A Smart Bandage Equipped with Miniaturized Needle Arrays and Wirelessly-Controlled Drug Delivery System for the Treatment of Chronic Wounds

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A Smart Bandage Equipped with Miniaturized Needle Arrays
and Wirelessly-Controlled Drug Delivery System
for the Treatment of Chronic Wounds

By

Hossein Derakhshandeh

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Chronic wounds are one of the most challenging complications of diabetes and are the leading cause of non-traumatic limb amputation. Despite the progress in identifying factors and promising in vitro results for the treatment of chronic wounds, their clinical translation has been limited. Given the range of disruptive processes necessary for wound healing, different pharmacological agents are needed at different stages of tissue regeneration. This requires the capability of wearable devices to deliver agents to critical layers of the epidermis with minimum invasiveness. Here, for the first time, I have engineered a programmable platform which is capable of actively delivering a variety of drugs with independent temporal profiles through miniaturized needle arrays (MNAs) into deeper layers of the wound bed. The delivery of vascular endothelial growth factor (VEGF) through the MNAs demonstrated that, in addition to the selection of suitable therapeutics, the delivery method and their spatial distribution within the wound bed is equally important. Administration of VEGF to chronic dermal wounds of diabetic mice using the programmable platform showed a significant increase in wound closure, re-epithelialization, angiogenesis, and hair growth when compared to standard topical delivery of therapeutics.
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Chapter 1- Introduction

Skin provides a decent protection against environmental hazards which threatens internal organs. In case of injuries like cuts or burns an orchestrated cascade of physiological events would be followed by skin to heal the wound. This is because of regenerative capacity of skin which heals acute wounds in a short period of time (not longer than 90 days). However, in some conditions the skin regenerative capacity becomes overwhelmed and the wound does not heal in the expected time which transit wound’s status from acute to chronic. These chronic wounds endanger patient’s health as they are prone to infection and usually need surgical treatment. The treatment of chronic wounds is expensive, challenging and time consuming. Just in the US, approximately 4.5 million people need to be treated for chronic wounds which cost over $25 billion annually. Considering the increasing rate of obesity and diabetes the burden of chronic wounds is expected to grow. Taking care of wounds dates back to several millennia. The first types of wound dressings were probably made from leaves, fabrics, and natural ointments to stop infection and reduce pain. Still, the treatment of wound has not changed fundamentally, and the use of regular bandages and topical ointments is very common. In the case of chronic wounds, conventional bandages are not sufficient to induce healing. Also, using regular wound care strategies for the treatment of deep cuts may result in forming permanent scars. Therefore, numerous studies have been done to restore the regenerative properties of the skin. The strategies pursued can be divided into following groups: 1) Identifying biological agents available in wound bed which cause healing and those which showing abnormal behavior in the chronic wounds and thus finding a therapeutics that can handle the disorganized situation in chronic wounds; 2) devolving drug delivery systems which can deliver appropriate therapeutics at the right time and dosage to the wound bed; 3) developing specific materials which can be applied as a scaffold for
expediting the tissue growth; and 4) advanced diagnostic dressing system which can monitor wound bed and send the status of wound as a feedback.

Drug delivery systems are of particular importance as the ineffective vasculature in wound bed can prevent effective delivery of drug to the healing tissue if when the drug is administered systemically. In addition, the side effects of some of the drugs, the low half-life of biological factors, and the dynamicity of the wound environment which requires complex drug delivery systems that can deliver the active factors in proper dosage to the appropriate location. Over the past decade, significant progress has been made in the field and many different systems and platforms have been developed.

In this thesis, the recent progress in various areas of wound care with particular emphasis on drug delivery aspects are critically reviewed. In the Introduction chapter, we discuss the physiology of wound healing and the pathophysiology of chronic wounds. In chapter two, systems designed for controlled release of drugs and factors are discussed. In addition, we discuss the new class of dressings that are smart and can sense the wound environment and can provide information essential for active wound care. Later in chapter three, the hypothesis of the research project will be explained followed by the detail of system design and the fabrication process. In chapter four, experimental setup and results for confirming the capabilities of our system will be explained followed by in vivo study which is located at chapter five. At the end, conclusion and future look is discussed in chapter six.
1.1 Acute Wound Physiology

Wounds are divided into two different categories including acute and chronic types. If the healing process of wound follows a regular process, it is called an acute wound. Different cell types, growth factor, and physiological agents are responsible for dermal wound healing. Wound healing can be divided into four processes including hemostasis, inflammation, proliferation, and remodeling. The first body reaction against injury is hemostasis to stop bleeding by clotting the blood. The second phase is inflammation which takes about three days. This stage prepares the wound bed for growing the new tissue by destroying pathogens and dead tissue by white cells followed by macrophages. At the proliferation phase of wound healing, three stages will occur that takes between four to 24 days. First, wound will be filled with shiny and deep red granulation tissue, and new blood vessel would be formed. Next stage is contraction where the wound margins will contact. After that, epithelization happens by covering the wound with epithelium. When the wound was closed then remodeling phase complete the wound healing process as the last step. During this phase collagen fibers remodel and increase the tensile strength of tissue which causes more flexibility and strength for the new tissue.

1.2 Pathophysiology of Chronic Wounds

In spite of the excellent capacity of skin for regeneration, in some cases the healing cascade gets disrupted impairing the wound healing and as a result, wound becomes chronic. Chronic wounds have diverse etiologies and thus diverse signatures. Despite their molecular and clinical heterogeneity, chronic wounds can be divided into three main categories: venous leg ulcers (VLUs), diabetic foot ulcers (DFUs), and pressure ulcers (PUs). VLUs are open lesions that occur between the ankle joint and the knee in patients with venous disease. These ulcers occur in
advanced forms of chronic venous disorders such as varicose veins and lipodermatosclerosis. DFUs are nonhealing full-thickness wounds that extend through the dermis, below the ankle, and are often caused by repetitive injury to the site. PUs are injuries to the skin and underlying tissue due to prolonged pressure on the skin. PUs commonly occur on the skin over bony areas of the body, such as the hips, heels, tailbone, and ankles. 

There is still much unknown information about the pathophysiology of chronic wounds. However, these wounds do not pass all the healing phases and are typically stuck in the inflammation phase. Also, hypoxia, impaired vascularization and the inability of immune cells to fight against infection are other challenges which occur in chronic wounds. Hypoxia results in the necrosis of the tissue, which prepares the wound bed to be suitable for bacterial growth and biofilm formation. Then, biofilm worsens the inflammation and tissue repair stops. All these conditions together threaten the patient’s health status and, in most cases, wound debridement or limb amputation is needed by the surgical process.

Prolonged and overexpression of various interleukins and other inflammatory cytokines (such as TNF-α) prevents the healing process from advancing to the proliferation phase. Hyper-inflammation also affects the expression of MMPs that play an important role in wound repair by degrading and removing damaged ECM molecules from the injured tissue. However, their excess proteolytic activity is associated with chronic wounds because they destroy growth factors, cell surface receptors, and temporary ECM essential for cell migration. In addition, the lack of growth factors and the presence of too many senescent cells in the injured area may result in the inability of these wounds to heal. Inadequate microvasculature can lead to chronic non-healing wounds that are especially common in diabetic patients. Most chronic wounds do not heal through regeneration but fibrosis forming excessive amounts of connective tissue. Fibrosis also
follows chronic inflammation and elevated amounts of pro-inflammatory mediators (such as TGF-β) have been found in the wounds that heal by fibrosis. Growth factor activity is poorly regulated causing unnecessary fibroblast proliferation, neovascularization, and increased collagen and fibronectin synthesis. In addition, excessive and prolonged wound contraction occurs resulting in a formation of fibrotic scar tissue. Pathological scars after an injury can be categorized into keloids and hypertrophic scars. Keloids are an abnormal overgrowth of the scar tissue. They extend beyond the boundaries of the original wound and do not regress spontaneously over time. Hypertrophic scars are more common and do not get as big as keloids by not expanding over the borders of the wound. They may also spontaneously regress over time.

Current strategies for wound care are shown to be outdated and ineffective. The treatment of chronic wound is based on a list of therapies including cleansing, debridement, oxygenation, antibiotics, and surgery which are expensive and time consuming. These strategies will be discussed in the next chapter in detail.
Chapter 2- Current Wound Care Systems

Based on the wound type including acute and chronic the approach for the wound management would be different. The goal of normal wound care therapies for the healthy patients having acute wound would be to: 1) not allowing to external agents such as bacterial infections or mechanical stress affect the wound negatively, 2) expediting the wound closure rate by keeping the wound moisture at suitable level, and 3) preventing the wound from scarring.

