AMERICAN UNIVERSITY OF BEIRUT

ENHANCEMENT OF PHYSICAL AND MECHANICAL PROPERTIES OF GEL-MA TOWARDS APPLICATION IN WOUND HEALING PROCEDURES

by
GEORGE DAVID DEEB

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Engineering
to the Department of Mechanical Engineering
of the Maroun Semaan Faculty of Engineering and Architecture
at the American University of Beirut

Beirut, Lebanon
January 2018

AMERICAN UNIVERSITY OF BEIRUT

ENHANCEMENT OF PHYSICAL AND MECHANICAL PROPERTIES OF GEL-MA TOWARDS APPLICATION IN WOUND HEALING PROCEDURES

by

GEORGE DAVID DEEB

Approved by:

Dr. Samir Mustapha, Assistant Professor — Advisor
Department of Mechanical Engineering

Dr. Ali Tehrani, Assistant Professor — Member of Committee
Department of Chemical and Petroleum Engineering

Dr. Mohammad Harb, Assistant Professor — Member of Committee
Department of Mechanical Engineering

Date of thesis defense: January 25, 2018

AMERICAN UNIVERSITY OF BEIRUT

THESIS, DISSERTATION, PROJECT RELEASE FORM

Student Name: Deeb (Last) George (First) David (Middle)

● Master's Thesis ○ Master's Project ○ Doctoral Dissertation

☑ I authorize the American University of Beirut to: (a) reproduce hard or electronic copies of my thesis, dissertation, or project; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

☐ I authorize the American University of Beirut, to: (a) reproduce hard or electronic copies of it; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes
after : **One ---- year from the date of submission of my thesis, dissertation, or project.**
Two ---- years from the date of submission of my thesis, dissertation, or project.
Three ---- years from the date of submission of my thesis, dissertation, or project.

George Deeb 13 February, 2018

Signature Date

ACKNOWLEDGMENTS

Special thanks are to Drs. Samir Mustapha, Ali Tehrani and Mohammad Harb for their great help and guidance to conclude my thesis and submit it with significant quality.

I would also like to thank Dr. Marwan Sabban and his lab members for their guidance and assistance with the anti-microbial studies required for my thesis.

Also, I would also like to thank Dr. Anwarul Hasan for his guidance during my first few years in the masters program and presenting me with many opportunities to learn and improve myself.

Finally, I would like to thank my family and friends who always encouraged me to pursue my dreams.

AN ABSTRACT OF THE THESIS OF

George David Deeb for Master of Engineering
Major: Mechanical Engineering

Title: Enhancement of Physical and Mechanical Properties of Gel-MA Towards Application in Wound Healing Procedures

Gelatin methacryloyl (GelMA) is a hydrogel that has found extensive use in the field of biomedicine, especially tissue engineering. GelMA hydrogels' structure closely resemble the structure of the extra-cellular matrix (ECM) found in tissues and organs. Also, the fabrication process of GelMA enables the customization of its mechanical properties as seen fit for its use. This study investigates the potential of GelMA for application in regenerative medicine. The study will concentrate on the potential for use as a wound healing patch for patients suffering from chronic wounds. Various GelMA hydrogel compositions will be fabricated and mechanically characterized. Nanoparticles (NP) will be added to the GelMA mixtures to evaluate their effect on the hydrogel's mechanical properties. Some of the NPs will contain a growth factor so as to study its release rate from the NP.

Cotton gauze will be used to simulate wound dressings and GelMA will be used as a medium to deliver antibacterial components onto the cotton. The tensile properties of cotton gauze was first evaluated, GelMA was then added to the cotton gauze to study if it will alter the strength of the cotton. Anti-bacterial tests were also conducted by mixing curcumin, chitosan and silver NP into the GelMA crosslinked on the cotton gauze. Agar diffusion and broth microdilution assays were performed to determine the efficacy of the various additives in combatting bacterial infections. Two strains of bacteria, *Escherichia coli* and *Staphylococcus aureus* (to model Gram negative and Gram positive bacteria respectively), were used in the tests.

It was found that GelMA hydrogel's can be significantly improved by varying its fabrication parameters as well as adding NP. GelMA was found to have minimal effect on strengthening cotton scaffolds and that cotton can be loaded with GelMA containing antibacterial components to adequate effect.

CONTENTS

FIGURES

ABBREVIATIONS

DPBS	Dulbecco's phosphate buffered solution
ECM	Extracellular matrix
ELISA	Enzyme-linked immunosorbent assay
EPC	Endothelial progenitor cells
GelMA	Gelatin methacryloyl
MA	Methacrylic anhydride
NP	Nano-particles
PCL	Polycaprolactone
PEG	Polyethylene glycol
PI	Photo-initiator
PLGA	Poly(lactic-co-glycolic) acid
PVA	Poly(vinyl alcohol)
SEM	Scanning electron microscope
SDF-1α	Stromal derived factor – 1 alpha
UTS	Ultimate tensile strength
UV	Ultraviolet

CHAPTER I

INTRODUCTION

Chronic health wounds affect more than 2% of the people in the US, costing an estimated 20 billion dollars in health care annually (1). The key characteristics of chronic wounds is that they do not heal quickly and inflammation persists, in addition to impairment of the extracellular matrix (ECM) (2). Chronic wounds can be a result of several diseases and injuries, from diabetes and vascular insufficiency to compromised immunological state and scars from surgeries and burns (3). Due to their high susceptibility to microbial infections, such as *E. coli* and *S. aureus* among others, chronic wounds do not heal as easily and as smoothly as normal wounds (4,5). Conventional healing methods, like skin substitutes and autografts, do not lead to the restoration of the chronic wound to normal homeostasis and functionality and might in severe cases lead to necrosis, sepsis and ultimately death (6).

Diabetes mellitus is a very common congenital disease that affects 450 million people, 8.3% of the world population and impacting a global economic cost of over 600 billion USD in 2014 (7,8). The number of people with diabetes has increased from 80 million in 1980 to 422 million individuals in 2016 and is expected to rise to 552 million by 2030 (9). Diabetes is especially prevalent in the Middle East, with the eastern Mediterranean region showing the highest increase in individuals affected with diabetes from 1980 to 2016 (10). Diabetes modifies the vasculature of a person considerably, affecting angiogenesis and the ability of the body to effectively recuperate from flesh wounds (7). Diabetes is severe and lethal with a high prevalence and many secondary effects that increase its lethality. The world health organization reports that diabetes alone is responsible for killing approximately 3 million people per year (11).

Diabetes mellitus is of two types. Diabetes mellitus 1 is characterized by the pancreas' failure to produce insulin due to an autoimmune problem whereby the body attacks the pancreas' insulin producing β-cells (12). Any person may be affected with this form of diabetes, however type 1 diabetes usually manifests in children and young adults (9). Diabetes mellitus 2 is characterized by insulin resistance, whereby cells do not respond to insulin as expected. Diabetes mellitus 2 may be further complicated with an inability to produce insulin (13). As is the case with type 1 diabetes, the causes of type 2 diabetes are also unclear, however several factors such as ethnicity, family's diabetic history, age and healthy lifestyle, do make a person more prone to diabetes. Diabetes mellitus 2 usually manifests later in life. For diabetes mellitus 1 and 2 life expectancy is reduced on average by 20 years and 10 years respectively (14).

In 1922, insulin was discovered in a dogs pancreas, and this is a milestone in diabetes therapy (15). Insulin has since been used to alleviate the strains of the disease and improve the quality of life of those affected. People inflicted with type 1 diabetes usually require a daily insulin shot. On the other hand, type 2 diabetes patients might not need daily insulin shots if they follow a healthy lifestyle as recommended by doctors.

Dozens of physiological factors act together to diminish the body's wound healing capabilities in diabetic people. Among them is the decreased and impaired production of growth factors (16–18), functionality of macrophages (19), angiogenic response (18,20), accumulation of collagen, functionality of epidermal barrier, granulation tissue quantity, keratinocyte and fibroblast migration and proliferation (18) as well as epidermal nerve ending quantity (21).

In a murine model of diabetes mellitus, the phosphorylation of endothelial nitric oxide synthase in bone marrow is impaired, and this leads to the limitation of endothelial progenitor

cells mobilization from the bone marrow into the blood stream. The study also showed that stromal derived factor-1α is expressed less in epithelial cells and myofibroblasts in diabetic wounds, which also prevents the homing of endothelial progenitor cells (EPCs) to wounds, thus preventing healing and reducing angiogenesis (22).

Since diabetes affects the body's cell signaling pathways that lead to wound healing and closure, in our experiments, we will study the potential of the use of NP to encapsulate and deliver a consistent dose of the growth factor stromal derived factor (SDF-1α) to aid in wound healing. Specifically, we want to study the potential of encapsulating SDF-1α in poly(lactic-co-glycolic) acid (PLGA) NP and imbedding them in a hydrogel which in turn is put on cotton gauze. This whole setup will be the wound healing patch.

The objective of this master's research is to study the potential of using GelMA hydrogel as a medium for drug delivery of growth factors and anti-bacterial constituents. A wound healing patch would be made up of cotton gauze as a scaffold immersed with GelMA. The GelMA would be loaded with growth factors that would aid in the regeneration of vascular and dermal tissues. It will also be loaded with antibacterial components to aid the body in combatting bacterial infections. The potential of using the aforementioned combination as a wound healing patch for patients suffering from chronic wounds was the primary focus.

In this study, GelMA was fabricated with varying concentrations of methacrylic anhydride (MA) and photo-initiator (PI) concentration. The various GelMA hydrogels will be mechanically tested for tensile strength, swelling and degradation properties. Nanoparticles were fabricated and added to GelMA to study their effect on the tensile strength. PLGA NP were also used to encapsulate SDF-1α and the release rate was studied. Cotton gauze was procured its physical and

mechanical properties were characterized. GelMA was added to gauze to study its effect on the tensile properties. Antibacterial studies were conducted by covering the gauze with chitosan, curcumin and silver nanoparticle loaded GelMA. The efficacy of the antibacterial effects were evaluated with agar diffusion tests and broth dilution assays.

CHAPTER II

LITERATURE REVIEW

A. Hydrogels

Hydrogels are three dimensional polymer networks that contain a large amount of water. The fact that hydrogels contain a very large amount of water is one reason why they are very appealing as biomaterial in the engineering of soft tissues. Also, hydrogels are open systems capable of exchanging chemicals, substances and energy between the hydrogel itself and its surrounding (23).

Hydrogels provide an adequate environment for the proliferation of cells in that they give support by their polymer chains for cellular attachment, and a suitable surrounding environment for the exchange of nutrients due to their high water content. These two factors are the major appeal for using hydrogels in regenerative medicine. The use of hydrogels is predominantly used in the tissue engineering of soft tissues as well as investigating cell-material interactions (24,25).

There are many different types of hydrogels. Hydrogels are categorized based on the origin of the polymer chain that forms it, whether natural or synthetic. Natural hydrogels are composed of chains formed from collagen, fibrin, hyaluronic acid and gelatin among many others. Some examples of synthetic hydrogels used today are poly-ethylene-glycol (PEG) and poly vinyl alcohol (PVA). Composite hydrogels use a mixture of synthetic and/or natural polymers (26). Figure 1 is a schematic of a typical hydrogel fabrication method.

