue-engineered product in phase III study. Fact about the Dermagraft® is its allogenic nature of treatment. It uses human neonatal derived dermal fibroblasts for treatment. Although the cells remain encapsulated in self-derived extracellular matrix, the possibility of low immunogenicity still exists. In addition, $500/piece valued Dermagraft® needed bacterial infection control is its disadvantage [9]. Both Apligraf and Dermagraft® also need supportive products, which can control moisture and re-epithelialization at the wound site. Spray-on films and hydrons consisted of an advanced technology have been used for this purpose, but they also possess some drawbacks. Spray-on films are easy to apply and can cover whole area of wound, but they are not effective against bacterial infection in 3rd degree wounds. Hydron is an example of *in-situ* forming dressing consisting of poly(2-hydroxymethyl methacrylate) and poly (ethylene glycol) (PEG); however, it is very expensive [12].

Preventive and alternative therapies have also been investigated to improve the wound healing for patients of complex wounds. Braden scale and prolonged bed rest are some preventive measures. Based on sensory perception, moisture, activity, mobility, nutrition, friction and shear stress; Braden scale predicts the possibility of the pressure sore risk. Some of ongoing researches include electrospun nanofibers with immobilized epidermal growth factor

(EGF) [11]. This system showed good wound closure rates at initial stage only. After approximately seven days, effect of EGF was not noticeable. This happened due to degradation of the growth factor in highly proteolytic environment at the wound site [11]. Another example of lab scale researches is chitosan crosslinked collagen sponge, which was successful in accelerating the wound healing rate with mild improvements but fail to hold the cytokine for a longer time [10].

1.3 Overview of the project

As discussed above, current treatments and some ongoing projects do posses positive aspect towards the wound healing process; however, at the same time they do have some drawbacks. For example; allogenic nature of the Dermagraft® treatment can increase the chance of immunogenic response. Requirement of additional or supportive treatments for controlling moisture, debridement, infection; is another setback for current advanced technologies used to treat complex wounds. And last but not the least, cost of the treatment which is causing burden on the pocket of individuals as well as the US government. This necessitates development of a wound healing system, which is less expensive and easy to apply. In addition, it should be self-contained in order to fight with bacteria and maintaining the moisture at the wound site. The system should also support growth and/or recruitment of the cells involved in order to enhance wound healing.

Hydrogels are ideal solution for designing such kind of wound bed as they possess a wide variety of unique properties such as swelling, crosslinked networks, *in situ* forming capacity, and hydrophilicity, which are important to design an effective complex wound dressing. For instance, swelling will avoid formation of fluid filled pockets eventually minimizing the risk of bacterial infection. Crosslinked network provides a good delivery platform for drug. *In*

situ forming nature will allow easy applicability and complete closure of the wound. Hydrophilic nature will maintain the moisture at the wound site necessary for enhancing epithelial cell migration and supporting necrotic tissue debridement [4, 6, 8, 12].

1.3.1 Research goal

The main goal of this project is to develop a hydrogel system for complex wound therapies by using a blend of a novel citric acid derived biodegradable elastomer poly (ethylene glycol) maleate citrate (PEGMC) and well-studied biocompatible polymer poly (ethylene glycol) diacrylate (PEGDA). The hydrogel system will possess a control over the drug release profile as needed due to the degradation of PEGMC and can be delivered and formed *in situ* to cover the wound site.

1.3.2 Specific aims

Aim 1: To study the biocompatibility of the basic formation factors of hydrogel and their effects on properties of the hydrogel and optimize the composite hydrogel system.

Aim 2: To enhance properties of the hydrogel to support healing of complex wounds and to combat the infection.

1.3.3 Proposed design

Previously some hydrogel systems have been developed for use in the wound healing [12, 13]. Hydrogel systems based on the PEGDA and PEGMC were also studied previously in our lab for their application in protein delivery and wound healing [14]. Based on these designs, I developed a hydrogel system, with a novel formulation, for complex wound healing. The

system has prepared by a blend of PEGMC and PEGDA polymers to provide a control of both degradation and hydrophilic properties (Figure 1.3). The system consists of antibacterial peptides, 14 amino acid derivative of Human β Defensine 3 (HBD 3), conjugated to the PEGMC polymer, which will add a bactericidal property to the system. This system is easy to prepare and apply, while it can maintain moisture to support epithelial cell migration and autolytic debridement. Additionally, it is having antibacterial property without using any conventional antibiotics, which can solve the major problem of antibiotic resistance of the bacteria [15, 16].

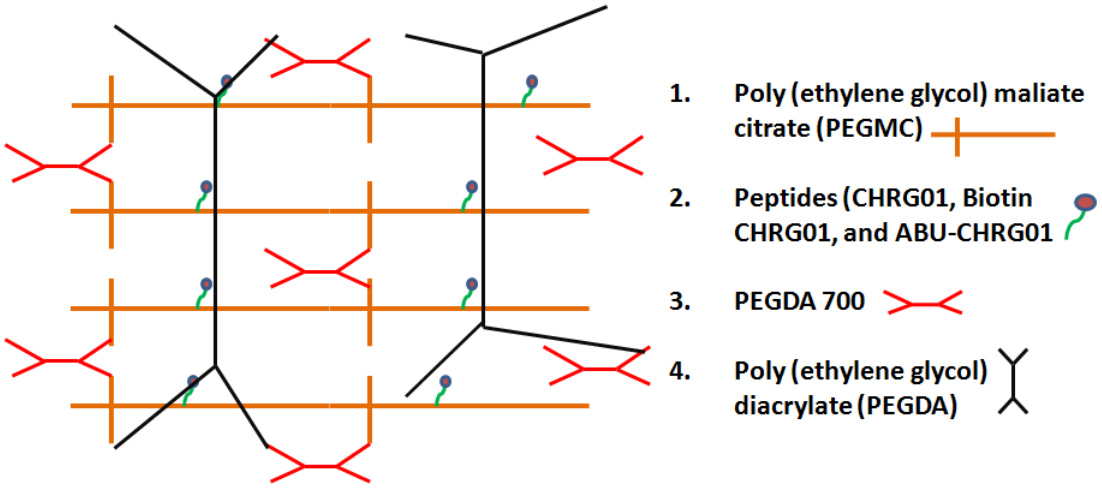

Figure 1.3 Schematic presentation of the proposed design, containing two polymers: (1) PEGMC and (4) PEGDA, antibacterial entity: (2) peptides, and crosslinker to form network: (4) PEGDA 700.

CHAPTER 2

TO STUDY BIOCOMPATIBILITY OF BASIC FORMATION FACTORS OF HYDROGEL AND THEIR EFFECTS ON PROPERTIES OF THE HYDROGEL

2.1 Introduction

Different types of hydrogels have been investigated for their biomedical applications over the last decade [17]. PEG-based hydrogels are among the few well-studied hydrogels. PEGDA hydrogel is a very good example of PEG-based hydrogels. These hydrogels are biocompatible because the base material, PEG, is resistant to protein adsorption and biologically inert as well as does not release any acidic byproducts [18]. "Tunable physical properties" is another advantage of using PEGDA hydrogels. Simple change in molecular weight of PEGDA, allows tailoring of stiffness, swelling ratio, and diffusivity [19]. Although the crosslinked network of PEGDA hydrogel serves as a good delivery platform for drugs, non-degradable nature limits its use in many biomedical applications.

On other hand, citric acid derived biodegradable elastomers (CABE) possess advantage of degradability as well as unique mechanical and optical properties. They also consist of additional pendent functionality provided by citric acid, a metabolic product of Krebs cycle. CABEs have been tested for their *in-situ* crosslinking capacity, which opens a vast area of biomedical application of this cytocompatible entity [20, 21]. Poly (ethylene glycol) maleate citrate (PEGMC) is a novel member of CABE family, developed in Dr. Yang's lab at the

University of Texas at Arlington (UTA). The *in situ* crosslinkable CABE has been analyzed for its drug and cell delivery applications [21].

However, previous unpublished work in our lab, especially on *in situ* crosslinked PEGMC hydrogels, had showed that their degradation and mechanical properties did not suite for complex wounds. This imposes further work on the modification of PEGMC hydrogels for complex wound healing applications. Complex wound healing needs a system, which has capability to maintain moisture, mechanical properties comparable to skin tissue, enough swelling capacity, and longer degradation period. In this study, hydrogels were made from a blend of PEGMC and PEGDAs with different molecular weights. In order to optimize the hydrogel system properties including degradation, we studied the effects of the formation factors on the properties of the system. Different combinations, obtained with the help of Design-Ease 8, were employed for this study. PEGMC concentration, PEGDA molecular weight, APS concentration, and PEGDA 700 concentration were analyzed for their effects on the system's properties. After studying the effects of the factors, biocompatibility of the system was analyzed. To analyze the biocompatibility of the hydrogel system, we checked biocompatibility of basic formation factors and effluents of the system on human dermal fibroblasts using the MTS cell viability assays. PEGDAs, used in this study, were excluded from the biocompatibility study due to their well-known biocompatibility (its biocompatibility was also previously studied by other students in our group).