Many dressings have been developed that are indicated for specific types of chronic wounds based on wound conditions, such as being dry or exuding, superficial or deep, and clean or infected. These dressings are associated with various limitations. As the wound healing process continues to be better understood, systems that actively control the spatial and temporal profile of drug release would be extremely beneficial for wound care treatment.

For the patient who deals with chronic wounds, there are three more goals in addition to what mentioned for the ones listed for the acute including 1) debriding necrotic tissue and biofilm, 2) regulating inflammation, and 3) helping the reparative step of healing, for instance, helping with angiogenesis or tissue blood perfusion. In 2002, a guideline was proposed to list some factors to be considered for wound care called TIME which stands for tissue, infection or inflammation, moisture balance and the edge of the wound. However, it should not be forgotten that other factors including decreasing the pain, cost and the burden of frequent wound dressing would be important for having an ideal wound care system.

The majority of existing wound care dressings are passive and cannot actively respond to variations in the wound environment. In some cases, these passive dressings are able to release anti-inflammatory drugs, antibiotics or antibacterial compounds, and angiogenic factors and uptake excessive exudate. Some of the more advanced dressings passively release biological...
factors and compounds to facilitate tissue healing. One of the major limitations of current wound care products is their inability to provide information about the status of the wound bed and its healing rate. As a consequence, patients must be frequently screened to assess the healing process and inspected for potential infection. Increased visits, stemming from the necessity to continuously monitor the healing process, add to the cost of the treatment and enhance the stress on medical centers. In addition, frequent visits to medical centers can be a major challenge for patients who are living in remote areas. Another important limitation of passive wound care products is their inability to recognize the difference between various stages of wound healing. In general, the rates of physiological processes are different throughout the healing process; thus, the concentration of the required factors and drugs may vary over time. Other challenges include the correct utilization of antibiotics, where improper and prophylactic use can lead to the development of antibiotic-resistant bacteria.

One of the main approaches for advanced wound dressing has been to stop bacterial infection by sealing the wound environment from contaminations. Vacuum assigned closure for the wound is listed as one of the popular approaches for wound care. In this method, a negative pressure is generated and by getting help from foam or sponge the suction would be applied on wound site for removing exudates which are enriched with pro-inflammatory cytokines and proteins. In addition, this approach has other advantages including decreasing the chance of infection and formation of biofilm, and also improving the blood supply for the wound bed. Despite the mentioned advantages of this method and even the majority of current products for wound care are acting passively, and it is not clear how effective are these approaches. Current wound care systems deal with two fundamental challenges. Firstly, they cannot detect infection properly. Secondly, in most cases, they apply topically and cannot target specifically.
As a result, detection of infection is late usually and so, therapeutics are less effective. In addition, unnecessary delivery of antibiotic or antimicrobial agents leads to the development of antibiotic resistance and systemic toxicity. Still, there is an outdated belief at current wound care system that “one treatment fits all” which is a mistaken concept

Other important feature about wound dressing is the maintenance of physiologic wound moisture and gas exchange. Having a moist wound environment increases the rate of migration and proliferation of keratinocytes and helping with wound closure. It also supports endothelial cells migration, angiogenesis and remodeling of ECM, and causing less intense fibrosis. Adopting a non-specific approach to increase wound moisture is one of the key strategies in many of currently wound care systems.

Chronic wounds are usually known with the existence of a thick layer of necrotic tissue that is a barrier for healing and an ideal place for bacterial growth. Therefore, wound detriment is another approach for wound care. It is performing with different strategies such as surgery, autolytic, enzyme substance, and mechanical methods. There are some dressings which can support with embedded detriment capability. Comparing to other methods like surgery this approach looks less-painful, cost-effective and more physiologic debridement.

In the U.S Food and Drug Administration (FDA) has approved a variety of wound healing products which can be divided into three different categories including passive, medicated, or interactive dressings. A passive dressing which is not able to elute drug works just as a physical barrier. Medicated dressing has the capability to provision healing directly by enhancing wound treatment agents or indirectly by removing the necrotic tissue. In medicated dressing, there are active agents which contain growth factors, antimicrobial agents, and mono-terpenes. These agents often are loaded into hydrogels, alginates, hydrocolloids, silicon gels, and polyurethane foam films.
In summary, current wound care products have meaningfully improved patient care. However, still more studies are needed to improve their capacity.

2.1 Passive Drug Delivery Systems

The existence of enough growth factors and cytokines in wound bed can expedite wound healing processes. But, in some cases, these factors are not adequate to support the healing process. In this situation, therapeutics can be effective in balancing the rate of physiological steps which cause to wound healing. Regarding chronic wounds, there are some abnormal conditions which need to be considered before acting on choosing the therapeutics and delivery method. 1) vascular system does not work properly which reduces the bioavailability of agents directed either orally or intravenously; 2) possibility of side effects from therapeutics; 3) existence of pro-inflammatory cytokines in the wound bed can stop the effect of drugs; 4) precedence of therapeutics during the right time needed for time-consuming physiological process. Therefore, local delivery looks more suitable comparing to systemic delivery since it decreases the chance of undesired side effects including the toxicity. A variety of drug delivery systems are developed to control the release of therapeutics or directly deliver the agents to the targeted tissue. Local controlled releasing agents have this advantage to provide spatial and temporal control over the drug dosage. As a result, the chance of deactivation of drugs is less over a long term of drug administration. A proper drug delivery system should be able to selectively and sequentially control the release of agents. Chronic wounds suffer from delayed angiogenesis, which results in hypoxia. In response, immune cells provide less reactive oxygen species, leading to more pro-inflammatory cytokines are secreted to employ more immune cells. The process of infiltration of immune cells will result in preventing tissue regeneration. Therefore, sequential and selective release of anti-inflammatory...
agents followed by pro-angiogenic growth factors, epidermal growth factors are needed to disrupt the impaired cycle at chronic wounds. Here a variety of drug carries are discussed including their release mechanism.

### 2.1.1 Controlled-release Drug Carriers

One approach for controlled-release in drug delivery system is to encapsulate therapeutics into a substance which can release drugs actively or passively. In active delivery, the release occurs in response to environment incentives including pH, enzymes, temperature, chemical reactions and so on; or release can be triggered by external stimuli such as light, magnetic field, ultrasound, etc. Passive delivery is based on the diffusion of the drug from a carrier matrix to nearby medium. This carrier matrix can be either organic including hydrogels, lipid-based system, multi-layer systems; or be inorganic such as ceramic, carbon-based nanotubes mesoporous particles, and metal-organic frameworks. Factors that affect the delivery rate through these agents are carrier size, porosity, shape, degradability, and electrostatic charge. Choosing an appropriate substance for engineering the drug carrier is very significant. Aspects such as charge, water solubility, the stability of carrier and effect of wound environment on the carrier play a crucial role in developing an effective platform. So, it is important to know about the techniques that can be applied for fabricating different drug carries.

### 2.1.2 Methods for Fabrication of Drug Carriers

The importance of tools that are used for the fabrication of drug carriers is mostly in terms of their robustness and their reproducibility. One of the most common methods for fabrication of drug carries is emulsification which is the mixing of two immiscible fluids together resulting in
the formation of spherical droplets. This process can be emulsified again to form double emulsion 56,57. This method is usually used for administering polymeric, hydrogel-based, and lipid-based drug carriers. Parameters for tuning the size of the particle are solutions viscosity and the mixing speed.

Another method which can be used for particle fabrication is micro- or nano-molding. The size of the generated particles is larger than those fabricated by emulsification, and it can be used for both hydrogels and polymeric. Advantages of this method are: 1) achieving a predictable release profile; 2) fabrication can be done in sterile and straightforward conditions; 3) stability of the drugs can be conserved. While the fabrication of spherical particles is challenging using this approach, rod-shaped, sheet-like, and planar-constructs can be feasibly produced.

Another popular tool for fabricating drug carriers is microfluidic systems 58. One major advantage regarding the microfluidic platforms is their ability to fabricate multicompartamental droplets 59,60. Particles made from hydrogels, polymers, and lipid-based materials can be formed by flow-induced shear stress. Particles are larger than the emulsification method 61. One of the limitations about this system is that fabrication rate of particles is slow and forming the size and shape of droplets is challenging. Electrospraying and self-assembly are other approaches that have also been used for the engineering of drug carriers. Detailed information about the fabrication of drug carriers using these systems can be found elsewhere 62,63.