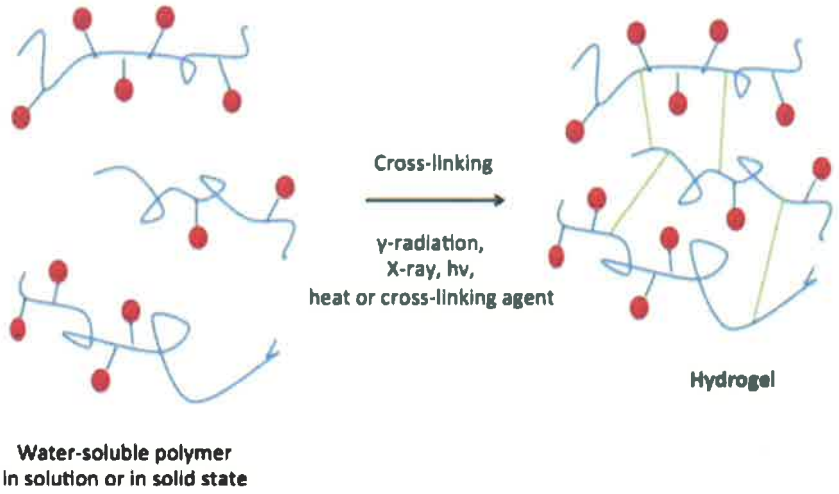

Figure 1 The basic principle of hydrogel fabrication. Polymer chains form links and bonds with each other to form a network that entraps water (27).

Another difference among hydrogels is the method in which they are crosslinked. Crosslinking is when the chains of polymers are exposed to a certain catalyst or reactant that causes the polymers to link together. There are two main methods for crosslinking hydrogels, physical crosslinking and chemical crosslinking. Physical crosslinking is when the hydrogel polymers form bridges and connection points among themselves without the addition of an external chemical agent. The degree of crosslinking depends on the time duration of crosslinking, the temperature at which crosslinking is initiated and sustained, and the concentration of the solvent (28).

Chemical crosslinking requires the addition of a catalyzing agent that would initiate the crosslinking of the polymer, either with itself or with the chemical that is added. In GelMA, chemical crosslinkers can be used to form bonds among gelatin and MA molecules. Also, PIs can be used to the same purpose. The degree of crosslinking would depend on the concentration of the chemical added and the time that it is left to take effect. For the PI method, crosslinking degree depends on the concentration of the PI, the

wavelength/energy in the light source, the time of exposure and the distance from the hydrogel to the light source (29). The various types of crosslinking for physical and chemical crosslinks can be seen in Figure 2. Crosslinking in hydrogels is a key concept and factor in their fabrication. The hydrogel's mechanical strength and structural rigidity are strongly dependent on the degree of crosslinking that as they are directly proportional to the crosslinking degree (28).

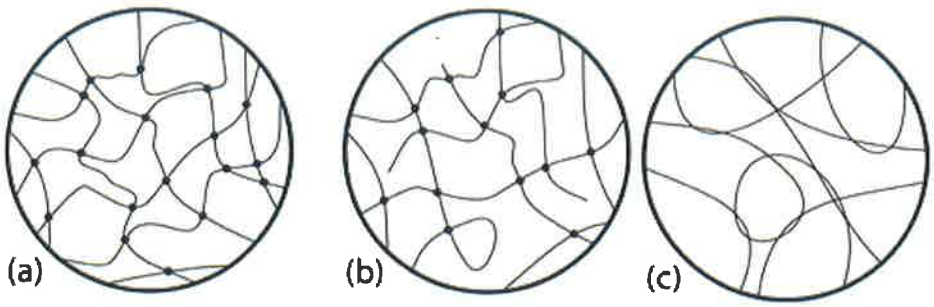

Figure 2 Schematic of different types of crosslinking in hydrogels. (a) an ideal cross-linked network; (b) cross-linked network with molecular ends and loops; (c) a physically entangled network (24).

To fabricate GelMA, gelatin fibers are mixed with MA. The MA reacts with the amine and hydroxyl groups on the gelatin chain, resulting in GelMA macromers. A PI is then added to the mixture and the resulting mixture is exposed to UV light so that crosslinking occurs. This bombardment by UV light results in the generation of free radicals, resulting in methacrylamide and methacrylate side groups on the GelMA chains, which polymerize by radical addition-type polymerization resulting in gelatin chains connected to each other by polymethacryloyl bondings (Figure 3). Different concentrations of GelMA and PI will be mixed and the mechanical properties of the resulting hydrogel will be characterized.

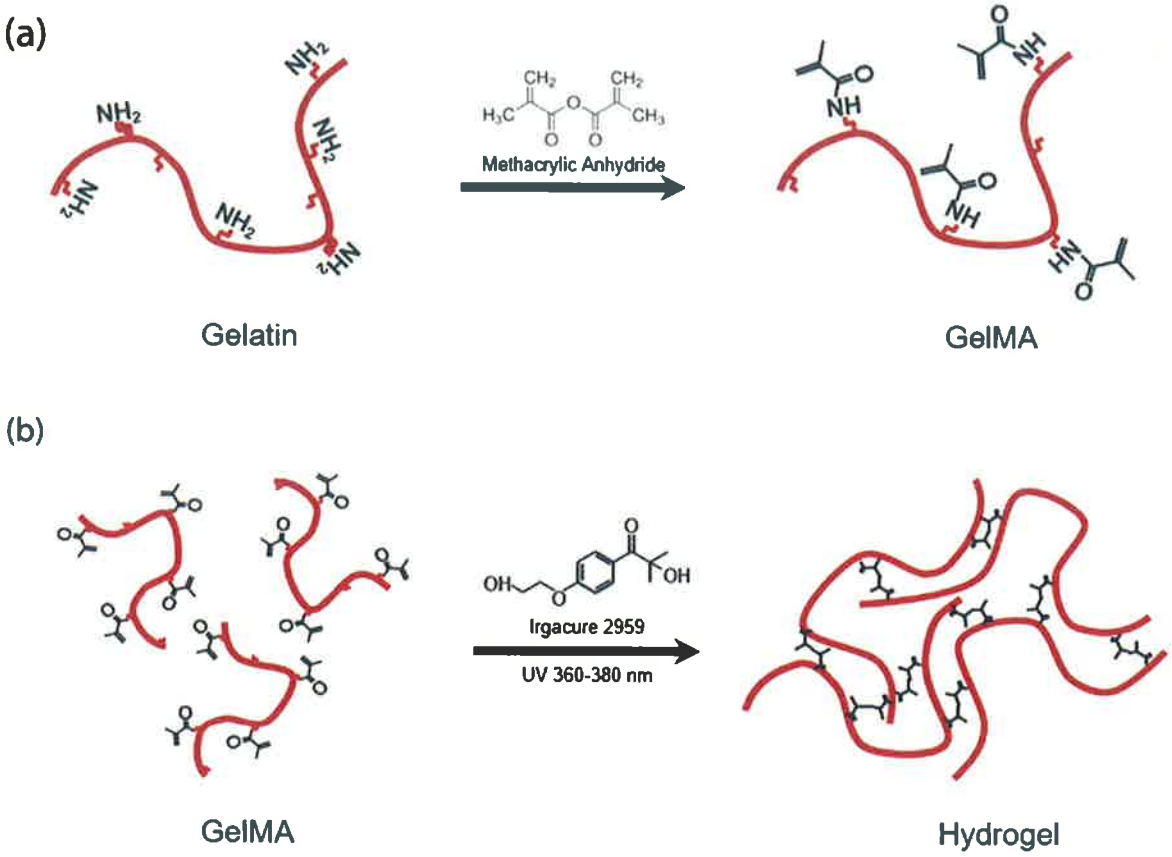

Figure 3 Synthesis of GelMA; (a) Gelatin is reacted with MA so that amine and hydroxyl groups on gelatin are methacrylized; (b) GelMA photo cross-linking with PI and UV radiation to form GelMA hydrogel (30).

B. Nanoparticles

The field of NP has had an increasing impact on the biomedical field, especially with the improvements in manufacturing and the different methods of administrating therapy to patients. NP are defined as solid (usually) spherical structures with dimensions from 1 to 1000 nanometers (31,32). They could be prepared from natural or synthetic materials. Natural materials such as metals (gold and silver) and nonmetals (silica

compounds, carbon, hydroxyapatite), or synthetic materials such as polymers made from acids and other compounds could also be used (33,34).

NP have many diverse applications. They have been used as a mechanism for drug delivery, protein delivery, thermal ablation, radiotherapy, biosensors and biological imaging (31,35). In this research we will study the potential of PLGA NP as carriers of growth factors.

PLGA is a synthetic polymer that has been used extensively in the biomedical field, as well as the nanoparticle domain, since it can be hydrolyzed into its two constituent acids, lactic and glycolic acid, by the human body. The breakdown of the polymers is shown in Figure 4. PLGA compounds used are defined by the ratios of the constituting monomers. For example, PLGA 25:75 denotes a copolymer with a composition of 25% lactic acid and 75% glycolic acid.

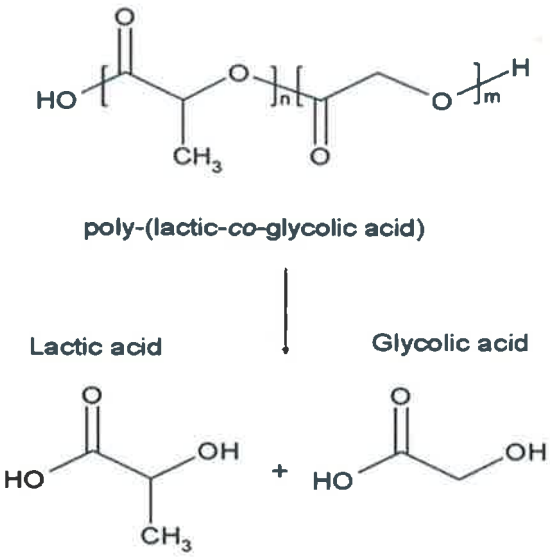

Figure 4 Degradation of PLGA into its constituents, lactic acid and glycolic acid (36).

PLGA has an advantage when proteins are required to be incorporated in tissue engineering and regenerative medicine therapy. PLGA has the advantage of giving the opportunity to control the spatial and temporal release of growth factors in scaffolds as well as their protection (37). Using PLGA to bind proteins to the scaffold relieves doctors from repeatedly injecting growth factors into a lesion, which has an adverse effect on the body's natural healing mechanism (38). PLGA nanoparticle have the ability to protect the protein from degrading as well as deliver the bioactive molecule in a controlled sustainable manner, ensuring that the concentration of the required protein/growth factor/therapeutic drug is actually delivered at the required therapeutic doses, minimizing the requirement of repeated injections. The localized and targeted effect of the bioactive molecules is doubly advantageous in that it would result in a faster recovery time, and also in the fact that some drugs/bioactive molecules while therapeutic at the injury site, may have a negative effect in other healthy non-targeted areas of the body (39,40).

One of the biological obstacles NP face in practice is the fact that hydrophobic particles, like PLGA, are considered to be foreign by the body (41,42). Hydrophobic particles are removed from the blood stream by the reticulo-endothelial system and sent to the liver or spleen. In the blood stream, opsonin proteins bind to the particles which leads to the particles being attached to macrophages, and ultimately their degeneration by phagocytosis (43). One method of circumventing the detection of the particles by the reticulo-endothelial system is to modify the surface of the particles by covering it with hydrophilic material. This can be achieved by using the polymer PEG. Using PEG to conceal the actual hydrophobic nature of the NP increases their half-life in the blood by

several orders of magnitude (43). In this study we used PVA to mask the hydrophobic nature of PLGA.

Another aspect of NP that plays a significant part in their interaction with the body environment and their uptake, is their charge. NP with a positive charge exhibit a larger degree of internalization by cells, due to the attraction to the negatively charged cell membrane of the cells (44,45).

One of the hardships encountered when using NP is achieving a high encapsulation efficiency and a high drug loading efficiency. Encapsulation efficiency is having a high percentage load amount of drug in the NP relative to the total amount of drug used in the formulation. Drug loading efficiency is having a high amount of encapsulated drugs with respect to the total amount of drugs used when fabricating the NP (41).

Another difficulty encountered with the use of many NP is the high burst release of the drug from NP. This phenomenon is also observed in PLGA NP as well as polycaprolactone (PCL) and alginate NP.