2.2 Materials and Methods

2.2.1 Materials

PEG 200, Citric acid, Dichloromethane, Acryloyl chloride, Magnesium Sulfate, PEG DA 700 (all from Sigma Aldrich), PEG (anhydrous), Maleic acid (Fluka), Potassium carbonate (Alfa

Aesar), Triethylamine (TEA) (MP Biomedicals), incomplete Dulbeco's Modified Eagle Media (DMEM) (Invitrogen), Adult human dermal fibroblast (HDFa) (ATCC).

2.2.2 Poly (ethylene glycol) maleate citrate (PEGMC) synthesis

PEGMC was synthesized by random polymerization technique mentioned elsewhere [21]. Briefly, 20 ml of PEG 200, 6.96 gm MA, and 7.68 gm of CA (molar ratio for PEG:MA:CA: was 0.6:0.4:0.1) were mixed together in a 100 ml round bottom flask, and a stopper was fitted on the flask. An oil-bath was preheated to 160℃ an d the round bottom flask was immersed into it. The mixture was allowed to dissolve with stirring at 360 rpm (revolutions per minute) on a magnetic stirrer plate. After dissolution of the mixture, stopper was removed and temperature was reduced to 140℃, keeping the same rpm. After f irst twitching of the stirrer, stirring rate was reduced from 360 to 200 rpm, while keeping temperature the same, 140℃. The same pattern was followed for second and third twitching to reduce stirring rate from 200 to 100 and then 0. After third twitch, reaction was stopped, and the impure PEGMC was dissolved in approximately 60 to 80 ml of DI water. This solution was filtered in order to remove any clumps resulted from cross-linked polymers. Filtration was followed by an overnight dialysis of the solution with 100-500 molecular weight cut off (MWCO) dialysis membrane to remove non-reacted materials. This purified PEGMC was then freeze-dried to remove any water content. FTIR was carried out to confirm the PEGMC synthesis.

2.2.3 Poly (ethylene glycol) diacrylate (PEGDA) synthesis

PEGDA synthesis was carried out according to a protocol described by Durst et al. [19]. Briefly, 12 gm of PEG, either 4.6 K or 8 K, was dissolved in to DCM and 1.3 ml of triethyl amine (TEA) was added to the solution. 0.61 ml of Acryloyl Chloride (AC) then separately dissolved in

DCM and added to the reaction drop-wise. This reaction then kept for 2 days in an ice bath. Continuous stirring, low light, and inert environment maintained throughout the reaction. After two days, the solution was washed with K_2CO_3 (2M) to remove hydrochloric acid and dehydrated by using 2 gm of anhydrous $MgSO_4$. Cross-linking test was performed in order to confirm the successful synthesis. Crosslinking test was carried out by treating 20% of PEGDA solution with APS and TEMED, to form a hydrogel system.

2.2.4 Scanning Electron Microscopy (SEM) for studying the structure of hydrogel

Hydrogels were prepared with detailed formulations (used for degradation study) mentioned in Table 2.1. These hydrogels were then kept in 5ml DI water for swelling and degradation. On pre-determined time points, hydrogel samples were collected and freeze dried. SEM analysis of these hydrogels was performed to study the structural changes taken place in the hydrogel over a period.

2.2.5 Studying effects of formation factors on the properties of the hydrogel system

Different formulations/runs (Table 2.1) were studied for the purpose of system optimization. Design-Ease 8 (Stat-Ease Inc.) was used to minimize the number of runs required for checking the effects of various formation factors on various properties of the hydrogel system. Four factors, consisting of PEGMC concentration, PEGDA molecular weight, APS concentration, and PEGDA 700 concentration, were analyzed. These factors are selected because PEGMC is a major monomer used in the hydrogel formation, PEGDA molecular weight is used to tailor the properties of the hydrogel, APS is acting as an initiator in the crosslinking, and PEGDA 700 is a major crosslinker. Instead of using single PEGDA, use of two different PEGDAs with higher molecular weight (4.6 and 8K) will allow us to fine tune the properties of

the hydrogel with better control. Two different levels, of each formation factor, were used to carry out the experiment. Table 2.1 shows the two levels used for each factor and responses evaluated based on those formulations.

Table 2.1 Formulations used to study the effects of the formation factors on different properties of the hydrogel system

Sr. No.	Formation Factors				Response evaluated
	PEGMC (mg/ml) Factor A	PEGDA (MW) Factor B	APS (mg/ml) Factor C	PEGDA 700 (mg/ml) Factor D	
1	200	4.6	32	96	Mechanical property (Young's modulus) Curing time Swelling ratio
2	200	4.6	16	48	
3	200	8	32	48	
4	200	8	16	96	
5	400	4.6	16	96	
6	400	4.6	32	48	
7	400	8	16	48	
8	400	8	32	96	
1	200	4.6	16	64	Degradation of the system
2	200	4.6	32	32	
3	200	8	16	32	
4	200	8	32	64	
5	400	4.6	16	64	
6	400	4.6	32	32	
7	400	8	16	32	
8	400	8	32	64	

Design-Ease 8® (Stat-Ease, Inc.) was used to analyze the effects of formation factors on various properties of the hydrogel system. The effects of formation factors on the Young's modulus were evaluated by performing elongation test with the help of MTS® Insight™ II mechanical tester equipped with a 500N load cell (MTS, Eden Praire, MN). For this test hydrogel was prepared in the form of strip. Thickness, width, and length for each strip (n=3 per run) were fed into the software, used for MTS® Insight™ II, and the test was performed. Young's modulus was directly obtained from the software.

For analyzing effects of the formation factors on the swelling ratio, hydrogels were prepared (n=4 per run), and each sample was freeze-dried in order to obtain dry weight (WD). After measuring the dry weight, every sample was immersed in 5 ml of DI water and allowed to swell up to 24 hrs. After 24 hrs, swollen weight (WS) was obtained for each sample. Equation 1 was used in order to calculate the swelling ratio.

$$\text{Swelling Ratio} = \frac{WS}{WD} * 100 \qquad \text{Equation 1}$$

Curing time was measured using magnetic stirrer technique. Briefly, after addition of every hydrogel component, the hydrogel solution with a small magnetic stirrer already in it was kept on magnetic stirrer plate with ~120 rpm. Stirrer was carefully observed. Time required for very 1st twitch of magnetic stirrer was taken as curing time.

For analyzing effects of formation factors on degradation of the system, slight changes were made in the PEGDA 700 concentrations, as shown in Table 2.1, but the number of runs remained the same. For this purpose sample size used was 3 (i.e. n=3) for every run and for every time point. Study was carried out with 8 time points over the period of 28 days. Initially, dry weights (WD) were measured for all the samples and all of them were immersed in 5ml of DI water and incubated over the time. For every time point, hydrogels were taken out of the water and dried. Weights of these dried hydrogels were measured (wd) and degradation was calculated in terms of percentage weight loss (Equation 2).

$$\%\ \text{Weight loss} = \frac{WD\text{-}wd}{WD} * 100 \qquad \text{Equation 2}$$

2.2.6 Biocompatibility testing for the system components

MTS assay

Human dermal fibroblasts were used for testing the biocompatibility of the system. MTS ((3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)) assay was performed on the treated cells. For MTS assay, 96-well plates were used with cell seeding density 5000 cells/well. Before performing the MTS assay, cells were allowed to attach on the surface of the well for 24 hrs with complete cell media (Dulbecco's Modified Eagle's Medium with 10 % Fetal Bovine Serum and 1% Penicillin: Streptomycin solution). After the attachment, the cells were treated with fresh complete cell media containing respective hydrogel component for which cyto-compatibility was tested. MTS reagent was added to the wells after 24 hours of the treatment. Plates were incubated at 37℃ for 2 hours and then absorbance was measured at 490nm.

Cyto-compatibility study for PEGMC

Certain amount of UV sterilized PEGMC was added to the media. Serial dilutions were made for obtaining different treatment groups (0-10mg/ml of PEGMC in complete media). HDFa cells were treated with these treatment groups. MTS assay was performed after 24 hours following the manufacturer's instructions, and cyto-compatibility of the polymer was obtained.

Cyto-compatibility study for APS

To check the cyto-compatibility of APS; HDFa cells were treated with the media containing different concentrations of APS (0, 5, 10, 50, and 100µg/ml). Before adding to the media, APS sterilized with UV treatment. MTS assay performed to check the cyto-compatibility of the APS.

Cyto-compatibility study for effluents of the hydrogel system

Hydrogel, with optimized formulation, was prepared and incubated in DI water for 24 hours. Effluents were collected from that water by freeze-drying and UV sterilized before adding to media. Dilutions made to get different treatment groups (0, 0.25, 0.74, 2.22, 6.67, and 20 mg/ml). HDFa cells were treated with these treatment groups and MTS assay was performed after 24 hours.