Achieving different release profile for various compounds is desired in wound healing. This can be achieved through: 1) the use of multiple drug carriers with different sizes or compositions; 2) the use of multi-layer multicompartamental drug carriers. In one example, double emulsion systems were used to fabricate PLGA based drug carriers in which PDGF-BB were encapsulated inside PLGA containing chlorhexidine, which has antibacterial activity. This enabled them to
release one hydrophilic and one hydrophobic drug with different release profiles. Wounds treated receiving dual treatments showed reduced infection and enhanced healing \(^{56}\). In another example, PDGF-BB was encapsulated in PLGA nanoparticles and then the mixture of the fabricated particles and chitosan/poly (ethylene oxide) mixed with VEGF were electrospun to form nanofibrous scaffolds in which VEGF was released fast and PDGF-BB was released gradually \(^{64}\). The sequential delivery of the growth factors resulted in enhanced wound healing in diabetic animals. The capability of releasing drug with different profiles is beneficial for healing of complex non-healing wounds and scientists have developed various tools for that. The emergence of micro and nanotechnologies has facilitated the fabrication of uniformly sized and structured drug carriers with predictable release profile. However, one key limitation of these tools is their low throughput and scalability.

### 2.1.3 Passive Transdermal Delivery Systems

An important aspect affecting the outcome of localized drug delivery is the selection of point of delivery. Chronic wounds are covered with a layer of non-viable tissue, which separates the outside environment from the underneath tissue \(^{65}\). Thus, if drugs and factors are delivered topically, they should first pass through the dead tissue filled with pro-inflammatory cytokines to access the cells that are supposed to receive the therapy \(^{66}\). As a result, a significant amount of drugs or factors may get deactivated before reaching the growing tissue. In addition, the significant exudate production in chronic wounds can further reduce the rate of penetration of drugs administered topically. The most traditional way of drug delivery across the skin, hypodermic injections, is quite unfavorable as it is painful, requires professional assistance and can transmit diseases when the hypodermic needles get in contact with different patients.
Thus, there has been a significant push for developing tools that can deliver drugs transdermally. These tools range from micro/nanocarriers that could pass through the skin barrier and stratum corneum or microneedles that could painlessly poke through the barrier and deliver drugs to the viable tissue underneath. Among different drug carriers, lipid-based particles in the form of liposomes and polymeric particles have been widely used. In case of wound therapy, the skin barrier is breached and thus such drug carriers might not be as necessary as compared to the treatment of other skin disorders. Microneedles are arrays of short needles that were initially developed for painless delivery of therapeutics transdermally. They are sufficiently small in size to pass the stratum corneum but not hit the nerves underneath. These microneedles can be categorized into four groups: i) solid microneedles disrupting the epidermis barrier and enabling the penetration of topically administered drugs, ii) microneedles coated with drugs that can penetrate the tissue and deliver their payload in there, iii) dissolvable microneedles that penetrate the tissue and stay there and release their payload gradually as they degrade, and iv) hollow microneedles that penetrate the tissue and facilitate the active delivery of drugs into the region of interest.

Since the first transdermal drug delivery product, a three-day patch that released scopolamine for the treatment of motion sickness, was approved for commercialization on the United States market in 1979, many more transdermal drug delivery products have hit the market. Since then microneedle arrays have been used for a wide range of biomedical application including the delivery of insulin, vaccines, and pain medications. However, there has been little interest in them for the treatment of chronic wounds and burns. In one example, Takeda et al. developed microneedles using chondroitin sulfate as the base material loaded with basic fibroblast growth factor (bFGF), a growth factor that is released during the early stages of wound healing.
and triggers endothelial cells to exert behavior typical for wound healing processes. The needle arrays were tested on rat models with wounds inflicted using a surgical scalpel. ELISA assays on bFGF levels in the tissue showed initially elevated concentrations that slowly declined over time. Caffarel et al. developed a dissolving microneedle system incorporating a photosensitizing compound that has antimicrobial effects to treat infected wounds. This mechanism making use of a photosensitizing drug also termed photodynamic antimicrobial chemotherapy aims to produce highly reactive radicals in the tissue upon irradiation of the photosensitizing drug. Continuing the trend of the use of microneedles for antimicrobial treatment, Park et al. designed antibacterial microneedles loaded with green tea extracts. Green tea includes polyphenols which have shown to be potent antibacterial and anti-inflammatory agents. More specifically, the catechins present in green tea extracts exhibit inhibitory effects on various bacteria. To deliver the extract to the wound area, dissolvable microneedles made of hyaluronic acid were developed.

One interesting application of microneedles for the treatment of skin injuries has been proposed by the development of microneedle arrays with swellable tips. The system was designed in a way such that once upon penetration of the skin, the needles would swell and lock themselves in place. These needles were used for improving the adhesion of skin flaps frequently used for the treatment of burns and chronic wounds. In a follow-up study, these needles were loaded with insulin and were used for transdermal and long-term delivery. The array proved to be successful in reducing blood glucose levels in diabetic rodents.

In general, microneedles are an interesting tool that can be fabricated from polymers that are known for their excellent drug protection and the gradual release of their payload. In these cases, the drug can be released over time for completion of physiological processes. These microneedles
can be made as composites of different materials, and they can also be developed in a multi-layered way to enable drugs required for late stage wound healing to be released at a later time.

2.1.4 Systems for Intracellular Delivery

With recent progress in the field of biology and genetic, recent years have seen many successful examples in which the cells have been programmed to a desired phenotype. Chronic wounds are the product dysfunctioned cell populations, for example in diabetic patients, endothelial cells are less responsive and usually take longer to form functional vasculature in the wound bed ⁸⁰,⁸¹. In addition, in wound healing, macrophage phenotype plays an important role in tissue regeneration. At the inflammation phase, M1 macrophage polarization (the pro-inflammatory phenotype) results in the removal of debris and pathogens. During the proliferation phase, the phenotype will be polarized toward the anti-inflammatory M2 phenotype ⁸². However, in chronic wound, this change of phenotype does not occur and results in continuous inflammation. Thus, noticeable attention has been dedicated to transfer genes, plasmids, and active molecules directly into the cells and different tools have been developed for that. These drug delivery systems are usually active, but there are some passive systems which will be discussed here.

Passive systems usually should possess features smaller than cells that enable them to be internalized. Liposomes and nanoparticles have been frequently used for silencing or activating genes that are important for wound healing. In one example, 13 nm gold nanoparticles decorated with nucleic acid were used to downregulate ganglioside-monosialic acid 3 synthase (GM3) which is over expressed in diabetic wounds. The use of these nanovectors had superior effectiveness on the downregulation of GM3. Upon the treatment of diabetic wound models, superior healing was observed in comparison to free siRNA delivery ⁸³. However, the delivery effectiveness of these
nanoparticles in heavily exuding wounds can be compromised. Thus, other effective ways which are less prone to being washed away would be more robust and can be applied to different types of wounds. Recently, nanoneedles have emerged as a useful tool that can penetrate cell membrane and delivery plasmids directly in them \(^{84,85}\). In one example, mesoporous silicon nanoneedles were engineered and were successfully tested for delivery of a number of plasmids to cells both \textit{in vitro} and \textit{in vivo} \(^{85}\). In animal studies, VEGF-165 gene was delivered using the nanoneedle array to the cells and the results show a significant enhancement in vascularization in comparison the animals receiving the same gene via hypodermic injection \(^{85}\).

The area of cell reprogramming is emerging, and it is expected to play a pivotal role in the future of medicine. Along with the advancement in the field drug delivery tools are needed to more effectively deliver the therapeutics into the cells without exerting unwanted damage to cells or their phenotype. Although the current tools have shown promising outcomes, these systems are either hard to fabricate or are prone to dislocation from the targeted site.