C. Cotton fabrics and additives for antibacterial effect

Cotton has been used as a textile fabric since the Neolithic period between 6000 and 5000 BC in the ancient Indus valley civilizations (46,47). Since that time, cotton fabric has been used for clothing and textile fabrics, as well as a wound dressing. To heal the wounds systemic antibiotic administration is employed, as well as saturating cotton pieces with topical ointments and balms. However, over-prescription and misapplication of antibiotics has led to increased drug resistance among bacteria and other pathogenic organisms (48).

Adding natural antimicrobial and antibacterial dyes or additives to cotton fabrics is one option to aid in the fight against drug resistant pathogens and bacteria. Recently,

several studies have concentrated on the use of natural minerals and herbal extracts as antibacterial additives to cotton fabrics. One natural mineral that has been used frequently is silver in the form of NP.

Silver NP are able to penetrate the cell wall as well as be deposited on it. The presence of silver NP on the cell wall affects electrolyte and metabolite transport, interacts with membrane proteins, activates signaling pathways and inhibits cell proliferation (49,50), as well as shrinks the cytoplasm membrane or detaches it from the cell wall (51–53). As a consequence of the cytoplasmic membrane detachment, DNA molecules condense together, losing their ability to replicate (51,54).

Chitosan, a polysaccharide that is manufactured by treating the shells of shrimps and other crustaceans with alkaline substances, has also been studied for anti-microbial and immune enhancing effects. In acidic solvents, the amine groups found in chitosan become a quaternary amino group that inhibit the growth of Gram negative and positive bacteria (55). There are two proposed mechanisms as to the nature of the antibacterial properties of chitosan. One mechanism is that the polycationic nature of chitosan itself interferes with bacterial metabolism by stacking at the surface (55). The second mechanism requires that the chitosan be hydrolyzed to a molecular weight of less than 5000 so that it can penetrate the bacterial cell wall and bind to the DNA and prevent the synthesis of mRNA (56).

Curcumin is a natural herbal extract that is also being studied for antimicrobial and antibacterial effects. Scientifically called diferuloylmethane, it is the major phytochemical found in *Curcuma longa L.* of the Zingiberaceae family, commonly known as turmeric. Curcumin is yellow in color. It has been used to flavor food, dye clothes and as a treatment

for various human maladies (57). Curcumin has been found to have several antimicrobial, antidiabetic, anti-inflammatory, anticancer, and antioxidant properties (58–60).

CHAPTER III

MATERIALS AND METHODS

A. GelMA

There have been many ways reported to prepare gelatin with methacryloyl substitution, however, they are fundamentally the same method as presented by Van Den Bulcke et al. (61). In brief, 10.0 grams of porcine skin type A (Sigma-Aldrich, St. Louis, MO) was added to 100 mL of Dulbecco's phosphate-buffered saline (DPBS) (Invitrogen, San Diego, USA) and mixed by stirring at 60°C using a magnetic stirrer for 1 hour. MA, 4 or 8mL, was added to the gelatin solution. The resulting solution was stirred vigorously at 50°C for 3 hours using a magnetic stirrer. The methacrylation substitution reaction was then stop by performing a 5 fold dilution with 40°C DPBS solution. The new solution was then poured into dialysis membranes with 12-14kD cutoff which were then immersed in distilled water at 40°C. After changing the distilled water twice daily to remove the salts and unreacted MA, the final solution in the membranes was lyophilized, resulting in a porous white foam which was stored at -80°C until further use.

The freeze dried GelMA was then mixed with DPBS in the desired w/v percentage. PI (2-hydroxy-1-(4(hydroxyethoxy)phenyl)-2-methyl-1-propanon)(Igracure 2959, CIBA Chemical, Basel, Switzerland) was then added in 0.1 or 0.2% w/v to the mixture. The degree of methacryloyl substitution, GelMA concentration, PI and the UV power and exposure time allow the tuning of the physical properties of the resulting GelMA hydrogel (28,61).

To prepare specimen for tensile, degradation and swelling tests, the resulting GelMA mixture was pipetted into polydimethylsiloxane (PDMS) molds. The molds were then placed under UV light (360-480 nm) at a power of 7.9 Wcm-2 for 30 seconds (Figure 5).

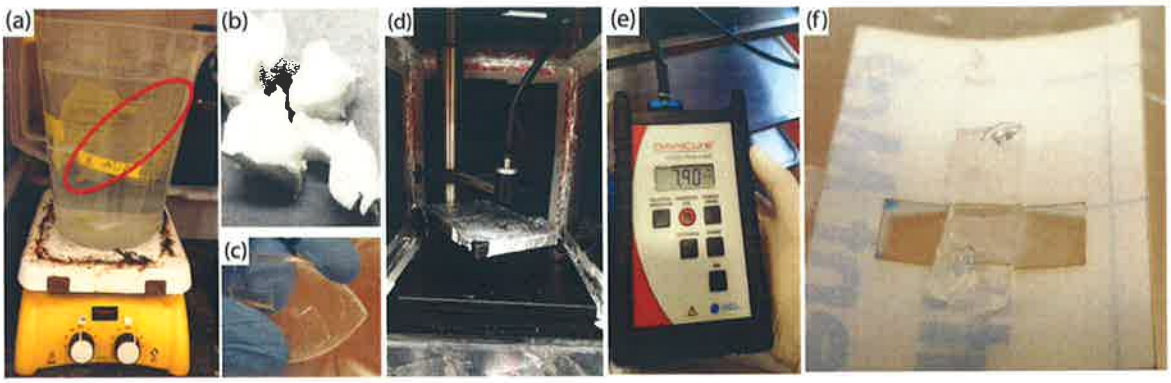

Figure 5 Preparing GelMA hydrogel for tensile testing; (a) Gelatin and MA are mixed then put in a dialysis membrane (circled in red) immersed in distilled water for all residual MA to diffuse out; (b) the solution from the membrane is then freeze dried giving a white foamy material; (c) the GelMA hydrogel solution is then prepared and pipetted into molds; (d) the mold is then placed under a UV lamp; (e) the sample is irradiated with 7.9W/cm² UV rays for 30 seconds; (f) the crosslinked sample is then removed from the mold and prepared for tensile testing.

Mechanical characterization of GelMA followed the general guidelines found in ASTM F2900. Tensile tests were conducted on rectangular samples using an Instron 5542 mechanical tester. The samples were stretched at a rate of 1mm.min-1 at room temperature. The samples were constantly hydrated with DPBS by using a pipette. Various repetitions showed that if left unhydrated, the hydrogel specimen will dry up during the test resulting in incorrect measurments. The constant hydration of the hydrogel also simulates the constant hydration of the wound environmet. The ultimate tensile strength (UTS) is the maximum stress in the speciment reached during the tensile test. The elastic modulus was calcualted as the slope of the linear region of the stress strain curves corresponding to 0%-10% strain (62)(Figure 6).

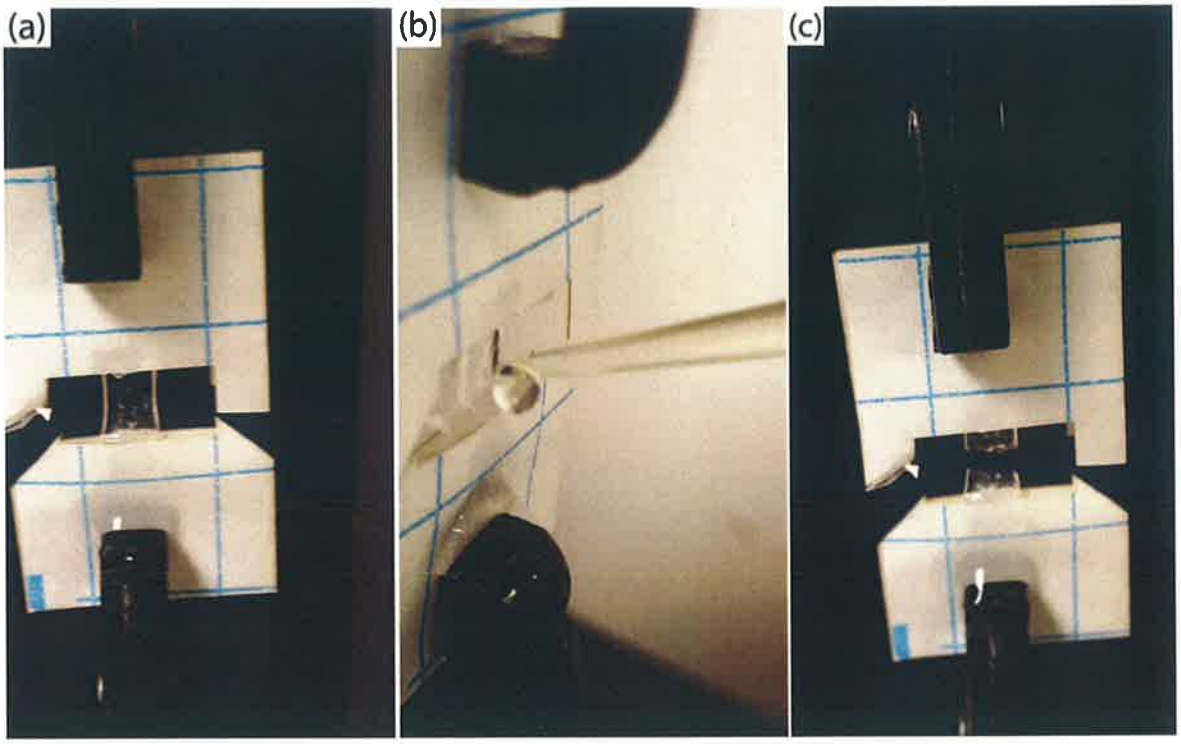

Figure 6 Mechanical testing of GelMA hydrogel; (a) the hydrogel specimen was glued to a laser cut lamination strips with a window and mounted on an Instron machine; (b) during the tensile test a pipette is used to constantly hydrate the GelMA specimen; (c) failure of the GelMA specimen with a clear tear in the middle of the sample.

The swelling ratio was determined by freeze drying cylindrical GelMA samples and measuring their dry weight. The samples were then placed in DPBS solution and an incubator at 37°C. Weight measurements of the sample were taken at 2, 4, 8, 12, 24 and 72 hours. The initial weight is the freeze dried weight of the samples. The swelling ratio is the dry weight subtracted from the measured swolen weight at the time points, then divided by the initial dry weight (63,64).

For degradation studies, cylindrical GelMA samples similar to the ones used for swelling tests were placed in well 24 plates with 500µL of DPBS with 2µg/ml of collagenase type II and placed in an incubator at 37°C. Measurements were taken at 12, 24, 48 and 72 hours. The degradation ratio was measured by subtracting the dry weight at the

measured time from the initial dry weight of the sample. Collagenase type II was added to the DPBS solution to simulate the wound environment (64).

B. Nanoparticles

1. PLGA nanoparticles

PLGA 50:50 lactide: glycolide (Durect Corporation, Birmingham, AL) was fabricated into NPs and loaded with SDF-1α (R&D Systems, Inc). The NPs were prepared by a double-emulsion/solvent-evaporation technique (Figure 7)(60,61). 2.5-3.0% w/v PLGA was dissolved in 2 ml of dichloromethane (DCM). Aliquots containing 1 μg of SDF-1α were dissolved in 100μL of DPBS. The aqueous SDF-1α solution was emulsified into the DCM/PLGA solution using a probe ultra-sonicator (Q500; QSonica LLC, Newtown, CT) for 1 minute on ice at 20% amplitude. The water-in-oil emulsion thus formed was further emulsified into 5ml aqueous 0.25% w/v PVA solution using the probe ultra-sonicator for 1 minute on ice at 20% set amplitude to form a water-in-oil-in-water emulsion. The second emulsion was then stirred using a magnetic stirrer for 16 h at room temperature followed by desiccation for 1 h under vacuum to remove any residual DCM. The NPs thus formed were recovered via 3 washing cycles by ultracentrifugation at 35,000 rpm for 5 min at 4°C. The NPs were lyophilized over 48 h to obtain a dry powder. The NP collected were put in an Eppendorf tube with DPBS and left in an incubator on a rotating shaker. At various timepoints the liquid is collected and washed again. An ELISA (enzyme linked immunoabsorbent assay) test was conducted to measure the growth factors released in each sample collected and a cumulative release of the growth factor SDF-1α was charted.