2.3. Results

2.3.1 Poly (ethylene glycol) maleate citrate (PEGMC) synthesis

PEGMC synthesis was carried out according to the method mentioned by Gyawali D et al. [21]. This is a simple poly-condensation and random polymerization reaction in which yellowish viscous PEGMC was obtained after 6-8 hours. This viscous fluid then purified and freeze dried to obtain pure PEGMC. FTIR result (Figure 3.4) confirmed the PEGMC synthesis by showing major peaks for functional groups in PEGMC similar to the previous study [21]. Peaks for functional groups from degradable ester bond, double bond, carboxylic group from maleic acid, and hydroxyl groups are visible in the FTIR spectra.

2.3.2 Poly (ethylene glycol) diacrylate (PEGDA) synthesis

White, powdered form PEGDA was obtained by vacuum drying. PEGDA synthesis was confirmed by crosslinking test. All of the PEGDA, irrespective of molecular weight, formed the hydrogel when treated with APS and TEMED. This means, PEGDA has the acrylate group on each side, which is able to crosslink in the presence of an initiator and accelerator.

2.3.3 Scanning Electron Microscopy (SEM) for studying the structure of hydrogel

SEM was performed on the hydrogel samples that were incubated in water for different periods. Figure 2.1 shows the degradation pattern of the hydrogel. SEM for the hydrogel incubated for one day (Figure 2.1 A) shows a wrinkled sheet like structure. As time passes, hydrogel degrades, which is reflected in the following images (Figures 2.1 B, C, D). From day 7 to day 28, the wrinkled structure keeps reducing due to surface erosion phenomenon.

2.3.4 Studying effects of formation factors on the properties of the hydrogel system

In order to tailor the hydrogel system better, we need to know the effects of the formation factors on properties of the composite hydrogel system. In previously done studies, it was shown that how composition and preparation method affected the morphology and swelling behavior of the hydrogel [22] and the protein release [14]. While considering for wound healing; applicability, high durability, swelling capacity of the hydrogel are important factors to be monitored and tailored [14, 23]. So in this study curing time (for applicability), Young's modulus and degradation (for durability), and swelling ratio (for swelling capacity) were taken as dependent variables.

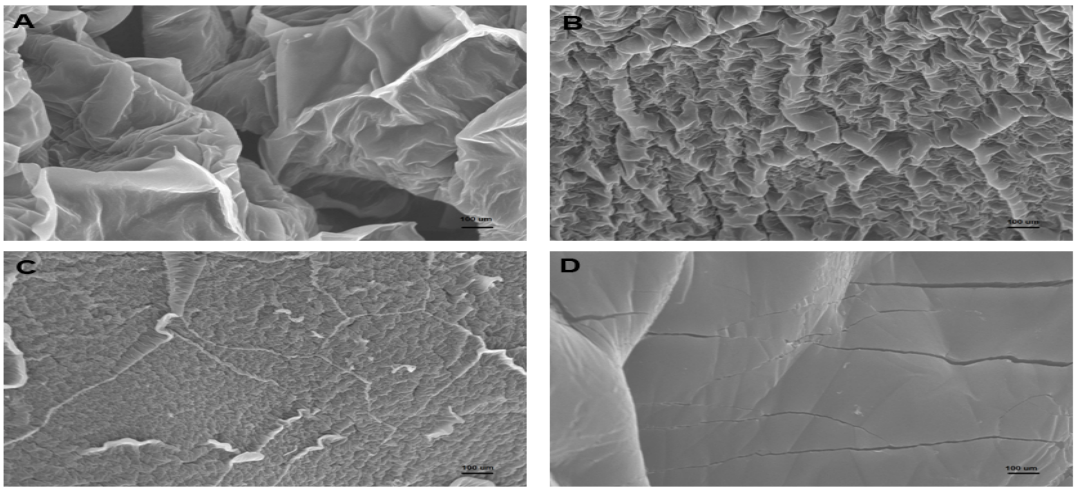

Figure 2.1 SEM images for the hydrogel surfaces degraded for (A) 1 day, (B) 7 day, (C) 14 day, and (D) 28 day.

PEGMC Concentration, PEGDA molecular weight, APS concentration, and PEGDA 700 concentration are the four factors studied as independent variables for their effects on the dependent variables, such as curing time, Young's modulus, degradation, and swelling ratio. As mentioned above, application of the system at the wound site is important. *In situ* forming gels can serve two main purposes in wound healing. First, easy applicability due to its ability to crosslink at the wound site and cover whole wound area. Second, local administration of the drug, as it can stay at the wound site through the treatment [14, 24]. To check the applicability of the system, curing time of the system was studied as a dependent variable. From the half normal plot (Figure 2.2A), it is clear that APS and PEGDA 700 concentrations have the highest effect on the curing time of the system in a negative manner, which means an increase in APS and PEGDA 700 concentrations will cause a decrease in curing time. At the same time, PEGMC concentration and PEGDA molecular weight are having positive effect on the curing time.

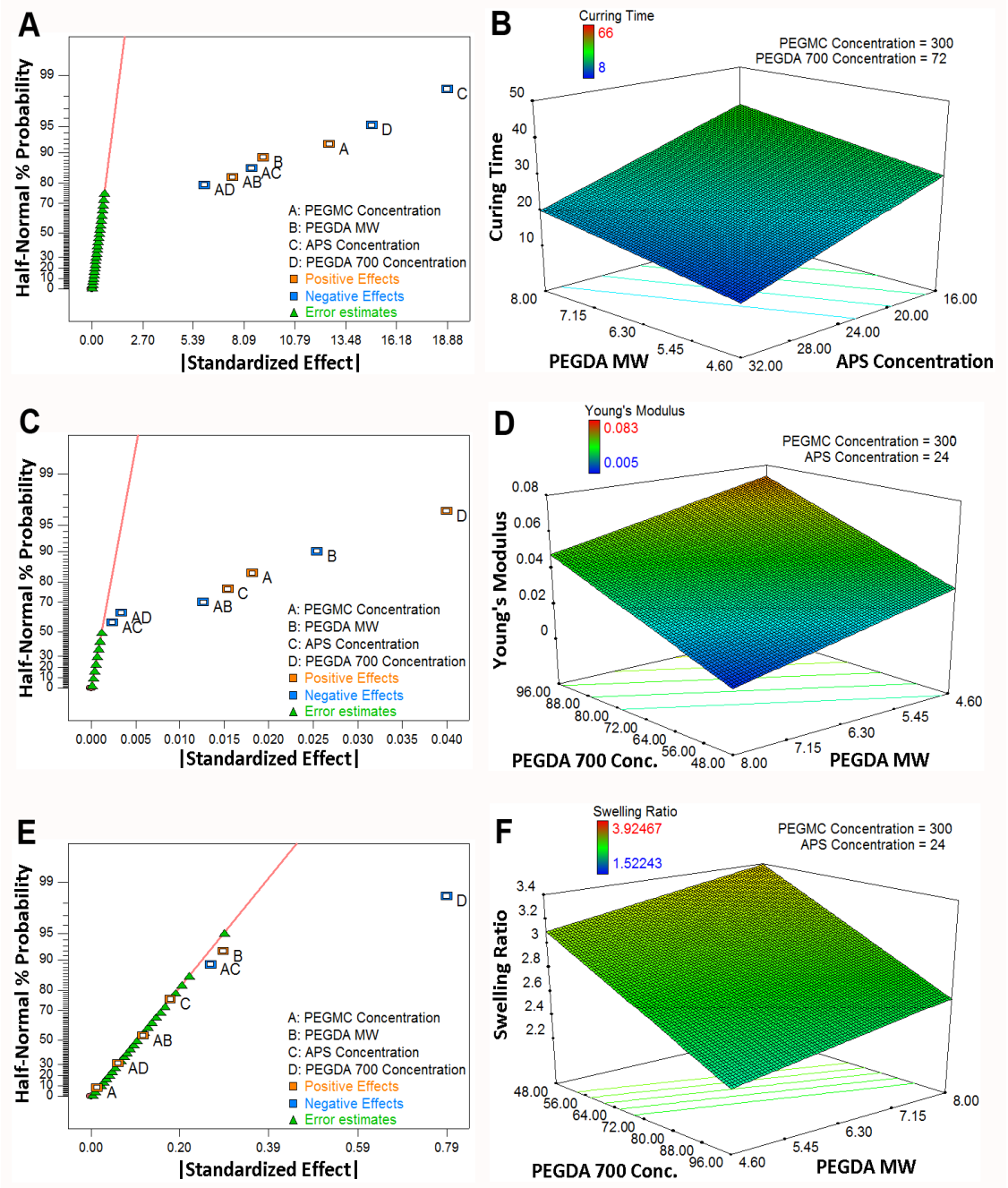

Figure 2.2 Half-normal plots for (A) Curing time, (C) Young's modulus, and (E) Swelling ratio. Three-dimensional (3D) surface graphs for (B) Curing time, (D) Young's modulus, and (F) Swelling ratio.