2.1.5 Kinetics of Controlled Release

The goal of drug delivery systems such as micro- and nano-sized particles, gels, and fibers is to increase the drug bioavailability and achieve a sustained biodistribution. Naturally-derived and synthetic macromolecules have been used to entrap drugs and provide a controlled release. The release rate is defined by at least one of the following mechanisms: (1) diffusion-based release, (2) degradation-based release, and (3) affinity-based release, and. Zero-order release kinetics is usually preferred since a steady drug concentration is maintained between the minimum effective concentration (MEC) and maximum toxic concentration (MTC) \(^{86}\). Although a zero-order release profile is desired, most drug delivery systems show a triphasic release profile. The initial phase
shows a rapid release of drug from the reservoir and is referred to as the burst release. This occurs due to the diffusion and migration of drugs to the surface of the drug carrier during the fabrication process or during storage. In phase two, the release is mostly governed by the diffusion of drug through the polymeric matrix or through the pores of the drug carrier. For biodegradable drug carriers, hydrolysis and degradation of the matrix are also initiated at this phase. In phase three, a faster release is observed due to the erosion of the drug carrier. Parameters such as matrix chemical structure, molecular weight, swelling degree, and porosity, as well as drug-carrier interactions, and drug-drug interactions can affect the release profile. Upon exposure to water, the pores and channels of the reservoir become filled with water. Driven by chemical potential gradient and osmotic pressure, drug molecules diffuse through these water-filled pores and randomly move towards the releasing medium. Apart from diffusion through the pores, drug molecules can diffuse through the polymeric matrix and can also be released due to the erosion of the carrier matrix. Polymeric biodegradable materials have been widely used as drug carriers. These polymers have labile bonds such as esters, amides, and anhydrides in their backbone which will break by hydrolysis or enzymatic degradation resulting in erosion of the drug carrier. Degradation occurs either from the surface or the bulk of the polymeric matrix. As water penetrates through the matrix of the drug carrier, hydrolysis takes place resulting in pore formation and enlargement which will ultimately alter the kinetics of drug release. Affinity-based systems rely on transient interactions between the drug and the polymeric matrix. Originally inspired by the controlled release mechanisms observed in the extracellular matrix, affinity-based drug delivery systems use transient interactions between the drug (small molecules, proteins or DNA) and the polymeric matrix. Such transient interactions result in a slow diffusion-based release of drug from the polymeric matrix, reducing the chance of burst release.
divided based on type of interaction between the drug and polymeric matrix. Most common systems rely on electrostatic interactions (heparin-based, heparin-mimetic or non-heparin interactions), while other novel systems rely on hydrophobic interactions (cyclodextrins interaction with small hydrophobic molecules) or multiple interaction systems (protein-protein or aptamer-based interactions) \(^9_4\). In general, understanding the release kinetic and proper modeling of drug diffusion from these carriers can help with the rational design of more effective drug delivery tools with predictable results.

Overall, passive drug delivery tools are excellent choices for enhancing the healing rate in chronic wounds. However, the unpredictability of these wounds and the fact each wound has its own signature will be a major obstacle against achieving optimal therapy using passive systems. Thus, systems that can be used for on-demand drug delivery has been developed that enable active intervention in the dysfunction healing cycle. These systems will be reviewed in the next section.

2.2 Active Drug Delivery Systems

Controlled release drug delivery systems that allow for steady passive drug release over time already provide more effective treatment options than traditional drug delivery methods. These traditional methods, such as hypodermic injections, often result in an elevated plasma concentration of the drug that is outside of the therapeutic window which can result in side effect and reduce the effectiveness. The ability to passively control release enables the system to contain larger amounts of drug while maintaining a drug concentration in the blood within the limits of the therapeutic window, thus allowing it to be used for a longer period of time and in a more efficient way \(^9_5\).
Wounds are dynamic environments and the proper timing of administration of active compounds is important. The treatment of some pathophysiological complications, however, might only require drug release at the right time. For example, infection is a serious source of complications associated with chronic wounds. Administration of antibiotics as prophylactic therapy has been suggested to prevent infection. However, the excessive use of antibiotics negatively affects the healing process and can result in the formation of antibiotic-resistant strains. The emergence of antibiotic-resistant strains so called “superbugs” is one of the biggest challenges that medicine is facing in the next few years. Current clinical practice includes the treatment of infection through systemic or topical delivery of antibiotics once infection detected by clinicians and confirmed by culture of wound swabs. Thus, for treatment of infected wounds, drug delivery systems should be designed that can release antibiotics only as needed with the correct dosage. This can be achieved through the use of systems that can either be triggered externally or self-respond to changes in physiological conditions such as pH, temperature or other microenvironmental changes in the tissue. Such systems are called stimuli-responsive and over the past two decades, various stimuli-responsive drug delivery systems have been developed. Polymers are the most used material for engineering stimuli-responsive systems due to their tunable character allowing for precise control over mechanical and physicochemical characteristics of the material and sharp changes in material properties in response to stimuli 96.

On-demand and stimuli-responsive systems have many advantages compared to passive release systems. As a result of their ability for spatial, temporal and dosage control over the drug release, these systems require a lower drug loading while also decreasing the adverse effects to the patient. This makes drug delivery therapies more efficient, cheaper and safer 97. In this part, we will review both self-responding and externally controlled systems.
2.2.1 Self-responding Drug Delivery Systems

Developing systems that can respond to their environment and change their state has been attractive for many biomedical engineering applications and in particular drug delivery systems. To achieve this goal, hydrogels and materials that can swell or change their state in response to environmental and external responses have been developed. In the wound environment, physical and chemical properties such as temperature and pH are indicators of its status that fluctuate by variation in the level of inflammation, oxygenation, and infection. Usually, skin temperature is in the range of 32 °C to 34 °C; however, it may locally increase due to inflammation. After a skin cut, the exposure of blood and body fluids temporarily increases the local pH to about 7. This value will be reduced to a slightly acidic value of 4-5 during the healing process. However, bacterial infection can change the pH. It has been reported that the environment of infected wounds is either extremely acidic or slightly alkaline depending on the type of bacteria and wound condition. The level of oxygenation also affects wound pH. In chronic and non-healing wounds which contain significant necrotic tissue, for instance, the pH becomes alkaline locally. Thus, significant effort has been dedicated to the development of drug delivery systems that respond to variations of the environment temperature and pH.

Thermo-responsive polymers have been widely used for engineering self-responding drug delivery systems. Thermo-responsive polymers can be divided into two classes based on the way they respond to heat: lower critical solution temperature (LCST) polymers and upper critical solution temperature (UCST) polymers. The first class of polymers exhibits dissolution upon exposure to heat, whilst the second class becomes soluble when heated. These critical temperatures can be tuned and are dependent on many factors such as molecular weight, polymer concentration and, if applicable, any other materials that are added in the system such as drug
formulations. The critical temperature is important, it should be high enough to not get triggered in room temperature and not too high such that high temperatures that can negatively affect the healthy tissue and the activity of the encapsulated drug are required for the material’s state to change. Thus, the suitable range for critical temperature is 35 °C-45 °C.

Poly(N-isopropylacrylamide) (pNIPAM)-based polymers have been widely used for engineering thermo-responsive drug delivery systems. The critical temperature of pNIPAM is around 32 °C, which is close to skin temperature. pNIPAM is hydrophilic below its critical temperature and becomes hydrophobic above that, where the aqueous solution containing hydrophilic drug will be pushed out of the drug carriers. Due to the low critical temperature of pristine pNIPAM, it can be used for engineering systems that can release their content once placed on the wound surface. Tran et al. designed such a thermo-responsive system by electrospinning pNIPAM and PCL to create nanofibers with a high surface-area-to-volume ratio. After incorporation of ibuprofen into the nanofibers, their drug release was tested at 22°C and 37°C and a significant change in release profile was observed. The composite nanofibers showed a reduced burst release as well as a controlled release profile over 4 h compared to fibers made of only pNIPAM. However, the critical temperature of pNIPAM can be increased by grafting other monomers to the NIPAM chains and can reach over 37°C. In addition, it has been shown the generation of hybrid pNIPAM-based systems can increase their critical temperature. In one example, the critical temperature of hybrid particles of NIPAM-poly(ethylen diacrylate) (PEGDA) was increased to close to 37°C minimizing the passive release of antibiotics at room temperature. Other thermo-responsive drug carriers based on Pluronic F-127 and chitosan have also been successfully synthesized and tested in the literature for engineering self-responding systems. In
one example, curcumin and DsiRNA were loaded into Pluronic hydrogels and the effectiveness of the drug release for modulation of inflammation in diabetic wounds was demonstrated \textit{in vitro} \textsuperscript{102}.

pH-responsive materials, often ionizable polymers that are weak acids or bases, function through a change in their ionization state resulting in changes in the polymer conformational state \textsuperscript{103}. In the case of hydrogels, a change in conformational state means a change in swelling behavior which can be utilized to control drug release \textsuperscript{99}. Ninan \textit{et al}. developed a pH-sensitive hydrogels consisting of tannic acid-carboxylated agarose, in which tannic acid was used for its antibacterial properties \textsuperscript{104}. Additionally, zinc(II) chloride was crosslinked with the hydrogel. Zinc salts stimulate cell proliferation, have antioxidative properties, and have been shown to be beneficial for wound healing through promoting the synthesis of new extracellular matrix, reducing free radical activity, and limiting the growth of bacteria. To study the responsiveness of the hydrogel, the release of tannic acid at different pH values was studied and a minimal release was shown at a pH of 7.4 as opposed to acidic conditions where controlled release was observed. Furthermore, the constructs were minimally toxic to 3T3 fibroblasts as tested \textit{in vitro} and exerted antimicrobial effects that were comparable to gentamicin, a commercial antibiotic, as tested on \textit{E. coli} bacteria \textsuperscript{104}.