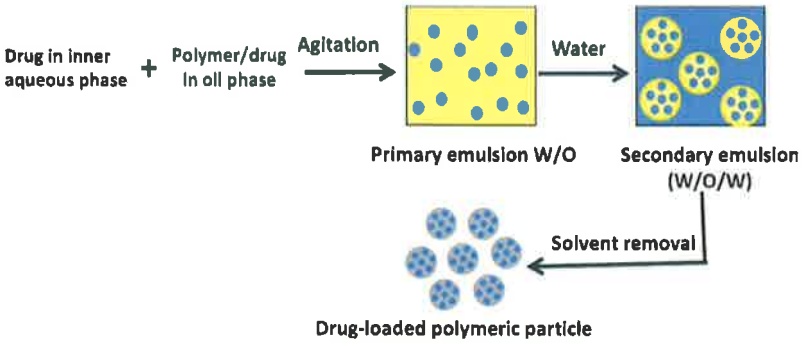

Figure 7 A schematic showing the W/O/W method used to fabricate PLGA-encapsulated SDF-1α molecules *(65)*.

2. *Alginate nanoparticles*

Alginate NP with rhodamine were fabricated to study the effect of alginate NP on the mechanical properties of the GelMA hydrogel in contrast to PLGA NP. First, 4mg of rhodamine B isothiocyanate was added to 4mL of distilled water. A solution of 4% w/v sodium alginate was prepared. 300 µL of the alginate solution was mixed with 100 µL of the Rhodamine solution. Another solution consisting of 4mL corn oil, and 400 µL of SPAN80 solution was prepared. The alginate/rhodamine solution was then added to the corn oil/SPAN80 solution and agitated to obtain a proper emulsion. The solution was then stirred for 15 minutes at 400rpm.

Separately, 200mg of calcium chloride was added to 5 mL of distilled water. From the new solution, 2mL were taken and added to 200 µL of TWEEN20. From this CaCl2/TWEEN20 solution, 1mL was added dropwise to the stirrring alginate/rhodamine/SPAN80 solution and left to stir for 15 minutes. Then, another 1 mL of the CaCl2/TWEEN20 solution was added to the stirring solution and left again to stir for 15

minutes. Another 2mL of CaCl2 solution was then added to the stirring solution, which was left to stir for 15 minutes (66).

The resulting soluution was then centrifuged at 2500 rpm until sedimentation was observed. Proper sedimentation is observed when one can observe an oil layer on top, a TWEEN20 layer below it, CaCl2 solution, and particles at the bottom. The oil, TWEEN20 and CaCl2 layers are removed, and what remains are the alginate NP (Figure 8). The alginate NP are resuspended in 500 μL of water and stored at -20°C until use. When intended for use, the suspended alginate NP were freeze-dried.

Figure 8 Fabricated alginate nanoparticles. The layers of CaCl2 and Tween 20 layers are still evident while the alginate particles forma sediment at the bottom of the tube.

C. Cotton testing

Cotton gauze (Compresses nonsterile gauze, BM Com Press) was procured from a local pharmacy. The gauze pads had dimensions of 7.5cm x 7.5 cm. The gauze was tested for thickness using MIRA3 LMU (TESCAN) and porosity measurements were taken using a capillary flow porometer (CFP-1100AH, Porous Materials Inc.).

For tensile testing (Figure 9), the cotton gauze was cut into strips of length 7.5cm and width 2.5cm. The strip was mounted on an Instron 5542 machine and pulled at a rate of 1 $mm.min^{-1}$. The elastic modulus was measured as the slope of the linear region of the stress strain curve over a strain of 0-10%. The UTS was recorded. The cotton gauze was then soaked with GelMA and crosslinked. The strips were then tested for any change in their mechanical properties in the same manner as described previously.

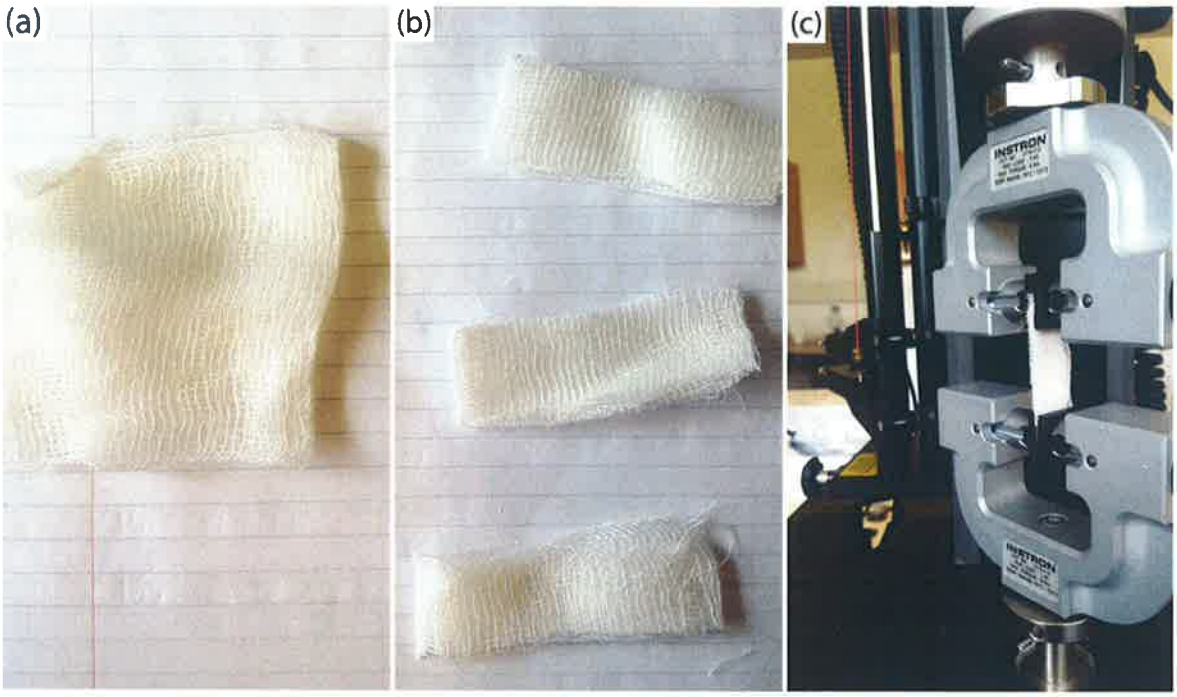

Figure 9 Cotton gauze tensile testing; (a) cotton gauze as procured from the pharmacy; (b) the cotton gauze is cut into strips of 2.5x7.5cm; (c) the c specimen is mounted onto the Instron machine and tested.

The antibacterial properties of cotton with GelMA and additives was evaluated with 2 tests, the agar diffusion test and the broth micro-dilution assay. For these tests, 10% GelMA was prepared after adding 8mL of MA, and 0.2 w/v% PI. Several GelMA solutions were prepared with curcumin, chitosan and silver NP as additives. Curcumin and chitosan were added to the GelMA-DPBS solution. In the case of silver NP (PL-Ag-S10-10mg, PlasmaChem, Germany), the NP were in a colloidal solution and so the GelMA foam and PI were directly added to the silver nanoparticle solution.

For curcumin and chitosan, 2 concentration of the additives were evaluated antibacterial activity, 100µg/ml and 200µg/ml. The silver NP were evaluated at 100µg/ml. The GelMA solution with both curcumin and chitosan was also prepared to evaluate if there is any synergy when both additives are combined with a concentration of 100µg/ml each.

The effect of the additives were evaluated against *E. coli* and *S. aureus* as models of Gram negative and Gram positive bacteria respectively.

Agar diffusion tests in accordance with AATCC 100-1099 were performed. Petri dishes were prepared with lysogeny broth (LB) agar (01-358-500, Scharlau, Spain). The *E. coli* and *S. aureus* bacteria were grown in separate LB broths (2-384-500, Scharlau, Spain) overnight. When the growth of the bacteria reached an optical density measurement at 600nm (OD600) of 0.6, 25μL were then pipetted onto the agar plate and spread using a bacterial loop. The cotton gauze was cut into squares of 0.5cm x 0.5cm. 60μL of the several GelMA solutions were pipetted onto the square specimen ensuring total coverage and then crosslinked with UV rays. For control, plain cotton specimen immersed with DPBS were used. The efficacy of the additives was evaluated against the antibiotic ampicillin by covering the square cotton specimen with 60μL of antibiotic at the concentration of 100μg/ml. The cotton specimen were then placed face down onto the agar plate. The plates were then placed upside down in an incubator at 37°C overnight. The next day, the plates were examined to see if there was a hindrance in the growth of bacteria around the various cotton specimen. A larger area of hindered growth is taken as an indication of a stronger efficacy against the bacteria. This test however is only a qualitative method of evaluating the potency and efficacy of the additives against the two species of bacteria used in the study.

For the broth micro-dilution assay, cotton specimens were prepared in a similar manner to the agar diffusion test. Individual specimen were placed into wells of a 24-well plate, and 0.5mL of bacteria broth with an OD_{600} of 0.6 were added to each well. The 24 well plate was placed in an incubator at 37°C for 24 hours. The broths from the well plates

were then diluted 10,000 times. 25μL of the diluted broth was then pipetted onto an agar plate and spread over the surface with a bacterial loop. The agar plates were then placed upside down in an incubator at 37°C for 24 hours. The plates were later removed and counted for colony forming units.

For all bacterial tests, the following specimens were tested: plain cotton (immersed in DPBS), cotton with GelMA (10% GelMA with 0.2% w/v PI), cotton with GelMA and curcumin additive (10% GelMA with 0.2% w/v PI, 100μg/mL and 200 μg/mL of curcumin), cotton with GelMA and chitosan additive (10% GelMA with 0.2% w/v PI, 100μg/mL and 200 μg/mL of chitosan), cotton with GelMA and silver NP (10% GelMA with 0.2% w/v PI 100μg/mL of silver NP), cotton with GelMA and curcumin and chitosan additives (10% GelMA with 0.2% w/v PI, 100μg/mL of curcumin and chitosan), and cotton with ampicillin (100μg/mL).

CHAPTER IV

RESULTS AND DISCUSSION

A. GelMA

1. Tensile tests

Two batches of GelMA containing 4 and 8 mL of MA were mixed with DPBS to prepare solutions containing 5, 7 and 10% GelMA. PI was added to each solution to have solutions with 0.1 and 0.2% (w/v) of PI. The results obtained show that the UTS and the elastic modulus increase as the amount of the GelMA concentration increases, and the PI concentration increases (Figures 10 and 11). This is due to the increase in the number of crosslinks present in the hydrogel. This result is in agreement with previous published work (64). We should also have seen that the amount of MA added in the initial mixing of the GelMA solution also increases the tensile strength. For the 5 and 7% GelMA solutions, that was not observed, however it did hold true for the 10% GelMA mixtures.

For GelMA mixtures with 4mL MA, the UTS of 5, 7 and 10% GelMA solutions with 0.1% PI were 1.41, 2.94 and 4.65 kPa respectively. When the PI concentration was raised to 0.2%, the resulting UTS were 2.31, 2.99 and 7.19 kPa for the same respective concentrations. This shows that the increase in PI resulted in changes of +63.83, +1.7, and +54.62% for each GelMA concentration. The elastic moduli were 1.75, 4.68 and 6.38 kPa for the hydrogel mixtures with only 0.1% PI, and 2.07, 4.3 and 12.23 kPa for mixtures with 0.2% PI. This means there was a change of +18.3, -8.12, and +91.7% in the elastic moduli. For the elastic modulus however, there was a general trend that as amount of MA added,

GelMA concentration and PI concentration were directly proportional to the elastic modulus.