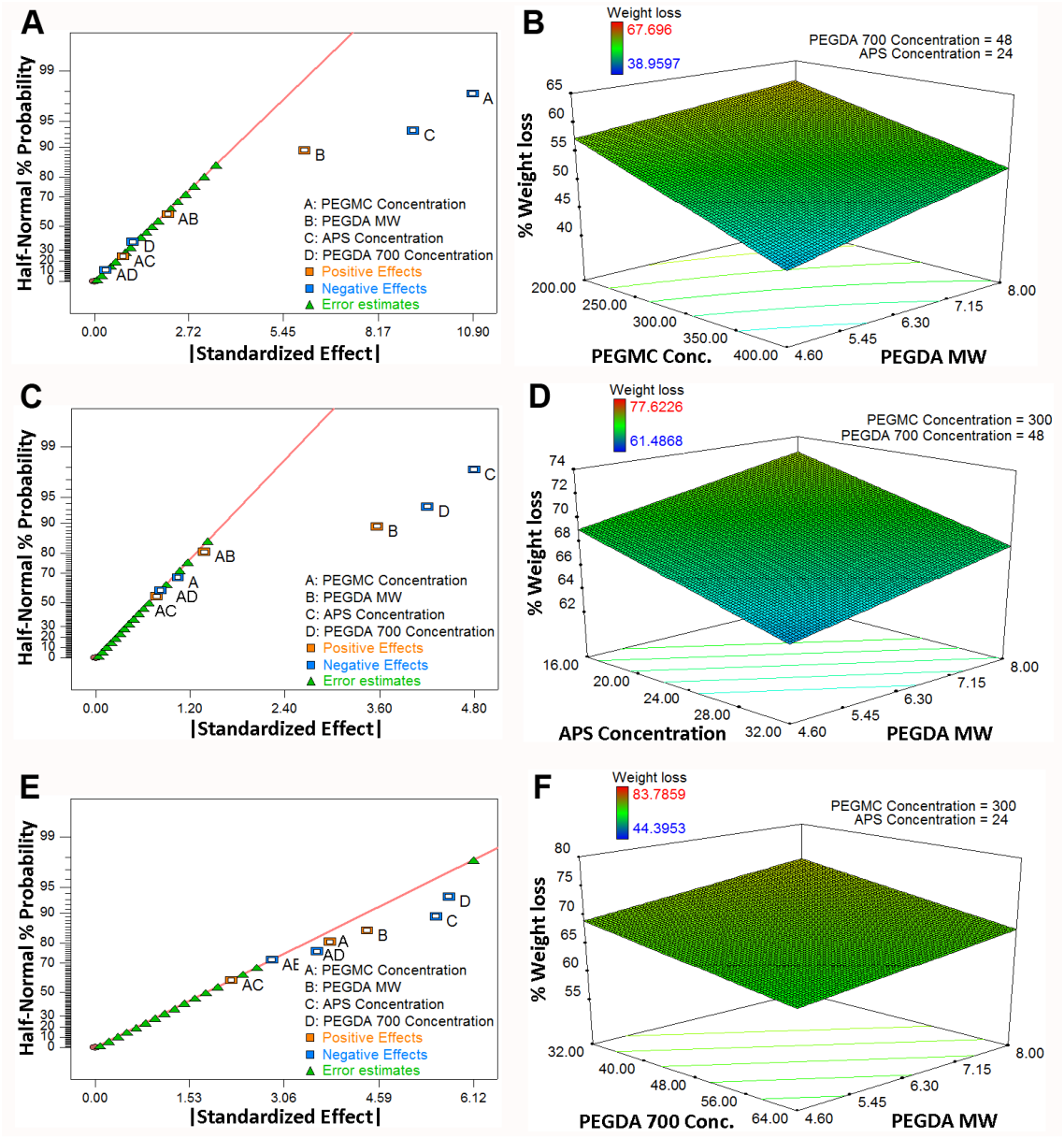

Figure 2.3 Half-normal plots for Degradation of the system after (A) Day1, (C) Day 14, and (E) Day 28. Three-dimensional (3D) surface graphs for Degradation of the system after (B) Day1, (D) Day 14, and (F) Day 28.

Of all the factors, molecular weight of PEGDA is the least affecting factor on the curing time. Slope in the 3D surface plot (Figure 2.2B) gives a clear idea about how APS concentration is having highest effect in a negative manner while PEGDA molecular weight is having the least

effect in a positive manner on the curing time. Equation 3 obtained from the factorial analysis software (Design-Ease 8®) can give formulation required for achieving the desired curing time, to serve the purpose of *in situ* formation of the system.

Curing Time = 16.22 + 0.14*A - 3.93*B + 0.41*C+ 0.07*D + 0.22*A*B - 5.31*A*C - 1.25*A*D

Equation 3

Where; A is the PEGMC Concentration, B is the PEGDA molecular weight, C is the APS concentration, and D is the PEGDA 700 concentration

Unlike curing time, APS concentration is showing least effect in a positive manner on Young's modulus of the system (Figure 2.2C). On the other hand, PEGDA 700 concentration is a dominating factor for Young's modulus in a positive manner (Figure 2.2C). PEGMC concentration is showing mild positive effect, while PEGDA molecular weight is the only one with a negative effect on the Young's modulus of the system (Figure 2.2D). Equations 4 can give the formulation required for specific Young's modulus of the system to achieve durable (strong) system.

Young's Modulus = $-0.12 + 4.10*10^{-3}*A + 3.68*10^{-3}*B + 1.41*10^{-3}*C + 1.04*10^{-3}*D - 3.71*10^{-3}*A*B - 1.48*10^{-5}*A*C - 7.03*10^{-6}*A*D$ Equation 4

In case of the system swelling ratio, PEGDA 700 concentration is the only dominating factor but in a negative manner (Figure 2.2E). PEGDA molecular weight is the other factor, which has very mild effect, whereas PEGMC concentration and APS concentration has no effect on the swelling ratio. It means that only a slight change in PEGDA 700 concentration can lead to a massive change in swelling of the system, which is clearly visible in 3D surface plot

(Figure 2.2F). Desired swelling ratio can be obtained using a formulation with predicted factors obtained by Equation 5.

$$\text{Swelling Ratio} = +2.92 + 1.03*A - 0.01*B + 0.06*C - 0.02*D + 3.37*10^{-3}*A*B - 1.66*10^{-3}*A*C + 1.23*10^{-4}*A*D \quad \text{Equation 5}$$

Analysis of effects of formation factors on degradation of the system at different time points was also performed. It was observed that at different time points, different factors are affecting on the degradation. For example, after Day 1, PEGMC concentration is the major factor, affecting on the degradation in a negative manner. At the same time PEGDA 700 concentration is not showing any effect (Figure 2.3A). Figure 2.3 B shows how PEGMC concentration is affecting in a negative way while PEGDA MW is affecting in a positive way. After day 14 (Figures 2.3 C-D), APS concentration showed maximum effect on the weight loss of the system. At this time point PEGDA 700 showed a negative effect on degradation of the system. Unlike Day 14, after day 28, PEGDA 700 and APS concentrations were having negative effects on the system. For this time point, no factor was, however, noticeably dominant. To obtain a desired degradation of the system after 28 days we can use equation 6,

$$\text{Weight loss} = 49.27 + 0.093*A + 3.83*B - 0.76*C + 0.16*D - 8.44*10^{-3}*A*B + 1.38*10^{-3}*A*C - 1.12*10^{-3}*A*D \quad \text{Equation 6}$$

2.3.5 Biocompatibility testing for the system components

Adult human dermal fibroblasts (HDFa cells) were used to test the cyto-compatibility of different components of the hydrogel since HDFa cells play a vital role in wound healing at proliferative stage via secreting collagen, which is an important factor for dermal regeneration

and contraction of the wound [6, 25, 26]. T-test was performed in order to check the significance of the results. There may be a possibility of incomplete crosslinking in the hydrogel system, because of which cells will encounter some PEGMC at wound site. Although the amount of free PEGMC will be very less, it is necessary to check the cyto-compatibility of the PEGMC. PEGMC showed cyto-compatibility with more than 90% cell viability up to 5mg/ml while after that concentration cell viability reduced to 75 % when compared with control, containing cells without any treatment (Figure 2.4A).

As a free radical, APS also takes part in crosslinking. There may be a possibility of remaining little traces of unreacted APS, thus it is important to check the biocompatibility of APS. After cytotoxicity evaluation, APS proved to be a cytocompatible up to a concentration of 50µg/ml (Figure 2.4B). At concentrations of APS larger than 50µg/ml, there was a significant cell death. Similar to unreacted components, hydrogel effluents are also released after hydrogel formation and degradation at the wound site. From 0 to 2.22mg/ml of effluent concentrations, there was no significant difference observed in cell viability. But at 6.67 and 20 mg/ml there was a significant reduction in the cell viability (Figure 2.4C).

2.4 Discussion

On the quest for achieving an ideal system for complex wound healing, we analyzed the hydrogel system for its properties including degradation and biocompatibility. SEM images of the degraded hydrogel showed the pattern of the degradation, which is surface erosion. When compared to the hydrogels made up of only PEGMC [20], the pattern of degradation in the PEGMC-PEGDA composite hydrogels is different. The previous one showed increased porosity while later showed decreased wrinkled surface. Diversifying the application of the PEGMC based hydrogels for tissue ingrowth to the controlled drug release.