To develop materials that respond to variation in both pH and temperature, copolymers of pH and temperature responsive materials have been developed and used for engineering drug delivery systems. For instance, Garbern \textit{et al}. synthesized NIPAM and propylacrylic acid (PAA) co-polymers through reversible addition fragmentation chain transfer meant for drug delivery in regions with an acidic pH \textsuperscript{105}. This copolymer response to pH changed in the range of 4.5 to 6 as well as temperatures ranging from 20\textdegree C to 50\textdegree C. Depending on modifications in the random copolymer as well as polymer concentrations, the transition characteristics of the hydrogel were
tuned. The hydrogel exerted a controlled release of the growth factor VEGF at pH values of 5 and 6, which are relevant in wound healing applications. Interestingly, drug release rate was not only controlled by polymer dissolution, but also by electrostatic effects between the protein and the polymer’s unreacted anionic groups. In a less acidic environment, fewer carboxylate groups were protonated leading to the possibility of electrostatic interactions with the VEGF, hindering protein release.\textsuperscript{105}

Another class of materials that have recently been explored as an option for responsive systems are materials that respond to the level of chemokines and cytokines in the wound bed.\textsuperscript{106,107} For example, the level of pro-inflammatory enzymes such as elastase and cathepsin have been demonstrated to be highly upregulated in chronic infected wounds.\textsuperscript{108,109} Thus, the use of protease cleavable peptides that can link suitable drugs to the polymeric backbones can result in the formation of polymers with a drug release rate that is proportional to the concentration of the targeted chemicals. In one example, hyaluronic acid capped-mesoporous particles were engineered to carry drugs.\textsuperscript{110} The capping hyaluronic acid could be degraded by hyaluronidase-1, resulting in the release of drugs. In another example, rhEGF was conjugated to dextran to be protected against environmental conditions. Upon delivery and exposure of the caged growth factor, dextran was degraded by α-amylase, exposing the protein. The protected growth factors had a better long-term effect on keratinocyte growth.\textsuperscript{111} Currently, such systems are widely being used as diagnostic tools for detection of hyper-inflammation and infection, but they may serve as an excellent tool for engineering self-responding materials that can modulate the level of inflammation or automatically eradicate the infection.

Self-responding systems are excellent drug delivery tools that are helpful for the treatment of patients with limited access to medical facilities and for diabetic patients in which any skin cut
can potentially turn into a chronic wound. However, the key limitation of these systems is their loading capacity, especially for systems responding to biological cues. These materials are also usually not FDA approved for internal use and they can be limited to temporary dressings. Thus, there will be a gap between the delivery point and the healing tissue, which can negatively affect the therapeutic outcome. Overall, these materials can potentially revolutionize wound care if these challenges are solved.

### 2.2.2 Externally Triggered Drug Delivery Systems

Another class of active drug delivery systems is those that can be triggered externally. Such systems ideally should offer zero passive release rate and the targeted drug would be only released once needed. Although such systems have numerous applications in medicine, in wound care they are mainly suitable for the delivery of antibiotics, anti-inflammatory drugs, and pain medication. In these systems, an externally triggered module drives the drug towards the skin \(^{112}\). Recent advances in the area of microfabrication and flexible electronics have further enabled the development of such systems. For example, the combination of stimuli-responsive drug carriers and flexible heaters has led to the development of wearable devices that can release drugs on demand. In one example, Bagherifard \textit{et al.} fabricated flexible heaters and cast a layer of alginate hydrogel containing pNIPAM microparticles on top of that. The platform was integrated with a driver that enabled the triggering of the heater and drug delivery \(^{113}\). In another example, nanofibrous meshes were fabricated in which thermoresponsive drug nanocarriers were embedded into elastic nanofibrous meshes \(^{114}\). These meshes possess morphology similar to paper and have been used as a substrate for the fabrication of flexible electronics. However, to avoid the triggering of the drug carriers a low temperature radio frequency sputtering was used to deposit metallic
heaters from various metals including gold, silver, magnesium, and zinc. It demonstrated that the release profile of the drugs could be controlled by the applied voltage and the generated heat. The released antibiotics were potent against the culture of different bacteria. The device could be triggered using a smartphone \(^{114}\).

Usually, skin disorders are multifactorial and different drugs at different stages of wound healing are required. Also, for patients who are leaving in remote areas, having a patch which is already loaded with potentially needed drugs in which they can be triggered as needed is helpful. However, releasing different drugs with independent profiles is challenging. In a recent study, Mostafalu \textit{et al.} formed multicompartiment fibers with a core thread heater coated by a layer of alginate-based hydrogel \(^{115}\). Thermoresponsive microparticles of pNIPAM-PEGDA were fabricated using a microfluidic systems and incorporated into the hydrogel coating. The fibers were then woven into a patch and each fiber was connected to a controller that enabled addressing them independently. It was shown that the fibers could be triggered one by one or together and the number of triggered fibers would result in the release of specific quantity of the drug. The system was effective for preventing bacterial growth. Also, the release of VEGF from the engineered patches helped with improved vascularization and wound healing both \textit{in vitro} and in diabetic animal models \(^{115}\).

One interesting example of active delivery for wound care is topical oxygen delivery \(^{116}\). It is known that tissue oxygenation can significantly improve the rate of wound healing. Currently, hyperbaric oxygen therapy (HBOT) and topical oxygen therapy (TOT) are being practiced for the treatment of diabetic and chronic wounds \(^{117,118}\). However, oxygen therapy usually requires the presence of sophisticated devices and which are not portable. In addition, these systems carry the risk of oxygen poisoning in patients. To address these challenges, recently a wearable dressing
was developed by Ochoa *et al.* in which hydrophobic porous substrates were coated with catalyst particles and were bonded to a PDMS layer containing microchannels. A manual pump was used for pushing H\textsubscript{2}O\textsubscript{2} through the channels. In contact with catalyst particles, H\textsubscript{2}O\textsubscript{2} was broken into water and oxygen. Only oxygen could permeate through the hydrophobic membrane towards the skin.

An alternative to thermoresponsive drug carriers for engineering externally triggered systems is the iontophoretic drug delivery platforms. In these systems, charged drug molecules or drug carriers are placed between two electrodes and are moved along the electrical field to penetrate the skin. These systems have been previously used for transdermal delivery of drugs. For instance, sweat generating drugs were delivered to enhance the rate of sweat generation in human subjects. These systems can also be applied to wound care as they enable precise control over drug delivery. Iontophoretic delivery of nitric oxide was compared with its subcutaneous delivery for wound healing after skin flap surgery. The results indicated the superiority of the iontophoretic delivery in improving flap survival.

In the past decade, the use of jet injections has gained attention for transdermal delivery of therapeutics. Jet injectors generate high-speed fluid streams to penetrate the skin and deliver compounds to deeper layers of the skin. Jet injectors have already seen use in a clinical setting in the fields of skin remodeling and rejuvenation and are being explored for use in wound healing. *Kobus et al.* showcased the capability of the AirGent, a microjet injection device that is used to deliver compounds into the skin in a minimally invasive manner, to induce wound healing. *Kwon et al.* used a similar injector called the INNOJECTOR\textsuperscript{TM} to investigate the process of skin rejuvenation through HA injection. They discovered that the microtrauma which stimulated
surrounding tissue to an increase in collagen synthesis was caused by the activation of vimentin, a protein that plays a role in wound healing \(^\text{125}\).

Different approaches such as gene transfer using jet injectors are also receiving attention. Gene therapy generally relies on the use of viral vectors or nanoparticles, which possess safety issues and are limited in efficiency. The use of needles for gene transfer into rats has previously been established, however jet injectors showed a 100-fold higher efficiency. Kunugiza \textit{et al.} used the Shima Jet, a jet injector that has already seen use in humans, to co-inject HGF and prostacyclin synthase (PGIS). Treatment of mice models using this approach resulted in promoted wound healing \(^\text{126}\).