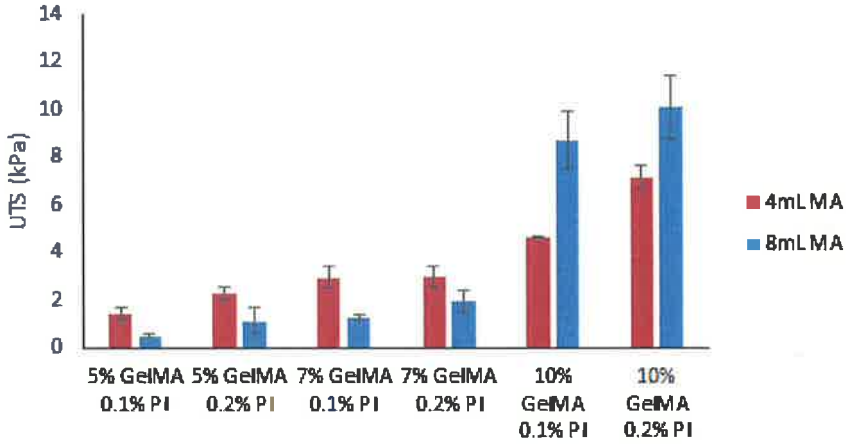

Figure 10 Effect of GelMA weight concentration, PI weight concentration and volume of MA during fabrication on UTS.

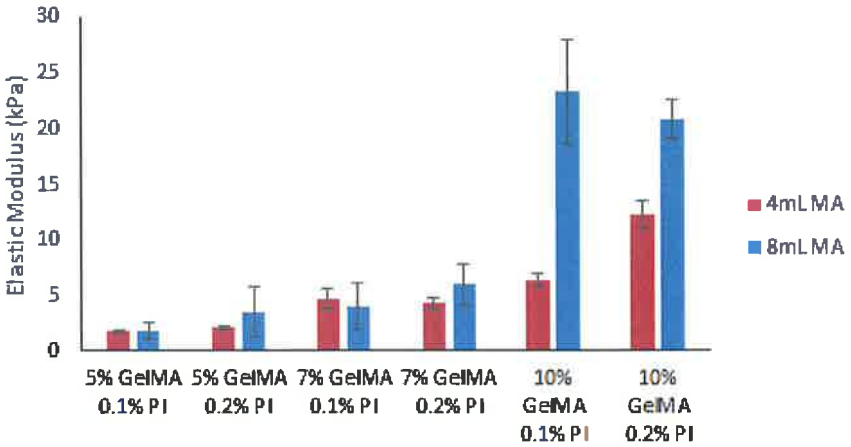

Figure 11 Effect of GelMA weight concentration, PI weight concentration and volume of MA during fabrication on elastic modulus.

PLGA NP were added to GelMA hydrogels. This resulted in the decrease in UTS from 1.95 kPa for plain GelMA with 7% GelMA and 0.2% PI to 2.00, 2.12 and 1.74 kPa after adding 0.1, 1 and 2 mg/mL PLGA NP. This represents a change of +2.56, +8.72 and -

10.77%. The elastic modulus decreased from 5.97 kPa to 3.53, 4.60 and 3.95 kPa, a decrease of 40.9, 23.0 and 33.8% (Figures 12 and 13).

The drop in the mechanical properties is expected due to the fact that PLGA is a hydrophobic particles added to a hydrophilic system. GelMA and PLGA do not have enthalpic interaction whereby bonds may be formed to strengthen the system. There is an increase in the entropic interaction causing a higher free volume in the system, ultimately resulting in a drop in mechanical properties.

Alginate nanoparticle solution of 0.3mg/mL solution was also added to GelMA (7% GelMA 0.2% PI solution, 4mL MA). Alginate is a hydrophilic compound, unlike PLGA. This leads to stronger bonds between the hydrogel and alginate, thus reinforcing the hydrogels strength. There is an increase in the UTS and the elastic modulus. The alginate particles improved the UTS from 2.99 to 3.43 kPa, and the elastic modulus from 4.30 to 4.97 kPa. This is an improvement of 14.7 and 15.6% in the UTS and elastic modulus respectively. Previous studies have shown that adding alginate to GelMA improves the mechanical properties of GelMA making it stronger (67).

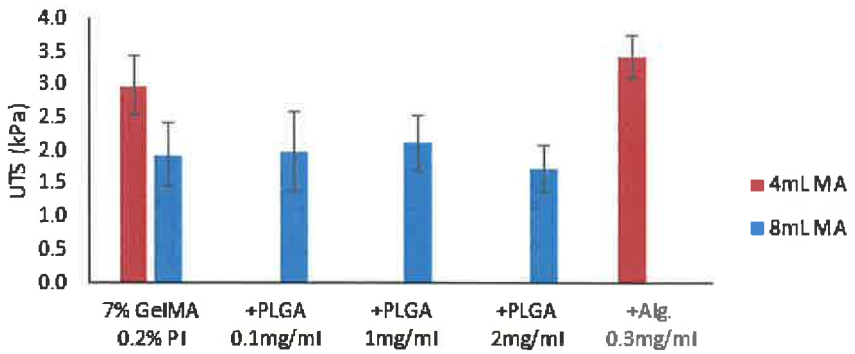

Figure 12 Effect of adding different concentrations of PLGA and alginate NPs to 7% GelMA and 0.2% PI on UTS.

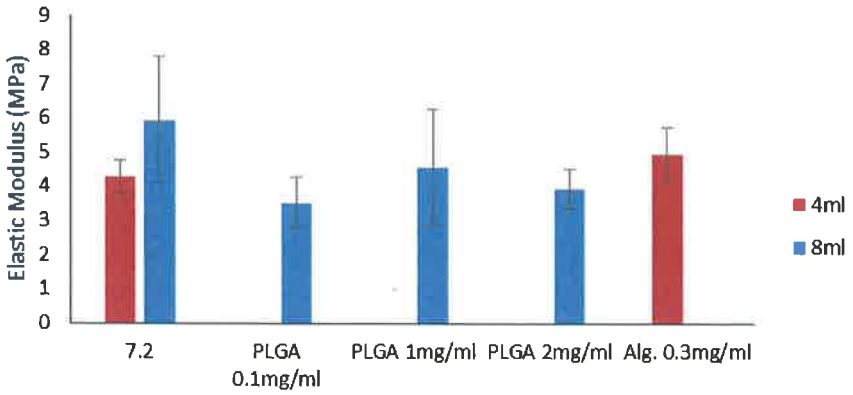

Figure 13 Effect of adding different concentrations of PLGA and alginate NPs to 7% GelMA and 0.2% PI on elastic modulus.

2. *Swelling and degradation*

The swelling and degradation tests were performed on cylindrical samples of GelMA with a diameter of 1cm and a height of 0.5cm. Swelling measurements were taken for GelMA that was mixed with 8mL of MA. The GelMA compositions tested were 5, 7 and 10% w/v GelMA and 0.2 w/v% PI. Measurements were taken at 2, 4, 8, 12, 24 and 72 hours. For the 5 and 7% GelMA hydrogels, there wasn't a significant increase in swelling ratio after 2 hours. This is a sign that most of the swelling occurs directly after immersing in water. For the 10% GelMA hydrogel, the swelling ratio increased 59%. However, we also observe that significant swelling occurred immediately after immersion in DPBS (Figure 14).

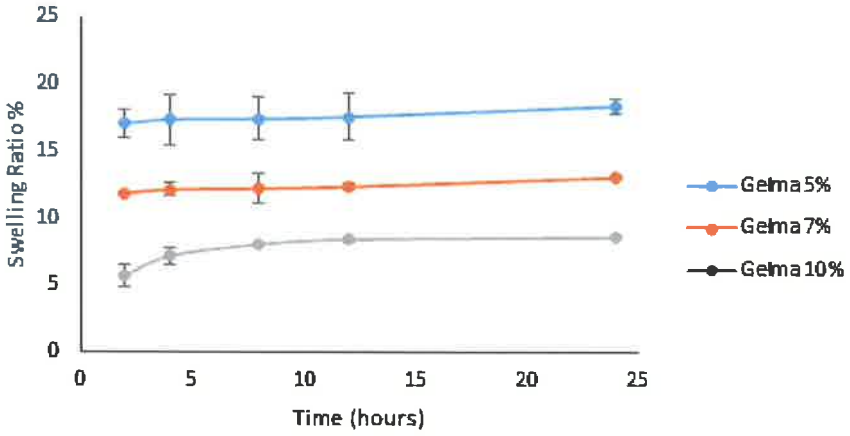

Figure 14 Swelling ratios of various GelMA concentrations with 0.2% PI.

Degradation measurements showed that as the weight percentage of GelMA increases, the degradation time increases. The time for 5% GelMA to degrade is significantly less than that of 10% GelMA. The 5% GelMA degraded within 1 day, while the 7% GelMA almost fully degraded by the end of the first day. The 10% GelMA did not degrade completely until after the third day (Figure 15).

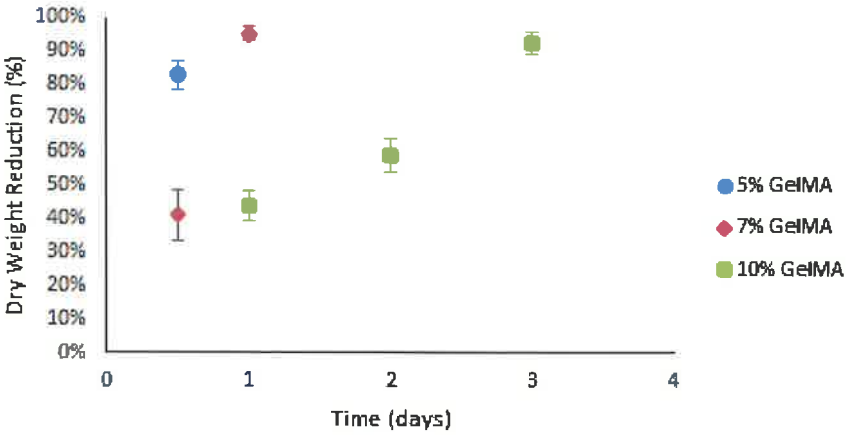

Figure 15 Degradation of various concentrations of GelMA hydrogel with 0.2% PI.

The results show that the lower the weight percentage of GelMA, the higher the swelling ratio. This is due to the higher crosslinking density found in GelMA hydrogel with higher GelMA concentrations which prevents and reduces the rate of water penetration.

The slower degradation rate is probably due to two factors. The first is that the higher concentration of GelMA will cause more crosslinks to have formed in the specimen. This will slow the penetration of the degradation solution from penetrating the specimen. The second factor, is that the higher concentration of GelMA means that there is more gelatin to degrade thereby taking more time to completely degrade.

B. PLGA nanoparticles and SDF-1α release

PLGA NPs were fabricated in the manner described previously. The resulting particles were observed under SEM to study their size distribution. The SEM images showed 72% of the fabricated particles were less than 2μm in size, and 41% were less than 1μm. The minimum particle was 185nm in diameter, and the maximum particle size was 19.4 μm in diameter. The average particle size was found to be 2.74 μm, with a standard deviation of 3.867. Figure 16 showing the distribution of the particle sizes and Figure 17 shows an SEM image of the fabricated NPs.