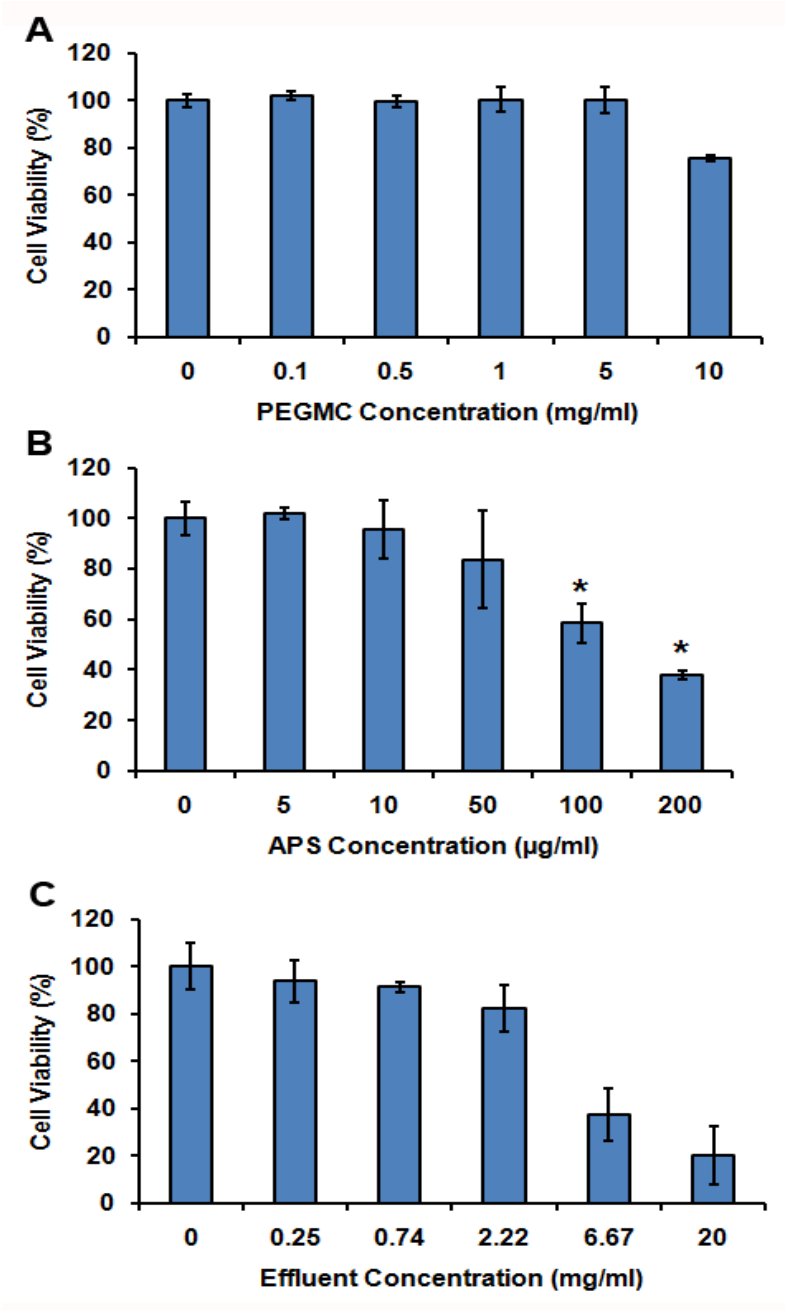

Figure 2.4 Cell viability study for testing the cytocompatibility of (A) PEGMC, (B) APS, and (C) Effluents of the hydrogel system.

After studying the degradation pattern, factorial analysis was done to check effects of the formation factors on different properties of the hydrogel. Curing time is very important factor when designing the *in situ* system because it will decide the ease of application at the wound site. According to the factorial analysis in this study, APS (initiator) concentration and PEGDA 700 (cross linker) concentration have showed negative effect on the curing time (66 to 8 seconds). Previous studies [27] have also shown that the PEGDA, used as a cross linker, has negative effect on the curing time. These results support the higher the number of binding sites the faster is the cross linking of the polymer to form the hydrogel. Similar to the cross linker, higher the initiator concentration leads to faster crosslinking in this study. At the same time, we have also studied the effects of the PEGMC concentration (main polymer) and PEGDA (additional polymer) molecular weight. The results suggested that both of them are having positive effects on the curing time of the system, which means the higher the polymer size/length the slower is the crosslinking or curing time.

There are several factors that might affect the mechanical properties of the hydrogel system. For example, Gyawali et al [20] have showed that monomer ratio in the PEGMC and type of crosslinker affect the mechanical properties of the hydrogel. According to their results when photocrosslinker was used, molar ratio of citric acid affected the tensile strength and elongation of the hydrogel in positive manner. In this study, we observed that the crosslinker concentration, initiator concenteration, PEGMC concentration and molecular weight of the polymer PEGDA (4.6 and 8K) affect on the mechanical properties of the hydrogel. The effect of molecular weight of PEGDA on the mechanical properties of the hydrogels was similar to the results obtained by Temenoff et al [28] for oligo (poly(ethylene glycol) fumarate (OPF), PEG derived polyer, hydrogels. In both the studies it was observed that molecular weight has a negative effect on the Young's modulus of the hydrogels.

Maintaining of the moisture at the wound site is very important for normal wound healing, thus factorial analysis was also performed to investigate effects of formation factors on the swelling of the system. In this study, PEGDA 700 concentration has maximum effect in negative manner, while PEGDA molecular weight has least effect in positive manner on the hydrogel swelling. The manner, in which the PEGDA molecular weight is affecting the swelling, is similar to what was observed by Sabnis et al but the intensity of the effect was different. According to Sabnis et al [14] molecular weight is most affecting factor while according to this study crosslinker concentration has the maxmum effect.

Degradation of the system was also studied to determine effects of the formation factors of the hydrogel. It was observed that, at different time points, different factors are playing a major role on the percentage weight loss of the system. Study from Gyawali et al [29] showed the effect of the monomer ratio of PEGMC on the degradation of PEGMC hydrogel while this study showed the effect of PEGMC concentration on the degradation of the PEGMC-PEGDA hydrogel. It was also observed that after day 14 and day 28 PEGDA 700 (crosslinker) and APS concentration (initiator) showed the effects on the degradation of the system. PEGMC concentration showed highest effect after day 1 while very mild or no effect after day 14 and day 28. This behavior suggests that as PEGMC is degraded, the effect of its concentration is reduced. On other hand crosslinker and initiator showed negative effects on the degradation, as time passes, suggesting that crosslinking is the major affecting factor on the percentage weight loss of the system. Gyawali et al. [14] also observed negative effect of crosslinker, on the percentage weight loss. Finally, cytotoxicity study for the system components supported the biocompatibility of the system similar to those observed by other studies for PEG based hydrogel system [29]

2.5 Conclusion

We successfully prepared different components of the hydrogel system. Hydrogel formation was achieved by radical polymerization. Wrinkled sheet like structure of hydrogel was observed using SEM. Sequential study of SEM revealed the degradation pattern of the hydrogel, which can be used for better understanding of the swelling and drug release properties of the hydrogel system. For complex wound healing applications, swelling, mechanical, and degradation properties of the system are very important. In this study, we successfully analyzed the effects of formation factors on the hydrogel system properties. For *in situ* formation of the hydrogel, curing time is very important. The effects of formation factors on the curing time were analyzed, and this analysis provided us the most formation factor affecting curing time with an equation for optimization of curing time (Equation 3). Using Design-Ease 8, we also obtained specific equations (Equations 4, 5 and 6) for tailoring the properties of the hydrogel to fulfill the needs of complex wound healing application. A series of biocompatibility studies for different formation factors also proved biocompatibility of hydrogel components, which will be available at the wound site. By using the data generated in aim 1, the system can be optimized according to an individual need.

CHAPTER 3

ENHANCEMENT OF THE HYDROGEL SYSTEM TO SUPPORT COMPLEX WOUND HEALING

3.1 Introduction

In addition to biocompatibility and tunable physical properties, some enhancing agents are required for proper wound healing of complex wounds. Controlling the drug/protein release, according to need, is important task in designing the drug delivery system. As shown in Figure 1.1, wound healing needs different drugs/proteins at different stages. Multilayer system can be a good option to provide a controlled release of therapeutic reagents for wound healing. A bilayer system has been tested previously for drug/protein delivery application [30] and skin reconstruction [31]. Based on these concepts, I proposed a design of a hydrogel system with two different layers that have different degradation properties, which in turn affect the drug/protein release from the system.