One important area of active delivery systems is the use of externally triggered microneedle-based platform. In one study, microfabricated tapered microneedles were used for insulin injection. The successful of these systems led to effective control in blood sugar level. These microneedles can be integrated with other actuation mechanisms such as ultrasonication devices, piezoelectric systems, and micropumps for active drug delivery \(^\text{127}\).

Iontophoresis is a well-established technology for transdermal delivery of compounds. Wearable iontophoretic drug delivery systems have already been fabricated and integrated with bandages and skin patches \(^\text{128}\). Similar to thermal stimulation, electrical stimulation has also been shown to positively impact the wound healing \(^\text{129}\). Iontophoretic drug delivery platforms, however, do not suffer from the risk of self-trigger due to changes in environmental temperature, which makes them more suitable for wound healing applications. In addition, these systems usually deliver therapeutics to deeper layers of skin and can enhance the drug concentration near the healing tissue. The application of these platforms for engineering smart bandages that enable active
control over the drug release kinetics is limited to a few examples. In one example, the benefit of electrical stimulation combined with the iontophoretic administration of zinc sulfate in the healing of abdominal surgical wounds in both diabetic and non-diabetic rats was demonstrated\textsuperscript{130}. In another study, the iontophoretic delivery of nitric oxide resulted in enhanced free flap survival in comparison to its delivery using hypodermic needles\textsuperscript{131}.

Overall, the active delivery of drugs for wound care is a relatively new and less explored area, which has significant potential for changing the current wound care practice. Identifying mechanism that can eliminate the unwanted drug release is important, but the realization of such platforms would significantly reduce the pain and morbidity of chronic wounds. Also, finding effective tools that allow the efficient transport of drug into deeper layers of skin and wound in a minimally invasive fashion could further improve the outcome of the utilized therapeutics.

**2.3. Smart Systems for Wound Monitoring**

Despite advances in wound healing technologies, there is still a need for devices that can provide diagnostic information, combat infection, and effectively heal chronic wounds by intervening in dysfunctional healing processes\textsuperscript{132,133}. Such systems could revolutionize the wound care practice and have profound effects on therapeutic outcomes. Smart systems, by allowing for sensing, responding, reporting, or a combination of such functions, can address many of the challenges associated with wound healing, particularly for chronic wounds. They also allow for better wound management, improving clinical outcomes by means such as detecting infections in a timely manner or providing alerts for patients. Sensors can be combined with active drug delivery systems to autonomously respond to potential infection or hyper-inflammation. These integrated
systems, which are summarized in the following sections, also have the potential to reduce healthcare costs for patients, hospitals, and insurance providers.

2.3.1 Dressings with Integrated Sensors

Current wound dressings are mainly designed to keep the injury site sealed and protected. Some of them release drugs or compounds that can prevent infection and help with faster healing. A key limitation of these dressings is their inability to provide information about the healing status and the conditions of the wound environment with regard to its pH, bacterial loading, tissue oxygenation, and level of inflammation. Sensors in the wound environment can provide important information that would expedite the decision-making process in wound care. In addition, they can decrease the frequent changing of the wound dressing. There are also significant benefits in reduction of healthcare cost and time of hospitalization. Chronic ulcers, lower infected acute wounds, and large full-thickness burns are the main variety of wounds that can be targeted by sensor technology. Several markers have been identified that provide information on important physiological processes such as vascularization and inflammation. In addition, another important condition that can prevent healing of wounds is infection; infection, if not treated at the early stages, can result in formation of established biofilms that are tough to treat. Once biofilm is established in necrotic tissue, surgical debridement and limb amputation might be required. Infected DFUs are the dominant cause of non-traumatic lower-extremity limb amputation. Sensors intended for use in wound care products should have specific properties, such as having suitable flexibility to conform to body contours; being non-toxic and immune-compatible; and being resistant to wound exudate, which has a varying pH and is rich with proteins and enzymes.
Researchers have developed many sensors that can measure important biomarkers in the wound environment. In this section, we review dressings engineered with integrated sensors.

2.3.1.1 pH Sensors

The pH of the wound bed is one of the significant biomarkers that can provide important information about the status of a wound throughout the healing process\textsuperscript{135}. It has been reported that more than 80% of chronic wounds with elevated pH are infected. Skin is naturally acidic, with a pH value in the range of 4.0–6.0. The acidic environment is thought to support proliferation of fibroblasts\textsuperscript{137,138}, promote angiogenesis and epithelialization\textsuperscript{137,138}, aid the release of oxygen from oxyhemoglobin\textsuperscript{139}, and control bacterial colonization\textsuperscript{140}. The pH of the tissue underlying skin is more neutral, with a pH of 7.4. Thus, wounding will cause exposure of this underlying tissue and alter the acidic environment at the site of injury\textsuperscript{138}. Also, chronic wounds often suffer from the cycles of ischemia-reperfusion injury; thus, their pH value is higher than that of regular healing wounds. Such wounds are also usually colonized with environmental pathogens and once infected may become more basic to further optimize bacterial growth and have an elevated pH of up to 10.0.

Various types of pH sensors have been fabricated for wound care applications. For example, several types of electrochemical pH sensors have been developed that can continuously monitor the wound environment. Electrochemical pH sensors usually utilize potentiometric measurement, conducting polymer pH sensors\textsuperscript{141,142}, metal oxide\textsuperscript{143}, ion-selective electrodes\textsuperscript{144}, and ion-selective field effect transistors\textsuperscript{145}. Potentiometric measurements have been used in engineering many wound dressings with integrated sensors. These sensors can be fabricated on stretchable, flexible substrates. In a notable study, a low-cost stretchable sensor was created by spraying
conductive inks and polymers on an Ecoflex substrate. The sensor had a Nernstian response to changes in pH response and showed reproducible data upon cyclic changes in the environmental pH. The sensor could form and maintain conformal contact with curved surfaces. Colorimetric sensors are another class of pH sensor that can be used to engineer bandages. These sensors are robust, easy to use, and can be used without integrated electronics. The readout of these sensors is usually based on image processing, although if the color change is sufficiently vivid, a naked eye view can be used for the estimation of pH value or identification of wound status. However, a key challenge associated with the utilization of luminescence systems is protecting the skin from the leached dye. In one attempt to combat this challenge, pH-responsive dyes were incorporated in mesoporous silica particles (MSPs) to prevent leakage during their use. The particles were then incorporated into flexible hydrogel fibers fabricated through microfluidic spinning. The fibers were then attached to a transparent medical tape for long-term monitoring of cutaneous wounds with the possibility of directly printing onto the medical tape. The pH of the wound was determined by processing images captured using smartphones. Image-based data analysis is susceptible to errors introduced due to lighting and image quality, so a hydrogel dressing was fabricated to solve this challenge where the hydrogel carried pH-responsive dyes. pH values were measured using an integrated photodiode that could wirelessly communicate with smartphones. Because integrating readout platforms or image analysis can be challenging, the idea of using dyes in which their color change is visible to the naked eye has been pursued. In one example, a dye was developed that could be loaded into wound dressings and would illuminate different colors upon exposure to UV light to predict the wound pH level.
2.3.1.2 Temperature Sensors

Measuring temperature can provide information regarding local blood flow and lymphocyte extravasation, along with wound infection and chronicity. A randomized study found a correlation between increased temperature and increased angiogenesis and fibrosis. A study of 35 patients with stage II–IV PUs found that ulcers with high temperatures healed more slowly than low-temperature ulcers, where the higher temperatures may be indicating the presence of critical colonization. Extreme hypothermia has also been correlated with unsuccessful wound healing and increased wound infection. Overall, temperature is a parameter that provides information on various factors relevant to healing, such as adequate blood flow, presence of infection, and oxygenation. As a result, temperature has been suggested as a marker that can be monitored to understand the level of inflammation or infection.