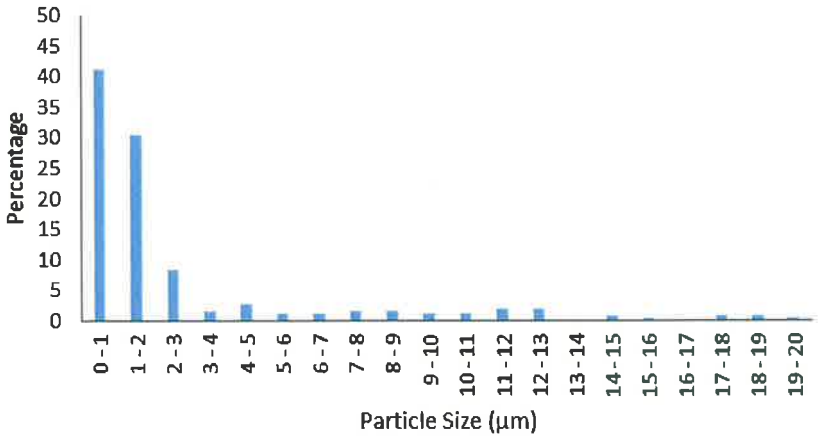

Figure 16 Size distribution of the fabricated PLGA NPs.

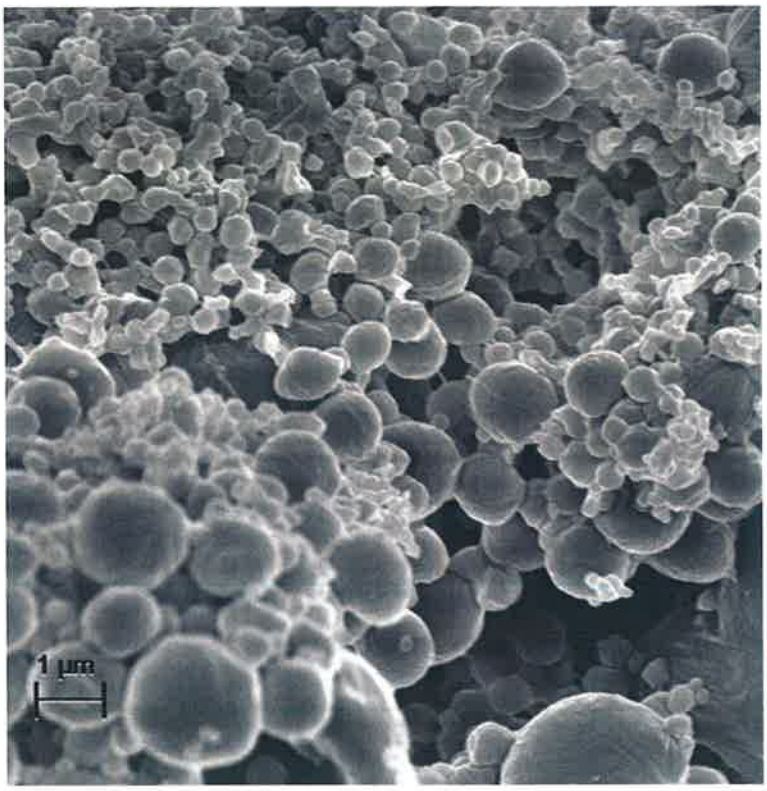

Figure 17 SEM picture taken of the fabricated PLGA NPs.

PLGA NPs loaded with the cytokine SDF-1α were immersed in DPBS and placed in an incubator on a shaker. DPBS was collected at various time points and the amount of SDF-1α in the DPBS was measured using an ELISA kit assay. The result showed a quick release whereby almost 25 ng of SDF-1α was released in the first 12 hours. The release became slower, releasing only around 10 ng more over the following 9 days (Figure 18). Ideally, in the case of chronic wounds, we desire a controlled and sustained release of the growth factor. A quick release of the SDF-1α would definitely lead to the signaling for EPC and other cells to be drawn to the wound site for tissue regeneration, however the excess release would mean that the homing mechanism that is achieved by the SDF-1α will be diminished significantly after a day or two. This would require the change of the wound dressing which would make the patient susceptible to new infections. Other polymeric NP could be used to achieve a quicker or even slower release. PCL has a slower release rate and a higher encapsulation efficiency than PLGA. A blend of PCL/PLGA NP can be used to attenuate the release of growth factors for a sustained and prolonged release as a wound dressing (68).

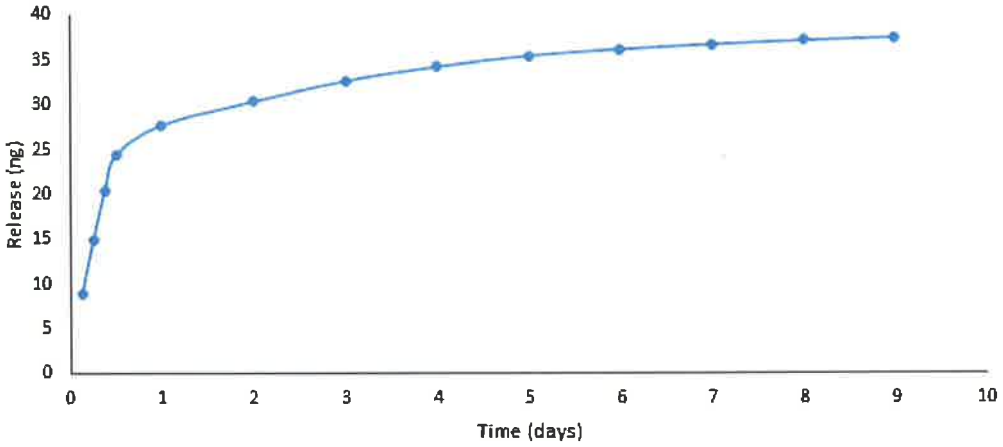

Figure 18 Cumulative release of SDF-1α from the PLGA NP in ng.

C. Cotton testing results

1. Cotton thickness and porosity

The cotton gauze thickness was measured. A total of 25 thickness measurements were taken to give an average of 1.15 mm, with a standard deviation of 0.03mm. Porosity measurements were also taken using the liquid Silwick (Porous Materials Inc., USA). The cotton gauze is 8 ply, and the measurements taken are when the gauze is placed as is in the machine. The average pore size is found to be 790 μm, with a range of pore sizes measured between 669 and 910 μm.

2. Cotton tensile tests

As described before, the cotton gauze was cut into strips 7.5 cm long and 2.5 cm wide. Tensile tests were done with plain cotton strips, and cottons strips covered with a GelMA mixture containing 10% GelMA and 0.2% PI. This was the concentrations at which the highest UTS and elastic modulus was measure for plain GelMA. GelMA with 4 mL and 8 mL of MA were used. The UTS of the various strips was 13.15, 12.84 and 14.33 MPa for

plain cotton, cotton with 4 mL GelMA and 8 mL GelMA respectively (Figure 19). This represents a change of -2.36% and +8.97% change with the different GelMA mixtures. The elastic modulus however, showed a consistent decline as the amount of MA increased. The measured elastic moduli were 161.6, 139.1 and 123.7 MPa for plain cotton, cotton with 4 mL GelMA and 8 mL GelMA respectively, showing a decline of 13.9 and 23.5% (Figure 20).

The decrease in the mechanical properties may be attributed to the wetting of the cotton fabric, as well as the damage it sustained under UV radiation while crosslinking the hydrogel. The increase in UTS however with the 8 mL MA hydrogel is probably due to the stronger GelMA bonds that form after crosslinking with cotton fibers. Previous research has shown that UV exposure may damage cotton and reduces its strength (69).

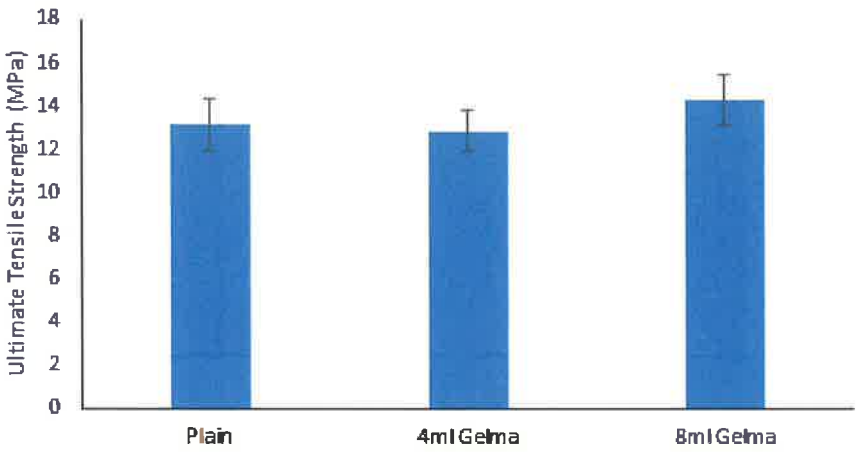

Figure 19 The effect of adding GelMA (10% w/v with 0.2% w/v PI) fabricated with 4 and 8 mL of MA on the UTS of cotton. The increase in the MA used to fabricate the GelMA does not show a significant effect on the UTS of cotton.

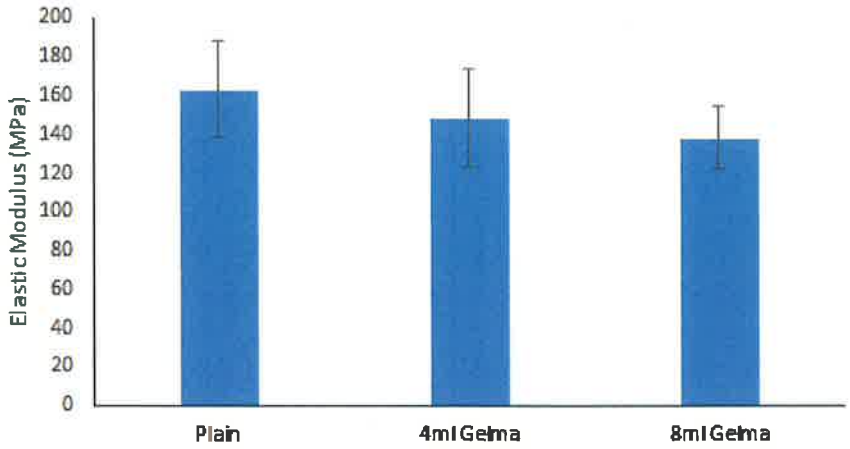

Figure 20 The effect of adding GelMA (10% w/v with 0.2% w/v PI) fabricated with 4 and 8 mL of MA on the elastic modulus of cotton. The graph shows a consistent decrease in the elastic modulus as the MA used in the fabrication of the GelMA increased.

3. Bacterial tests

As described previously, agar diffusion and broth micro-dilution assays were conducted using cotton covered with GelMA loaded with curcumin, chitosan and silver NPs. The effect of the additives was compared to the antibiotic ampicillin.

In the agar diffusion tests with *E. coli*, there was a clear halo around the cotton saturated with ampicillin, which is expected. There were lesser halos around the other cotton pieces which were loaded with GelMA and additives. Agar tests on *S. aureus* showed a significant halo around the ampicillin as expected, however there was minimal effect on the other cotton specimens (Figure 21).