Bacterial infection is another major issue in treatment of the complex wound. Many groups and institutes are trying to find different tactics to tackle the infection [32-36]. Conventional antibiotics are effective against bacterial infection; however, some of them are very toxic and can cause harm to normal cells while some of them are eventually become useless when bacteria begins to develop antibiotic resistance [7, 34]. To overcome these limitations, alternative methods and antibacterial drugs have been investigated. Nanosilver is an attractive option as an alternative bactericidal entity. It has good antibacterial activity as well as wound healing enhancement property. However, it can cause high toxicity if used in high

concentrations and can damage DNA by direct interaction [33]. Antimicrobial peptides (AMP) have been investigated as an ideal antibacterial entity for combating infection as they are part of innate epithelial chemical shield [37, 38]. Of these AMPs, β- defensins are most attracting because of their role in epidermal keratinocyte migration and proliferation as well as proinflammatory cytokine production [39]. Human β- defensin 3 (HBD3) is a member of the defensin family, and it has been studied extensively along with its derivatives for their antibacterial activity [40, 41]. As chemotactic and antibacterial properties are considered, HBD3 stands well as an alternative to conventional antibiotics. To improve the antimicrobial property, antibacterial peptides derived from HBD3, either CHRG01 (UTNA 2) or Abu-CHRG01 (UTNA 3) [41], were incorporated into the PEGMC hydrogel system.

In this study, we developed a dual layer system with two different layers having different degradation properties for controlling the drug/protein release profile. The rationale behind this design was to achieve a primary burst release of the drug/protein with faster degrading layer and sustained release with slower degrading layer. Similar to the controlled delivery of drug/protein, it is important to have good anti-microbial ability in the system. To achieve this goal, we incorporated antibacterial peptides to the main polymer of the system, PEGMC, and used these materials to form the hydrogel system. We performed the preliminary study to check the antibacterial activity of the system using 'area of zone of inhibition' technique.

3.2 Materials and Methods

3.2.1 Materials

Dichloromethane (DCM), Ethyl (dimethylaminopropyl) carbodiimide (EDC), N-hydroxysulfosuccinimide (NHS), and LB Broth (all from Sigma-Aldrich), CHRG01 (UTNA 2) and ABU-CHRG01 (UTNA 3) (AnaSpec), LB Agar (Research Product International Corp).

3.2.2 Formation of dual layer hydrogel system and drug/protein release study

Two different hydrogel systems, with different degradation rates generated from the factorial analysis, were formulated. Respective curing times were also calculated. For both the formulations 25% of PEGMC solution was used. For layer 1 (L1), degrading 72%, in 3 days, 50mg of PEGDA 6KDa, 4mg of APS, 19.61mg of PEGDA 700, and 50 mg of bovine serum albumin (BSA) as a model protein were added to 1 ml of PEGMC solution. And for layer 2 (L2), degrading 72%, in 7 days, 50mg of PEGDA 6KDa, 4mg of APS, 47mg of PEGDA 700, and 50 mg of BSA were added to 1 ml of PEGMC solution. For formation of a double layer (L2/L1), as shown in Figure 3.1, formulation for layer 1 (100 µl) was poured into mold. After 55 seconds, curing time for layer 1, formulation for layer 2 (100 µl) was poured on top of layer 1. Protein release study was performed to analyze the effects of double layer on the drug release profile. For drug release study, the dual layer system was kept in 6 well plates, and whole sample was immersed in 1 ml of PBS buffer except upper surface of L2. Drug release from single layer for each formulation (200µl/sample) was taken as a control.

3.2.3 Cyto-compatibility testing of peptides:

To check the cytocompatibility of the peptides PicoGreen® assay was employed. For PicoGreen assays, cells were allowed to attach for 24 hours. Then cells were treated with low serum media (Dulbecco's Modified Eagle's Medium with 2 % Fetal Bovine Serum and 1%

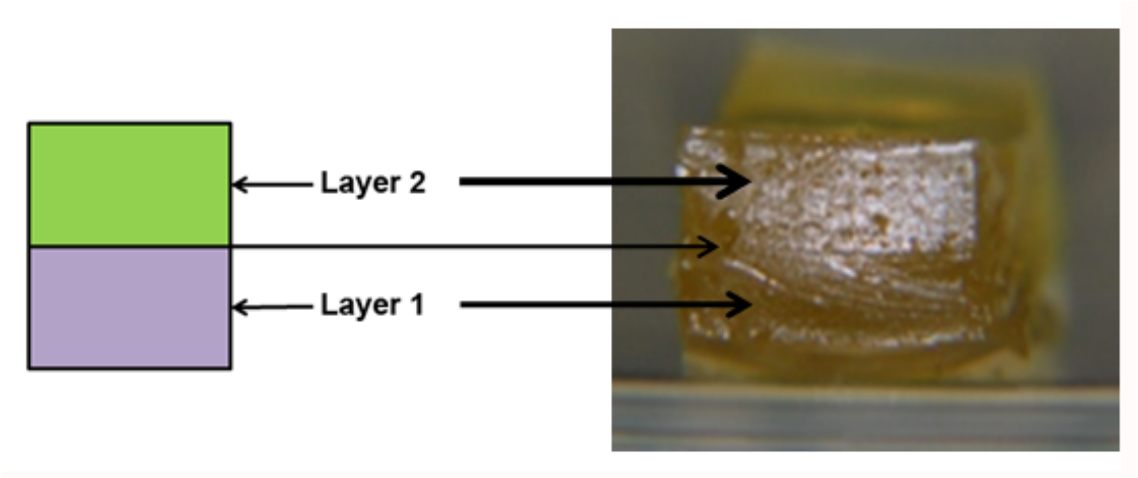

Figure 3.1 Schematic for dual layer system, in which Layer 2 (L2) is slow degrading, and Layer 1 (L1) is rapidly degrading.

Penicillin: Streptomycin (pen/strep) solution for 12 hours. After 12 hours, fresh complete cell media, containing either 160 µg/ml of UTNA 2, UTNA 3, and ampicillin, was used to replace low serum media. Treatment of the fresh medium was followed by an incubation period of 3 days. Then cells were lysed with 1X Triton. PicoGreen® reagent was added to the cell lysis samples, and readings were taken at excitation wavelength 480nm and emission wavelength 520nm. DNA standard curve was also established using DNA stock solution provided by the company. Cells treated with complete DMEM without any peptide and ampicillin, were used as control.

3.2.4 Formation and testing of antibacterial hydrogel system

PEGMC was conjugated with antibacterial peptides, either CHRG01 (UTNA 2) or Abu-CHRG01 (UTNA 3) [41], via EDC-NHC coupling technique as previously described [42]. Briefly, 1% PEGMC solution in MES was treated with 0.011 M of MHS and 0.023 M EDC. After 30 minutes of treatment, 1mg of peptide (either CHRG01 (UTNA 2) or Abu-CHRG01 (UTNA 3)) was added to the PEGMC solution. Final solution was kept overnight with a gentle agitation. Afterwards, the solution was dialyzed and freeze-dried to obtain pure PEGMC conjugated with peptides. Conjugation was confirmed by FTIR analysis. A formulation for hydrogel system with antibacterial property was decided with the help of factorial analysis. Briefly, 25 % of 1ml PEGMC conjugated with peptide, 64mg of PEGDA 700, 16mg of APS, and 50 mg of PEGDA 8K were added together to form the antibacterial hydrogel system. To test the antibacterial property, hydrogel system with peptide was kept on the freshly cultured *E. coli* agar plates. The plates were incubated at 37℃ for 16-18 hours. After incubation area of zone of inhibition was measured and compared with the controls. Hydrogels, with ampicillin (antibiotics), were taken as positive control, while hydrogels without any treatment served as a negative control.

3.3 Results

3.3.1 Formation of dual layer hydrogel system and drug release study

Drug release from a dual layer was successfully performed, and both layers showed different drug release profiles compared to those of single layers. Figure 3.2 shows the drug/protein release pattern for the dual layer. When compared to slow degrading layer, layer 2 (L2), dual layer system (L2/L1) showed faster drug release. There was a very short burst release in L2 only for half hour. On other hand the drug release from Layer 1 (L1), a fast

degrading layer, was fastest; showing initial burst release for one hour. In this phase, L1 hydrogel released almost 83% of its total loaded drug. As compared to L2/L1 (9%) and L2 (4.2%) burst release from L1 was too high. In case of L2/L1, burst release was also observed up to one hour, but only 9% of total loaded drug, then release was exponential as a function of time until 12 hours. After 12 hours, L2/L1 showed a sustained drug release.

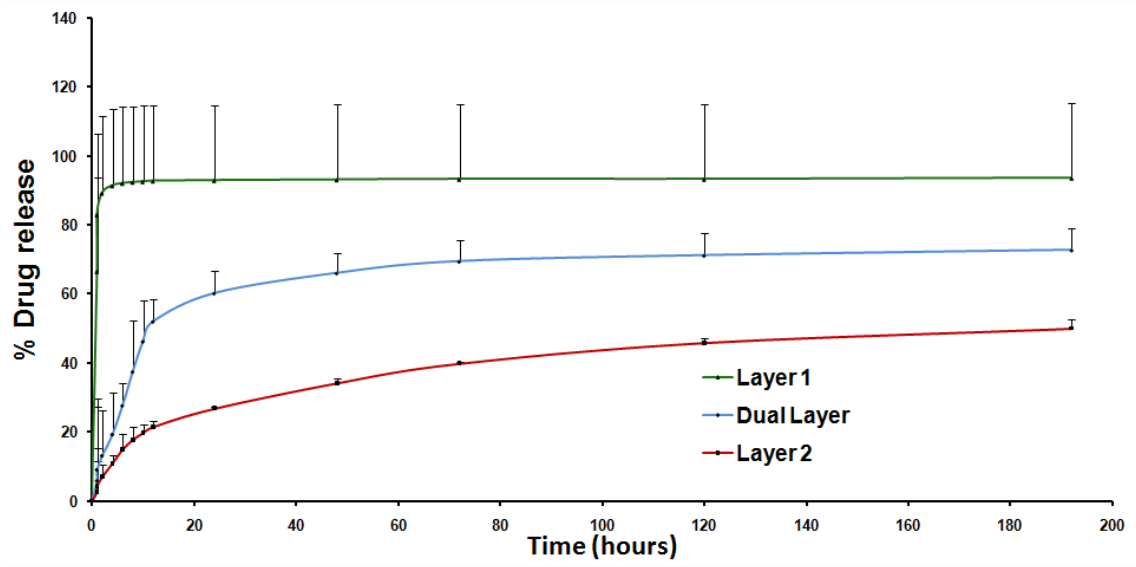

Figure 3.2 Drug release profile showing drug release for Layer 1 (L1), Layer2/Layer1 (L2/L1), and Layer 2 (L2).