Similar to pH sensors, flexible colorimetric and electrochemical temperature sensors have been widely used in biomedical application. Microfabricated metallic resistive sensors are the most common type of flexible temperature sensors. One example dressing was fabricated from microfabricated arrays of temperature sensors for skin temperature mapping of cutaneous wounds. The conductive lines were stretchable and were fabricated on a flexible substrate and could form a conformal contact to the skin. The platform was integrated and could provide a map of temperature distribution in the wound area. To reduce the cost of fabrication, material conductive inks have also been used for the fabrication of these sensors. One key challenge associated with conductive inks is the change in their electrical resistance due to mechanical strain that can interfere with the accuracy of the temperature sensors. However, using suitable porous substrates can alleviate this issue significantly. In one example, a nanofibrous substrate was used to fabricate flexible sensors based on silver inks. The results showed a relatively linear response.
in temperature variations in the range of 25–35 °C. In another study, a nanocomposite of poly(styrene-b-(ethylene-co-butylene)-b-styrene) and multiwall carbon nanotubes was fabricated and used to engineer a dressing for monitoring DFUs and VLUs. One of the factors that affects the accuracy of the sensor readings is its conformal contact with the skin. To facilitate the adherence of sensors to skin for long-term use, octopus-mimicking surfaces were developed and utilized as a substrate for fabrication of printed carbon-based sensors. In general, using carbon nanotube-based materials to fabricate sensors has enabled engineering low-cost yet highly sensitive devices. However, the use of carbon nanotubes in biomedical engineering has been controversial due to their potential toxicity.

2.3.1.3 Oxygen Sensors

Oxygen is a requirement for the effective progression of the wound healing process, supporting the wound bed with cell proliferation, angiogenesis, collagen synthesis, and bacterial defense. Previous work has found conflicting results on the effects of oxygen in wound healing, with some studies suggesting that hypoxia induces angiogenesis and others suggesting that hypoxia enhances angiogenic cytokines. Some studies suggested a minimum tissue oxygen tension of 20 mmHg to promote wound healing, although this value was observed to be significantly lower in non-healing wounds. Despite these varied statements, oxygen is important at different stages in wound healing and must be studied alongside other factors when assessing wounds.

Chronic wounds have insufficient wound oxygenation due to inefficient vascularization. As a consequence, acute hypoxia in a chronic wound may be detrimental to the healing process and result in unnecessary tissue loss. Hence, monitoring tissue oxygenation provides valuable data
on wound healing. One study developed a flexible and wireless smart bandage with a customized oxygen sensor from off-the-shelf electronic parts to monitor the wound bed in real time. The oxygen sensor was structured using an electrochemical galvanic cell on a flexible parylene C substrate. In this platform, silver and electroplated zinc electrodes worked as the cathode and anode, respectively. This system was tested on a setup simulating wound environment. Another study developed a colorimetric sensor that could be painted on the surface of the wound to form a thin and stable layer with conformal contact, allowing for oxygen level monitoring. This platform utilized a reference dye and porphyrin-dendrimer phosphor that fluoresced in the presence of oxygen. The platform was successfully tested both ex vivo and in vivo to monitor the progression of burns and integration of skin grafts and to sense tissue ischemia. A key challenge in designing oxygen sensors is distinguishing the tissue oxygen level from the oxygen introduced topically from air. These sensors usually use an oxygen-impermeable coating to address this challenge, but this coating, in turn, can negatively affect the wound healing.

2.3.1.4 Moisture Sensors

Inflammation resulting from the initial injury enhances capillary permeability and the leakage of fluid from blood vessels. The majority of the exudate is produced during the inflammatory and proliferative stages of wound healing, but its production rate varies depending on the stage of the healing and the characteristics of the wound. Wound healing in a moist environment also was found to have a greater rate of revascularization and dry wounds also had a slower progression to the remodeling phase of wound healing. However, since some bacterial species grow better in a moist environment, excessive wound fluid may also increase the risk of bacterial infection. Thus, measuring the level of moisture in the wound environment can
provide information on the wound status and the proper intervention for managing the exudate and moisture level.

In a notable study, electrochemical sensors were used underneath dressings to monitor the level of moisture at the time of bandage change. The results showed that more than 40% of dressing changes occurred before the optimal time, which indicated the need to improve current clinical protocols.

2.3.1.5 Mechanical and Electrical Sensors

The mechanical and electrical properties of skin change in response to skin pathologies. In addition, the mechanical and electrical stimulation of a wound has been proven to facilitate wound healing and prevent infection. Thus, sensors that can monitor the stiffness and impedance of the skin or wound environment can provide important data on the tissue conditions. In a notable study, impedance spectroscopy using flexible electrode arrays was used to predict the skin condition and the onset of skin damage in response to excessive pressure. The results showed the suitability of the approach for predicting PUs. In a study on a rat model, the sensor couple map the impedance magnitude, phase angle, and damage threshold, which otherwise could not be detected with a naked-eye inspection. Another study developed a flexible fabric-based electrical pressure sensor that could map the applied pressure on skin for assessment of the chance of formation or deterioration of decubitus ulcers. The sensor array wirelessly could communicate with external devices. In one other study, a flexible strain sensor was fabricated on elastic biodegradable substrates suitable for use as wound dressings. The sensor operated based on measuring varying magnetic flux through a known area, which occurred when the sensor was stretched. Such sensors could provide information on patient’s movements and their potential risk in developing non-healing wounds. In general, wound care products equipped with sensing capabilities can
significantly change the current clinical wound care practices. The generated information can provide valuable insight on the status of the wound, which is especially important in the case of diabetic wounds, where regular signs of abnormality and infection such as redness, swelling, and hyperthermia are not as severe as those in regular wounds. Thus, current clinical approaches used for wound inspection are usually insufficient; the engineered smart bandages reviewed here could bolster current clinical practices, given that they can actively extract information about the wound environment. Currently, however, the number of markers that are being monitored are limited and the majority of the markers are nonspecific. Thus, there is a need to identify more suitable markers as well as design platforms that can integrate multiple types of sensors. A recent study reported different thread-based sensors for medical diagnostics that could measure pH level, glucose, temperature, and strain of a targeted tissue\textsuperscript{173}. Similar platforms could substantially enhance the impact of these smart bandages. Beyond this, flexibility and longevity of currently engineered sensors are not ideal for wound care applications and require improvement. One of the key limitations of sensors for wound care is their durability, as the wound environment is moist and concentrated with various proteins that can affect the sensitivity of the sensors in a short time.

2.3.2 Automated Wound Dressings

The majority of existing medical devices are either equipped with sensors or actuators that can deliver therapies. In recent years, along with the advancement of flexible electronics and packaging, multifunctional medical devices have been developed that can both sense and deliver therapeutics. In the field of wound care, the complexity of the physiological events has made the decision-making process extremely challenging. As a result, there has been a preference toward leaving the decision-making process to medical professionals. However, the recent progress in the
field of wound biology has improved our understanding of physiological processes. This progress, combined with the realization of miniaturized data processing systems and controllers, has enabled researchers to design systems that can sense and interpret data and decide on the delivery of therapeutics. A key challenge in designing automated dressings is the identification of suitable parameters to be monitored and pathophysiological processes to modulate. There have been several markers that have been related to (patho)physiological processes. For instance, tissue oxygenation has been considered as a measure of vascularization, while inflammation and infection have been identified by varying wound temperature, pH, and enzyme levels. The two pathophysiological processes for which modulation can benefit from automated systems are inflammation and infection. While inflammation is essential for healing, as it helps with removal of debris and dead tissue, hyper-inflammation should be avoided. Infection is one of the major pathophysiological challenges that prevents wound healing and necessitates surgical intervention. Although the delivery of antibiotics at early stages of colonization is more effective, the prophylactic use of antibiotics has shown limited success, can retard the healing rate, and can lead to the formation of antibiotic resistance\textsuperscript{36}. Thus, antibiotics should be used when needed to eliminate the side effects associated with infection or antibiotic overuse. Among different biological markers, pH has been used to design automated wound dressings. There are only a few examples of automated dressings published in the literature. A fully automated wound dressing was recently engineered that utilized an electrochemical sensor that could measure the pH of the wound\textsuperscript{174}. Data were processed by an on-board controller to detect potential pH values indicating infection. In this case, the controller could automatically trigger the integrated flexible heater to heat the hydrogel that carried drug carriers that could then release antibiotics. The effectiveness of the platform in treating infection was demonstrated \textit{in vitro}\textsuperscript{174}. In another recent study, a hydrogel-
based dressing could continuously monitor wound pH, monitor for infection, and deliver antibiotics into the wound bed, if necessary. This semi-automated platform utilized an immobilized pH-sensitive dye that could be imaged using a smartphone to determine potential infection. The dressing also carried thermo-responsive drug carriers that could be triggered using a smartphone app to release antibiotics upon infection. Automated dressings can revolutionize wound care, significantly improve the effectiveness of therapies, and enhance a patient’s comfort. However, there is a need to engineer dressings that can integrate sensors to detect more specific markers for better interpretation of biological events. In addition, the ability of releasing multiple drugs with different release profiles could enhance the effectiveness of such platforms.
Chapter 3- Research Hypothesis, Design and Fabrication of the System

In this chapter, the hypothesis of this research is discussed. Furthermore, all the materials which were used for engineering the drug delivery system and designed experiment are listed followed by details for fabricating different parts of the smart bandage.