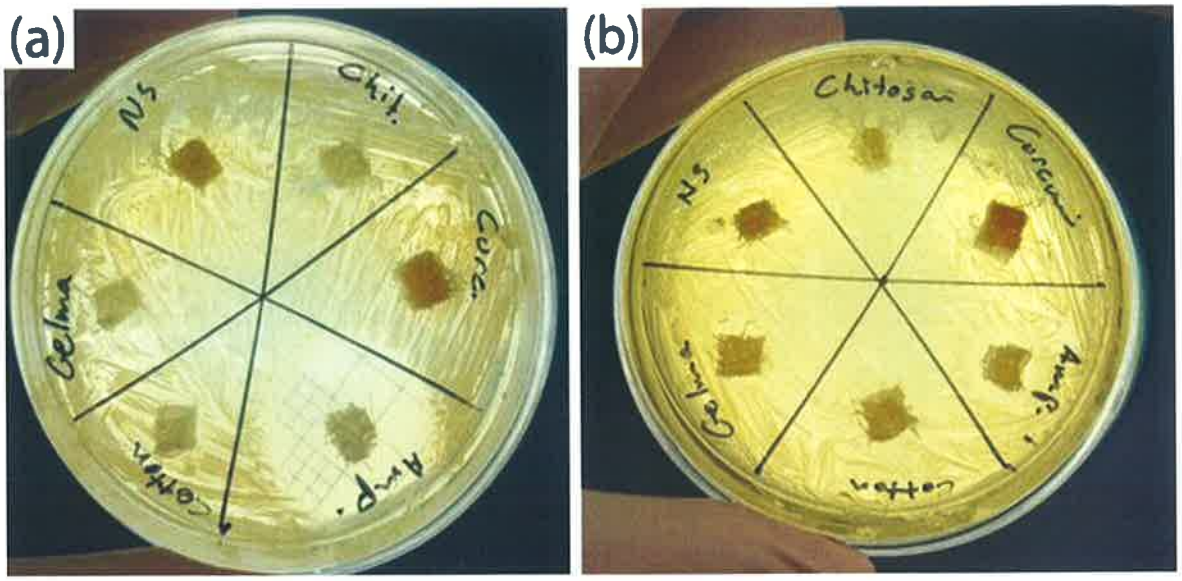

Figure 21 Agar diffusion test of (a) S. aureus *and (b)* E. coli. *In both species of bacteria, the most significant effect was observed for Ampicillin, while all other specimen showed minimal effect.*

In the broth microdilution assay test, there was a more clear and measurable effect of the additives on the bacteria (Figure 22). The tests are compared to the specimen of cotton immersed with DPBS and not containing any additives. The tests done on *E. coli* showed that cotton loaded only with GelMA reduced the number of bacteria by 9.56%. The adding of curcumin, chitosan and silver nanoparticles resulted in a reduction of 33.5, 49.7 and 75.4% of colony forming units. Ampicillin however is still most effective in that it killed 87.5% of the colony forming units.

The tests conducted on *S. aureus* showed that GelMA reduced the number of colony forming units by 28.3%. The addition of curcumin, chitosan and silver nanoparticles reduced the number of colony forming units by 32.6, 42.7, and 74.6%. Ampicillin was still most effective, reducing the colony forming unit number by 95.3%.

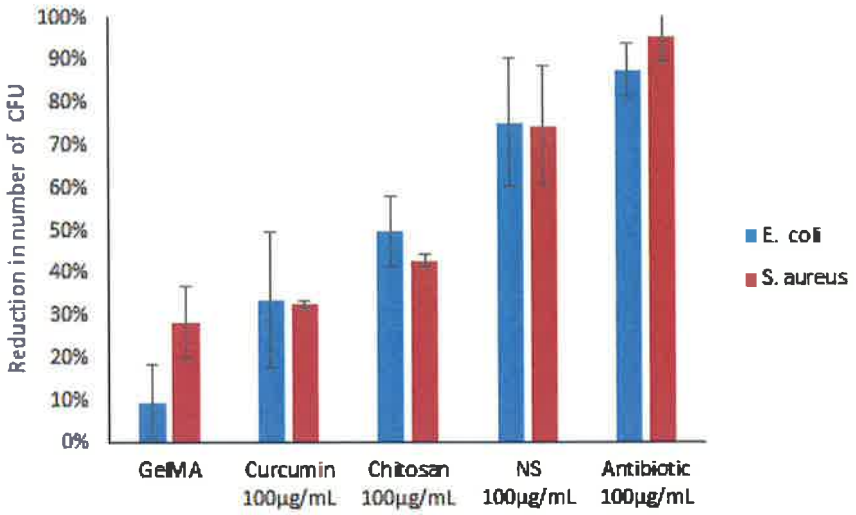

Figure 22 Broth microdilution assay results showing the percentage reduction in colony forming units compared to plain cotton for various GelMA compositions for E. coli *and* S. aureus.

Further tests were conducted to measure the effect of increasing curcumin and chitosan concentrations in the GelMA (Figure 23). The results showed that there is a significant improvement in antibacterial properties when the concentration was doubled from 100 µg/mL to 200 µg/mL. New batches of *E. coli* and *S. aureus* were prepared. These batches showed considerable resistance to the additives contrary to the batches prepared for the previous broth microdilution assays. The reduction in *E. coli* increased from 4.7% to 27.6%, and the reduction of *S. aureus* increased from 2.6% to 22.3%, when the concentration of curcumin was doubled. For chitosan, the reduction in *E. coli* increased from 11.5% to 52.2%, and the reduction of *S. aureus* increased from 5.7% to 19.5%. A GelMA solution containing both curcumin and chitosan at 100 µg/mL each was also tested. The result showed that curcumin and chitosan had a synergistic effect that was clearer against *E. coli* than *S. aureus*. The reduction in colony forming units with the combined solution was 25.3% and 9.0% for *E. coli* and *S. aureus* respectively.

The broth microdilution assays proved that curcumin, chitosan and silver NP have measurable and significant antibacterial capabilities. Their concentration plays a significant part in their efficiency. As their concentrations increase, their bacterial lethality also increased. This is in agreement with literature (49,70,71).

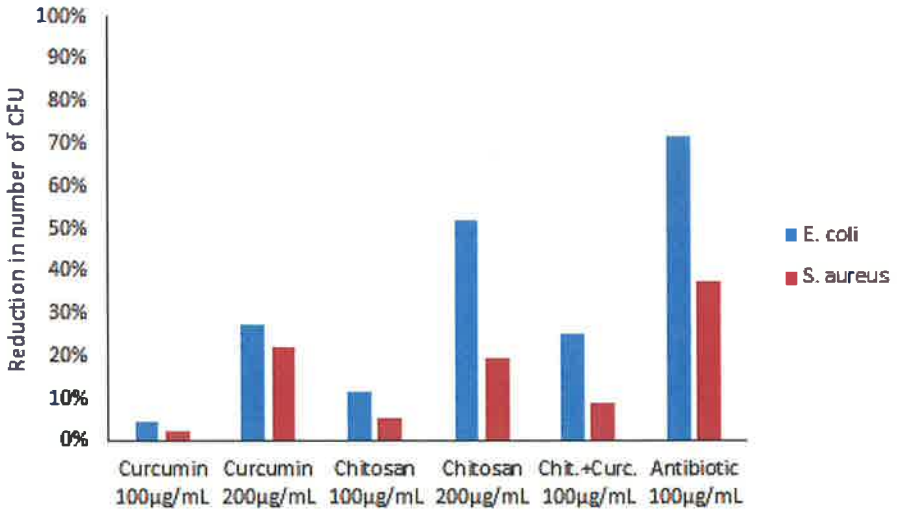

Figure 23 Broth microdilution assay results showing the percentage reduction in number of colony forming units in comparison to plain cotton when investigating the effect of doubling the concentration of curcumin and chitosan.

There is a discrepancy between the results of the agar diffusion test and the broth microdilution assay. In the agar tests, the results show minimum effect of the various GelMA specimens on both bacterial species, while the broth micro-dilution assays do show a significant decrease in the number of colony forming units. The probable explanation to this discrepancy is in the different conditions of the cotton specimens in each type of test. For the agar diffusion, only one side of the cotton is in touch with the agar. The degradation of the GelMA hydrogel will only occur on that interface. Also, in the agar diffusion assay, the cotton specimens are hanging upside down, attached to the agar on side. The diffusion of the antibacterial additives is reduced since gravity does not aid their diffusion into the

agar. On the other hand, in the broth microdilution test, the cotton is completely immersed in the broth, this encourages the degradation of the GelMA and release of the additives into the broth.

CHAPTER V

CONCLUSIONS

In conclusion, GelMA is a hydrogel with tunable properties. Its mechanical properties can be modified by varying the MA during its fabrication, or by varying the concentration of the GelMA itself and PI used in mixing the final hydrogel solution. The GelMA also has potential in being a medium for drug delivery using NPs encapsulating various drugs and growth factors, or as a medium for delivering antibacterial components such as curcumin, chitosan and silver NPs.

PLGA particles show a very quick release of SDF-1α in the first few hours and a very slow release in the following days. This will require the fabrication of a GelMA with a high GelMA and PI content so that it lasts for 4 weeks and more to sustain such a long release. The addition of alginate NPs could be used to counter the effect of PLGA NPs on the mechanical properties of GelMA.

Incorporating techniques investigated, a GelMA hydrogel of satisfactory mechanical strength and degradation properties, and using NPs to encapsulate growth factors, and loading the hydrogel with antibiotics or antibacterial components, GelMA has potential as a dressing for chronic wounds.

BIBLIOGRAPHY

1. Frykberg RG, Banks J. Challenges in the Treatment of Chronic Wounds. *Adv Wound Care* (2015) **4**:560–582. doi:10.1089/wound.2015.0635

2. Humphreys G, Lee GL, Percival SL, McBain AJ. Combinatorial activities of ionic silver and sodium hexametaphosphate against microorganisms associated with chronic wounds. *J Antimicrob Chemother* (2011) **66**:2556–2561. doi:10.1093/jac/dkr350

3. Werdin F, Tenenhaus M, Rennekampff H-O. Chronic wound care. *Lancet Lond Engl* (2008) **372**:1860–1862. doi:10.1016/S0140-6736(08)61793-6

4. Veiga AS, Schneider JP. Antimicrobial hydrogels for the treatment of infection. *Biopolymers* (2013) **100**:637–644. doi:10.1002/bip.22412

5. Edwards R, Harding KG. Bacteria and wound healing. *Curr Opin Infect Dis* (2004) **17**:91–96.

6. Ng VWL, Chan JMW, Sardon H, Ono RJ, García JM, Yang YY, Hedrick JL. Antimicrobial hydrogels: a new weapon in the arsenal against multidrug-resistant infections. *Adv Drug Deliv Rev* (2014) **78**:46–62. doi:10.1016/j.addr.2014.10.028

7. International Diabetes Federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation (2015).

8. Shi Y, Hu FB. The global implications of diabetes and cancer. *Lancet Lond Engl* (2014) **383**:1947–1948. doi:10.1016/S0140-6736(14)60886-2

9. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract* (2011) **94**:311–321. doi:10.1016/j.diabres.2011.10.029

10. WHO | *Global report on diabetes*. *WHO* (2016) Available at: http://www.who.int/diabetes/global-report/en/ [Accessed January 25, 2017]

11. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* (2004) **27**:1047–1053.

12. Rother KI. Diabetes Treatment — Bridging the Divide. *N Engl J Med* (2007) **356**:1499–1501. doi:10.1056/NEJMp078030

13. Tripathy B ed. *RSSDI Textbook of Diabetes Mellitus*. 2nd Edition. Jaypee Brothers Medical Publsihers (2012).

14. Gowthamarajan K, Kulkarni GT. Oral Insulin—Fact or Fiction? *Resonance* (2003) **8**:38–46. doi:10.1007/BF02867128

15. Banting FG, Best CH. The internal secretion of the pancreas. 1922. *Indian J Med Res* (2007) **125**:251–266.

16. Galkowska H, Wojewodzka U, Olszewski WL. Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers. *Wound Repair Regen Off Publ Wound Heal Soc Eur Tissue Repair Soc* (2006) **14**:558–565. doi:10.1111/j.1743-6109.2006.00155.x

17. Goren I, Müller E, Pfeilschifter J, Frank S. Severely Impaired Insulin Signaling in Chronic Wounds of Diabetic ob/ob Mice. *Am J Pathol* (2006) **168**:765–777. doi:10.2353/ajpath.2006.050293

18. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet Lond Engl* (2005) **366**:1736–1743. doi:10.1016/S0140-6736(05)67700-8

19. Maruyama K, Asai J, Ii M, Thorne T, Losordo DW, D'Amore PA. Decreased Macrophage Number and Activation Lead to Reduced Lymphatic Vessel Formation and Contribute to Impaired Diabetic Wound Healing. *Am J Pathol* (2007) **170**:1178–1191. doi:10.2353/ajpath.2007.060018

20. Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, Bunting S, Steinmetz HG, Gurtner GC. Topical Vascular Endothelial Growth Factor Accelerates Diabetic Wound Healing through Increased Angiogenesis and by Mobilizing and Recruiting Bone Marrow-Derived Cells. *Am J Pathol* (2004) **164**:1935–1947.