3.3.2 Cyto-compatibility testing of peptides

All peptides including UTNA 2 and UTNA 3 showed good cyto-compatibility. For all treatment groups significantly higher cell growth was observed than the control. Figure 3.3 shows that the cell growth for UTNA 2 was the higher compared to other treatment groups.

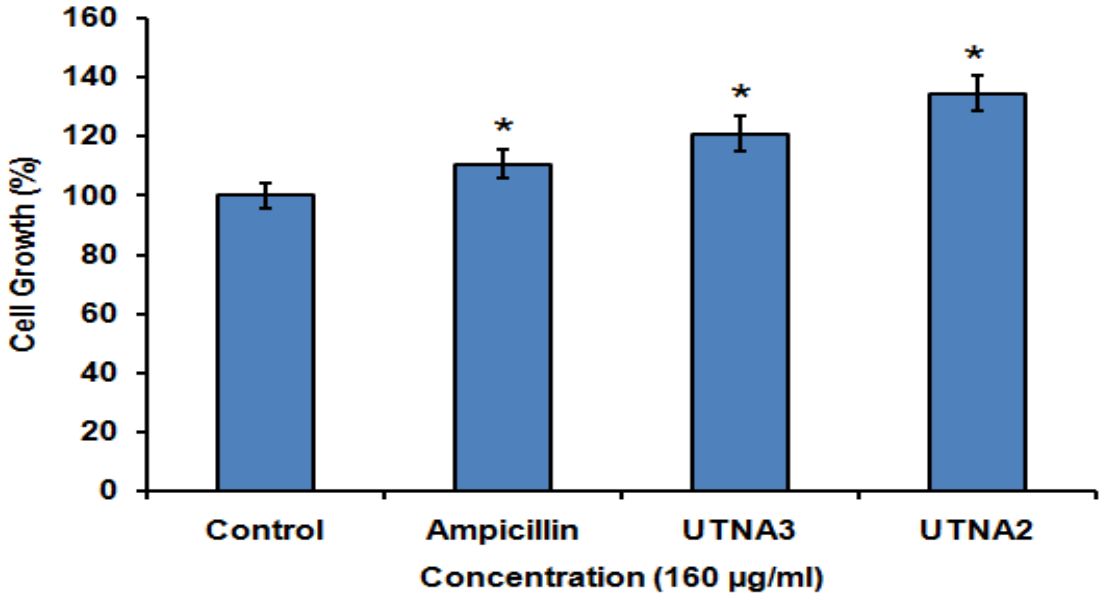

Figure 3.3 Cyto-compatibility study for peptides (UTNA 2 and UTNA 3) and ampicillin.

3.3.3 Formation and testing of antibacterial hydrgel system

Conjugation of peptides was confirmed by FTIR (Figure 3.4). FTIR for PEGMC shows its characteristic peaks for -OH, -CH_2, -C=C, and –C=O. FTIR for peptide conjugated PEGMC shows an additional peak for -N-H while retaining its characteristic peaks. After confirmation of the conjugation, the peptide conjugated PEGMC was used for hydrogel formation. Treatment with hydrogel sample showed zone of inhibition for each of the group (Figure 3.5 (A) only PEGMC, (B) PEGMC conjugated with UTNA 2, (C) PEGMC conjugated with UTNA 3, and (D) PEGMC with ampicillin. By measuring the area for zone of inhibition for three hydrogel samples, PEGMC with either peptide or ampicillin showed significantly higher antibacterial activity compared to hydrogel system made up of PEGMC only. System consisting of peptide (UTNA 1 or UTNA 2) showed comparable results with the system consisting of ampicillin. One reason behind the zone of inhibition for system with only PEGMC is swelling of the system. This

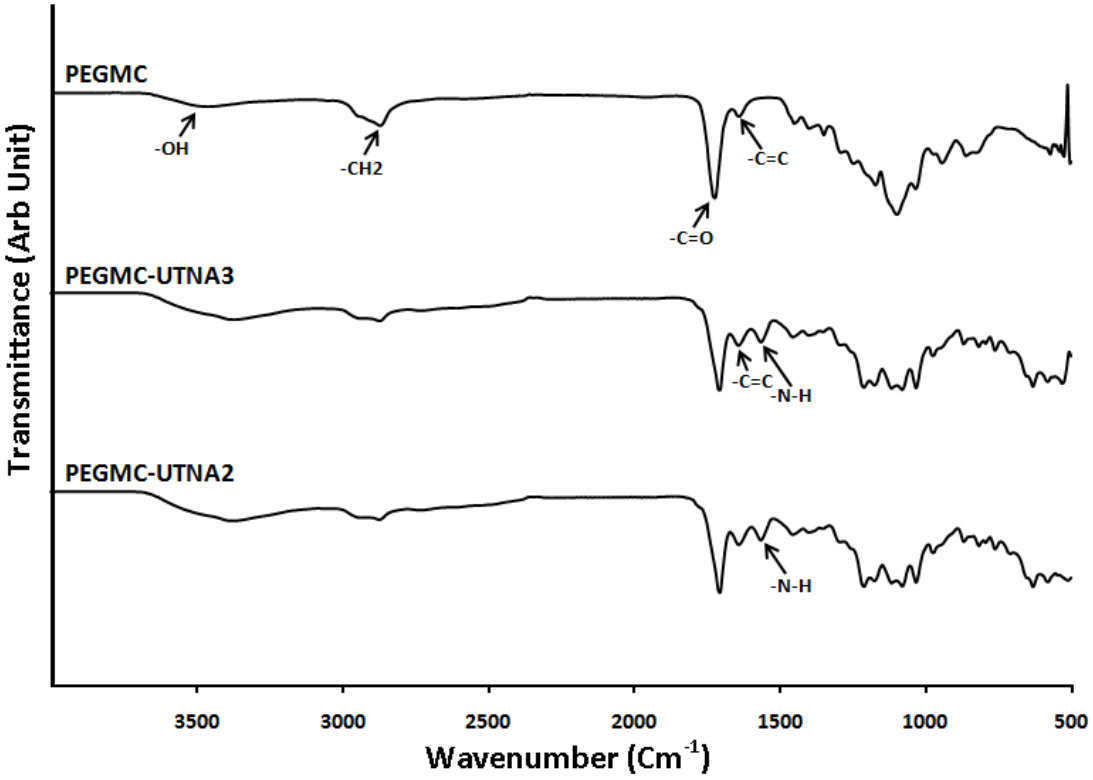

Figure 3.4 Comparison of FTIR spectra for PEGMC, PEGMC conjugated with UTNA 2 (PEGMC-UTNA2), and PEGMC conjugated with UTNA 3 (PEGMC-UTNA3), showing characteristic peaks for C=C, C=0, CH_2, and OH for PEGMC and an extra amine (-N-H) peek for peptide conjugated PEGMCs.

characteristic will avoid the formation of the fluid filled pocket at the wound site, which is an ideal area for bacterial growth. In addition to swelling, the antibacterial entity loaded into the system is also working. A significant difference between the areas of zone of inhibition is supporting the claim (Figure 3.6).