21. Gibran NS, Jang YC, Isik FF, Greenhalgh DG, Muffley LA, Underwood RA, Usui ML, Larsen J, Smith DG, Bunnett N, et al. Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. *J Surg Res* (2002) **108**:122–128.

22. Gallagher KA, Liu Z-J, Xiao M, Chen H, Goldstein LJ, Buerk DG, Nedeau A, Thom SR, Velazquez OC. Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1α. *J Clin Invest* (2007) **117**:1249–1259. doi:10.1172/JCI29710

23. Kamata H, Li X, Chung U-I, Sakai T. Design of Hydrogels for Biomedical Applications. *Adv Healthc Mater* (2015) **4**:2360–2374. doi:10.1002/adhm.201500076

24. Oyen ML. Mechanical characterisation of hydrogel materials. *Int Mater Rev* (2014) **59**:44–59. doi:10.1179/1743280413Y.0000000022

25. Brandl F, Sommer F, Goepferich A. Rational design of hydrogels for tissue engineering: Impact of physical factors on cell behavior. *Biomaterials* (2007) **28**:134–146. doi:10.1016/j.biomaterials.2006.09.017

26. Zhang X, Xu B, Puperi DS, Wu Y, West JL, Grande-Allen KJ. Application of hydrogels in heart valve tissue engineering. *J Long Term Eff Med Implants* (2015) **25**:105–134.

27. Caló E, Khutoryanskiy VV. Biomedical applications of hydrogels: A review of patents and commercial products. *Eur Polym J* (2015) **65**:252–267. doi:10.1016/j.eurpolymj.2014.11.024

28. Yue K, Santiago GT, Alvarez MM, Tamayol A, Annabi N, Khademhosseini A. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials* (2015) **73**:254–271. doi:10.1016/j.biomaterials.2015.08.045

29. Zhao L, He C, Gao Y, Cen L, Cui L, Cao Y. Preparation and cytocompatibility of PLGA scaffolds with controllable fiber morphology and diameter using electrospinning method. *J Biomed Mater Res B Appl Biomater* (2008) **87**:26–34. doi:10.1002/jbm.b.31060

30. Yoon HJ, Shin SR, Cha JM, Lee S-H, Kim J-H, Do JT, Song H, Bae H. Cold Water Fish Gelatin Methacryloyl Hydrogel for Tissue Engineering Application. *PLOS ONE* (2016) **11**:e0163902. doi:10.1371/journal.pone.0163902

31. McNamara K, Tofail SAM. Nanoparticles in biomedical applications. *Adv Phys X* (2017) **2**:54–88. doi:10.1080/23746149.2016.1254570

32. Kreuter J. Nanoparticles--a historical perspective. *Int J Pharm* (2007) **331**:1–10. doi:10.1016/j.ijpharm.2006.10.021

33. Hans ML, Lowman AM. Biodegradable nanoparticles for drug delivery and targeting. *Curr Opin Solid State Mater Sci* (2002) **6**:319–327. doi:10.1016/S1359-0286(02)00117-1

34. Hillaireau H, Couvreur P. Nanocarriers' entry into the cell: relevance to drug delivery. *Cell Mol Life Sci CMLS* (2009) **66**:2873–2896. doi:10.1007/s00018-009-0053-z

35. Choi J, Wang NS. Nanoparticles in Biomedical Applications and Their Safety Concerns. (2011) doi:10.5772/18452

36. Gentile P, Chiono V, Carmagnola I, Hatton PV. An Overview of Poly(lactic-co-glycolic) Acid (PLGA)-Based Biomaterials for Bone Tissue Engineering. *Int J Mol Sci* (2014) **15**:3640–3659. doi:10.3390/ijms15033640

37. Ladewig K. Drug delivery in soft tissue engineering. *Expert Opin Drug Deliv* (2011) **8**:1175–1188. doi:10.1517/17425247.2011.588698

38. Wang Y-C, Wu Y-T, Huang H-Y, Lin H-I, Lo L-W, Tzeng S-F, Yang C-S. Sustained intraspinal delivery of neurotrophic factor encapsulated in biodegradable nanoparticles following contusive spinal cord injury. *Biomaterials* (2008) **29**:4546–4553. doi:10.1016/j.biomaterials.2008.07.050

39. Nutt JG, Burchiel KJ, Comella CL, Jankovic J, Lang AE, Laws ER, Lozano AM, Penn RD, Simpson RK, Stacy M, et al. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology* (2003) **60**:69–73.

40. Kordower JH, Palfi S, Chen EY, Ma SY, Sendera T, Cochran EJ, Cochran EJ, Mufson EJ, Penn R, Goetz CG, et al. Clinicopathological findings following intraventricular glial-derived neurotrophic factor treatment in a patient with Parkinson's disease. *Ann Neurol* (1999) **46**:419–424.

41. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. *J Control Release Off J Control Release Soc* (2012) **161**:505–522. doi:10.1016/j.jconrel.2012.01.043

42. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids Surf B Biointerfaces* (2010) **75**:1–18. doi:10.1016/j.colsurfb.2009.09.001

43. Owens DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *Int J Pharm* (2006) **307**:93–102. doi:10.1016/j.ijpharm.2005.10.010

44. Foged C, Brodin B, Frokjaer S, Sundblad A. Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model. *Int J Pharm* (2005) **298**:315–322. doi:10.1016/j.ijpharm.2005.03.035

45. Vasir JK, Labhasetwar V. Quantification of the force of nanoparticle-cell membrane interactions and its influence on intracellular trafficking of nanoparticles. *Biomaterials* (2008) **29**:4244–4252. doi:10.1016/j.biomaterials.2008.07.020

46. McIntosh J. *The Ancient Indus Valley: New Perspectives*. ABC-CLIO (2008).

47. Moulherat C, Tengberg M, Haquet J-F, Mille B. First Evidence of Cotton at Neolithic Mehrgarh, Pakistan: Analysis of Mineralized Fibres from a Copper Bead. *J Archaeol Sci* (2002) **29**:1393–1401. doi:10.1006/jasc.2001.0779

48. Frieri M, Kumar K, Boutin A. Antibiotic resistance. *J Infect Public Health* (2017) **10**:369–378. doi:10.1016/j.jiph.2016.08.007

49. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J Colloid Interface Sci* (2004) **275**:177–182. doi:10.1016/j.jcis.2004.02.012

50. McShan D, Ray PC, Yu H. Molecular toxicity mechanism of nanosilver. *J Food Drug Anal* (2014) **22**:116–127. doi:10.1016/j.jfda.2014.01.010

51. Jeong SH, Hwang YH, Yi SC. Antibacterial properties of padded PP/PE nonwovens incorporating nano-sized silver colloids. *J Mater Sci* (2005) **40**:5413–5418. doi:10.1007/s10853-005-4340-2

52. Ki HY, Kim JH, Kwon SC, Jeong SH. A study on multifunctional wool textiles treated with nano-sized silver. *J Mater Sci* (2007) **42**:8020–8024. doi:10.1007/s10853-007-1572-3

53. Lee HJ, Yeo SY, Jeong SH. Antibacterial effect of nanosized silver colloidal solution on textile fabrics. *J Mater Sci* (2003) **38**:2199–2204. doi:10.1023/A:1023736416361

54. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *J Biomed Mater Res* (2000) **52**:662–668.

55. Zhang Z, Chen L, Ji J, Huang Y, Chen D. Antibacterial Properties of Cotton Fabrics Treated with Chitosan. *Text Res J* (2003) **73**:1103–1106. doi:10.1177/004051750307301213

56. Tokura S, Ueno K, Miyazaki S, Nishi N. "Molecular Weight Dependent Antimicrobial Activity by Chitosan," in *New Macromolecular Architecture and Functions* (Springer, Berlin, Heidelberg), 199–207. doi:10.1007/978-3-642-80289-8_21

57. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as "Curecumin": From kitchen to clinic. *Biochem Pharmacol* (2008) **75**:787–809. doi:10.1016/j.bcp.2007.08.016

58. Gupta SC, Prasad S, Kim JH, Patchva S, Webb LJ, Priyadarsini IK, Aggarwal BB. Multitargeting by curcumin as revealed by molecular interaction studies. *Nat Prod Rep* (2011) **28**:1937–1955. doi:10.1039/c1np00051a

59. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: A short review. *Life Sci* (2006) **78**:2081–2087. doi:10.1016/j.lfs.2005.12.007

60. Zorofchian Moghadamtousi S, Abdul Kadir H, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. *BioMed Res Int* (2014) doi:10.1155/2014/186864

61. Van Den Bulcke AI, Bogdanov B, De Rooze N, Schacht EH, Cornelissen M, Berghmans H. Structural and rheological properties of methacrylamide modified gelatin hydrogels. *Biomacromolecules* (2000) **1**:31–38.

62. Xiao W, He J, Nichol JW, Wang L, Hutson CB, Wang B, Du Y, Fan H, Khademhosseini A. Synthesis and characterization of photocrosslinkable gelatin and silk fibroin interpenetrating polymer network hydrogels. *Acta Biomater* (2011) **7**:2384–2393. doi:10.1016/j.actbio.2011.01.016

63. Occhetta P, Visone R, Russo L, Cipolla L, Moretti M, Rasponi M. VA-086 methacrylate gelatine photopolymerizable hydrogels: A parametric study for highly biocompatible 3D cell embedding: VA-086 GelMA Photopolymerizable Hydrogels. *J Biomed Mater Res A* (2014) **103**: doi:10.1002/jbm.a.35346

64. Zhao X, Lang Q, Yildirimer L, Lin ZY, Cui W, Annabi N, Ng KW, Dokmeci MR, Ghaemmaghami AM, Khademhosseini A. Photocrosslinkable Gelatin Hydrogel for Epidermal Tissue Engineering. *Adv Healthc Mater* (2016) **5**:108–118. doi:10.1002/adhm.201500005

65. Iqbal M, Zafar N, Fessi H, Elaissari A. Double emulsion solvent evaporation techniques used for drug encapsulation. *Int J Pharm* (2015) **496**:173–190. doi:10.1016/j.ijpharm.2015.10.057

66. Ong W-D, Tey B-T, Quek SY, Tang S-Y, Chan E-S. Alginate-Based Emulsion Template Containing High Oil Loading Stabilized by Nonionic Surfactants. *J Food Sci* (2015) **80**:E93–E100. doi:10.1111/1750-3841.12729

67. X. Chen Y, Cain B, Soman P. Gelatin methacrylate-alginate hydrogel with tunable viscoelastic properties. *AIMS Mater Sci* (2017) **4**:363–369. doi:10.3934/matersci.2017.2.363

68. Snehalatha M, Venugopal K, Saha RN. Etoposide-Loaded PLGA and PCL Nanoparticles I: Preparation and Effect of Formulation Variables. *Drug Deliv* (2008) **15**:267–275. doi:10.1080/10717540802174662

69. Yatagai M, Zeronian SH. Effect of ultraviolet light and heat on the properties of cotton cellulose. *Cellulose* (1994) **1**:205–214. doi:10.1007/BF00813508

70. Reddy N, Han S, Zhao Y, Yang Y. Antimicrobial activity of cotton fabrics treated with curcumin. *J Appl Polym Sci* (2013) **127**:2698–2702. doi:10.1002/app.37613

71. Goy RC, Morais STB, Assis OBG. Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on E. coli and S. aureus growth. *Rev Bras Farmacogn* (2016) **26**:122–127. doi:10.1016/j.bjp.2015.09.010

72. Cross DP, Wang C. Stromal-derived factor-1 alpha-loaded PLGA microspheres for stem cell recruitment. *Pharm Res* (2011) **28**:2477–2489. doi:10.1007/s11095-011-0474-x