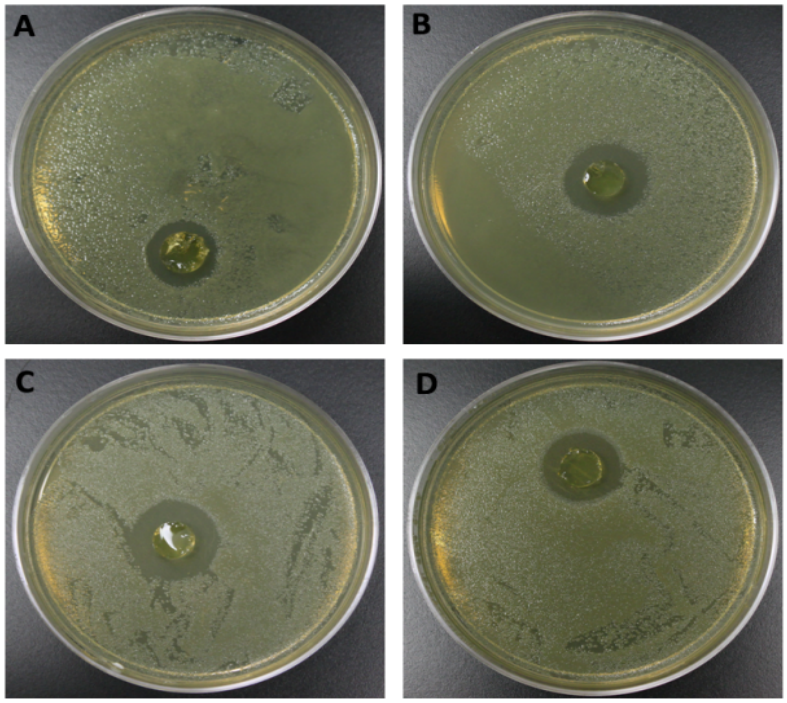

Figure 3.5 Zone of inhibition for hydrogel system containing (A) only PEGMC, (B) PEGMC conjugated with UTNA 2, (C) PEGMC conjugated with UTNA 3, and (D) PEGMC and ampicillin.

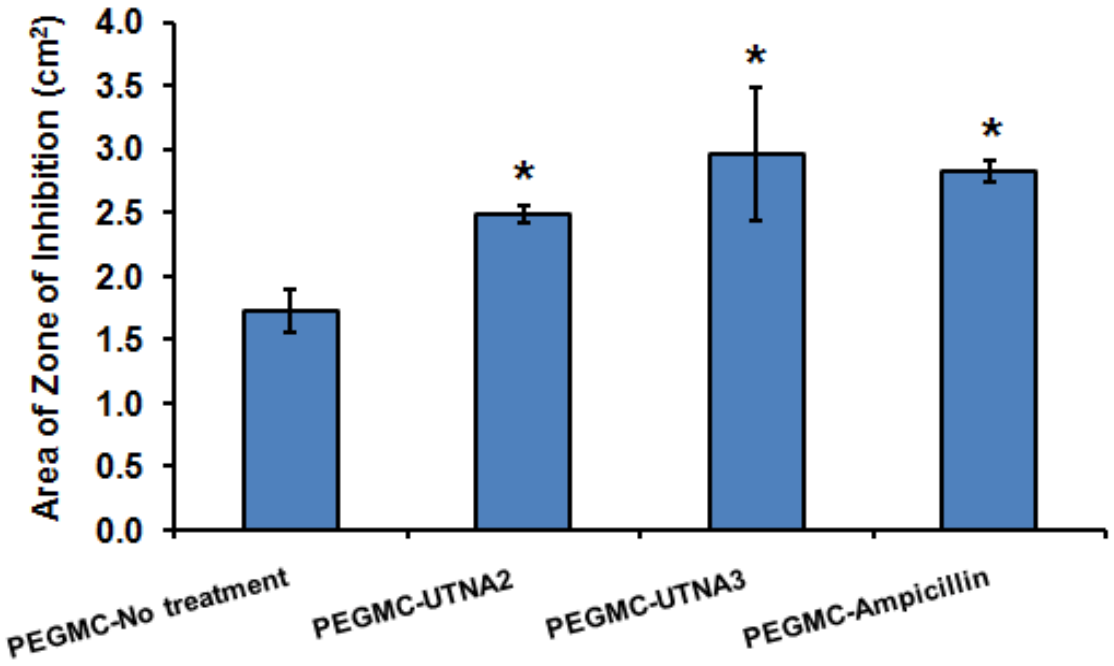

Figure 3.6 Comparison of area of zone of inhibition for system containing only PEGMC, PEGMC conjugated with UTNA 2, PEGMC conjugated with UTNA 3, and PEGMC and ampicillin.

3.4 Discussion

Drug/protein release profiles from the hydrogel system can be regulated by various factors including degradation rate. Holland T A et al [30] showed that by controlling the swelling ratio and mass loss one can control the drug release. While Sabnis et al [14] showed that a bilayer hydrogels can also be used to control the drug release. Based on these two designs we designed a hydrogel system with two layers having different degradations rates. The factorial analysis performed in Chapter 1 was used to optimize the bi-layer hydrogel such as, faster degrading layer and slower degrading layer that would lost up to 72% of their mass in 3 days and 7 days, respectively. Drug release study performed with this system provided us with expected results showing an initial burst release and later sustained release (Figure 3.2).

Bacterial infection is highly devastating for an effort to treat the complex wound. In order to tackle this problem we designed a hydrogel system consisting of an antibacterial peptide. Kazemzadeh-Narbat M et al. [43] demonstrated the effectiveness of cationic peptide loaded titanium against the bacterial infections associated with the implants. Similarly, we conjugated CHRG01 and ABU CHRG01 to the PEGMC and showed the effectiveness of the peptide conjugated PEGMC hydrogel system. Of alternative antibacterial materials, silver shows very good antibacterial activity and hence it is a part of many commercially available antibacterial dressing. According to one survey, performed by Dr. David Marx of University of Scranton, Johnson and Johnson Biopatch showed largest zone of inhibition after one day (1.58 cm^2) [44]. In comparison to this result, our hydrogel system showed better antibacterial activity showing up to 2.49 cm^2 and 2.96 cm^2 of zone of inhibition with CHRG01 containing and ABU-CHRG01 containing hydrogels, respectively. The results are also affected due to swelling capacity of the hydrogel as a zone of inhibition is observed for a hydrogel without any peptide (1.73 cm^2).

3.5 Conclusion

Dual layer hydrogel system, consisting of two different layers with different degrading properties, have been successfully prepared and studied. Drug release study for the system showed that the drug release profile can be easily controlled by changing the degradation of the hydrogel. Figure 3.1 showed that if the hydrogel is very fast degrading then it can release the drug faster and vice versa. Also from the release curve obtained for L2/L1, we can get an idea that, “how one can design a system useful for different drug/protein release profiles.” By using this property of a dual layer system, multi drug system can be designed which can release different drugs at different time points and/or release rates. This kind of drug delivery system will be very useful for complex wound healing, as wound healing needs different things/factors at different time points.

Apart from a successful design of dual layer, a system with an integrated antibacterial property was also prepared and characterized. Hydrogel system prepared with the antibacterial peptide conjugated PEGMC was able to resist a bacteria from growing in its proximity. Hydrogels, with CHRG01 (or UTNA 2) and ABU-CHRG01 (or UTNA 3), showed comparable antibacterial activity with hydrogel contained ampicillin. As controlling a bacterial infection with debridement is necessary in the complex wound healing, a hydrogel with integrated bactericidal property will be a useful option.

CHAPTER 4

SUMMARY, LIMITATIONS, AND FUTURE WORK

On the voyage of developing an ideal system for complex wound healing, we successfully studied the effects of various formation factors on the hydrogel system properties. Ideal system for wound healing should posses some basic characteristics. Such system should be biocompatible. Biocompatibility testing for the components provided us with exact concentrations of each component to maintain the biocompatibility for the hydrogel system. Some physical properties like swelling, mechanical, degradation, and curing are also important from the wound healing point of view. These properties are also affected by the system components. To understand these effects and optimize the system, factorial analysis was carried out. The effects of some of the major formation factors were analyzed in this study. Finally, we obtained equations necessary for fine-tuning the properties according to the specific individual needs.

We also studied a drug release from the dual layer hydrogel system. The results showed that we could control the drug release profile by combining two layers with different properties. This will serve as a programmed delivery system in wound healing. With the incorporation of antibacterial entity in the system, we were also able to form an antibacterial wound bed. Bacterial infection is one of the major problems related to different types of complex wounds, and the antibacterial hydrogel system will help avoid the bacterial infection at wound site.

While performing experiments for the research project, we encountered some limitations as listed below.

- We checked biocompatibility for basic un-reacted components such as PEGMC, APS, and the effluents, but not PEGDAs, as we assumed these components are biocompatible from previous studies in our group. In addition, the concentrations selected, for the study, were based on the consideration that after crosslinking, very little un-reacted factors will be available at the wound site. But, the exact amounts of un-reacted entities were not measured.
- The equations given by factorial analysis are able to optimize the system within the range given by the software only.
- Drug releases for dual layer system were carried out with the optimized systems. But they were designed to degrade in very short time interval, 3 days and 7 days. For complex wound healing purpose, drug release should be carried out from the system with longer degradation periods.

Based on the listed limitations and further requirements for an ideal wound healing system, future work will include:

- To perform drug release study for longer periods.
- To check the cytotoxicity of the effluents of the system, at different time points, especially for up to one month.
- To check the system for its ability to load and release the therapeutic agents (growth factors like PDGF-BB and VEGF) that can facilitate healing for complex wounds.

- To check the system for its ability to deliver multiple drugs/proteins at different time points by using a dual layer design.
- To enhance the system for better gas exchange at the wound site for reducing the pressure and improving the blood supply.
- To perform *in vivo* and *in vitro* studies for determining the effectiveness of the hydrogel system to treat complex wounds.

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BIOGRAPHICAL INFORMATION

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