3.1 Hypothesis

Many research and clinical methods have been tried so far to test a variety of single factors for enriching different physiological processes which will result in healing of chronic wounds \textsuperscript{176,177}. Because of the accessibility of cutaneous wound, usually topical delivery method has been applied for targeting the wound environment. In previous chapters, it was mentioned that various biological factors are needed at different stages of wound healing \textsuperscript{36,178}. Various advanced bandages have been designed with the capability of predicting the pathophysiological conditions and even actively delivering the drugs \textsuperscript{32,115,147,178,179}.

Despite the promising results which were achieved \textit{in vitro}, still in clinical transition, they fail to support the healing process properly. Passive dressing often does not directly affect the wound or eluting a drug into wound bed except making a physical barrier. Medicated dressing has this capability to enhance wound healing stages directly or indirectly. These medicated dressings contain some active agents which may help in cleaning or removing the necrotic tissue. One criterion which is ignored in their designs is the importance of the point of delivery on the efficiency of therapeutics in wound healing. In most cases, therapeutics are delivered topically in form of liquids, gels or ointments. It should be noted that usually there is a curst and a layer of necrotic tissue which covers the chronic wound. This layer significantly limits the accessibility of the wound bed. In other word, exitance of necrotic tissue which usually can be formed on chronic
wounds limits the healing and is an ideal location for bacterial colonization and growth. In addition, wounds discharge exudates which can wash out the topically delivered therapeutics or deactivate them because of the availability of different enzymes and protein into wound exudate. Therefore, it is expected from these conditions to reduce the bioavailability of drugs at the healing tissue. Here at this study hypothesis is to shorten the traveling distance of therapeutics through bypassing the necrotic tissue and doing direct delivery of drugs into the wound bed and live tissue to improve the effect of therapeutics on the healing process. A specific and unique type of bandage is designed and fabricated in this study. This wearable bandage is equipped with polymeric hollow miniaturized needle arrays (MNAs) for delivering vital pharmacological agents and growth factors into the wound bed. Figure 3.1 depicts the hypothesis of this study. Compared with the topical delivery of drugs, this innovative method is proved to be more effective in endorsing re-epithelialization and angiogenesis leading to wound closure and even hair growth.

*Figure 3.1: Schematic of the engineered bandage and its operation.*
Furthermore, the MNAs used in this study are minimally invasive, thereby induce minimal pain and inflammation compared to other invasive methods. In addition, this bandage is also equipped with a programmable electric driver for controlling the active drug delivery using micropumps. In addition, the system is capable to communicate wirelessly with smartphone and control the time point and dosage of different therapeutics simultaneously via an android application which is specifically designed for this approach.

3.2 Materials

Materials and reagents including NaOH, cefazolin, (3-Aminopropyl) triethoxysilane (APTES), HUVEC culture media, BCA Protein Assay kit 23225, Human VEGF 165, FBS, Geltrex matric, and Phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (MO, USA). Polydimethylsiloxane (PDMS) was purchased from Dow (MI, USA). 3D printing resins were obtained from Stratasys (MN, USA). Recombinant Mouse VEGF (VEGF 164) was purchased from Biolegend (CA, USA) and Anti-CD31 antibody ab28364 was obtained from Abcam (Cambridge, MA, USA).

3.3 Design and Fabrication of the System

The smart bandage is designed in a way to cost low and keep it flexible and light. As it shows in Figure 3.2, it was designed with two modules, a wearable bandage with integrated MNAs connected to the controlling module, which can communicate wirelessly with a smartphone in order to control the drug delivery rate. In next sections methods for designing/fabricating the different parts of the system is discussed.
Figure 3. 2: A representative photograph of the bandage for the delivery of multiple drugs.

3.3.1 Fabrication of Miniaturized Needle Arrays

Forming hollow channel inside the miniaturized needle in microscale is very challenging, and few studies have been able to fabricate a decent type of hollow MNAs. Micromolding as one of the major types of MNAs fabrication still deals with difficulties to form a hollow channel inside the miniaturized needle. Thanks to modern technology, 3D printing method is growing fast, and here MNAs were fabricated by a high-resolution FDM 3D printer (Objet 500 Connex3, Stratasys, USA). First, CAD model of MNA was designed in Solidworks (Figure 3.3). Then design was fabricated through 3D printing process.
This type of 3D printer coats the printing part with a flexible support material which needs to be removed. The support material of 3D printing is a gel-like soluble material for upholding the overhangs and helps for printing small cavities. Figure 3.4 shows the schematic of fabricating the MNAs. For removing the support material, 3% (w/v) NaOH was dissolved in DI water. Then the printed MNAs were placed in NaOH solution while the container was placed in ultrasonic water bath (Fisher Scientific) for 2 hr.
The material for printing the MNAs was a resin-based polymer (Vero clear, Stratasys, USA) and biocompatibility of this material is evaluated at this study and is discussed in next chapter. Using the 3D printing provided the capability for printing MNAs at different needle spacing (1.5-3 mm), needle length (0.8-3mm), base sizes (0.5-1.5 mm), and opening diameters (0.2-0.5 mm). Figure 3.5 depicts the microscopic view of printed MNAs with different arrangement and number of miniaturized needles.

![Microscopic image of sample MNAs.](image)

**Figure 3.5:** Microscopic image of sample MNAs.

A length of ~2mm was selected for MNAs to pass through the crust and part of the necrotic tissue, allowing drug delivery to the deeper layers of the wound. Noting the fact that patients with diabetic ulcers are typically suffering from neuropathy and lack of sensation in their limbs, MNAs insertion will not cause any pain in user patients. As it mentioned recently, forming an open hollow channel inside the solid miniaturized needle which is not clogged with some unwanted material is challenging. SEM images of MNA shows the hollow channel is formed properly (Figure 3.6).
Another interesting advantage of 3D printing was printing a MNAs having a mixture of material. As Figure 3.7 shows hard resin was used for printing the miniaturized needles and base is made from a flexible resin (Tango, Stratasys, USA).

**Figure 3. 6:** SEM images of i) front view and ii) top view of a single hollow miniaturized needle.

**Figure 3. 7:** Multi-material printing of MNAs for creating rigid needles on a flexible base.
3.3.2 Fabrication of Flexible Bandage Bonded with MNAs Islands

There are some studies which have designed drug delivery systems for having temporal control over the drug release. The point is they have not been successful in controlling the spatial distribution of medicine and getting proper access into the deeper layer of wound bed which is covered with necrotic tissue. In this study, a programmable integrated system is designed to have both temporal and spatial control on drug delivery by a flexible PDMS-based bandaged with two independent temporal profiles which is bonded to MNAs for having spatial control. PDMS is a well-known polymer which is popular to be used in many systems which deal with biomaterial. Here micromolding method was applied to make a flexible bandage which was made out of PDMS in a way to have two independent microchannels profiles. In addition, a flexible bandage was needed to have a conformal contact with the wound and also be biocompatible. PDMS was used to fabricate the flexible bandage due to its flexibility, low protein adsorption, biocompatibility, and oxygen permeability. The transparency of the bandage can also facilitate the visual inspection of the wound without the bandage removal. The mold for the bandage was made out of PMMA through laser cutting. Then the CAD model of microchannel was designed at Solidworks and was fabricated by the 3D printer. Finally, it was glued on PMMA-base mold. The mold was casted by PDMS (Sylgard 184) and considering the ratio of 1:10 (hardener: base) and heated for 2 hours at 80 C. Furthermore, a protocol was developed to bond the resin-based polymer on PDMS-based flexible bandage. Figure 3.8 depicts the schematics for making the PDMS bandage and bonding to MNAs islands subsequently.
Figure 3.8: Schematic showing the steps for fabricating the flexible bandage and bonding process between MNAs island and bandage.

For bonding MNAs on PDMS, first, MNAs were covered by (3-Aminopropyl)triethoxysilane (APTES) through the salinizing process. Bonding process of MNAs island on PDMS layer. Briefly 200 µL of APTES was dropped on the plate of the desiccator which is covered by aluminum foil, then MNAs was placed close to droplets and it was left under vacuum overnight. Covered MNAs was then activated (oxygenized) in the plasma cleaner chamber (Harrick Plasma, NY, USA) for 15 seconds at high mode, then stuck on a thin layer of PDMS which was made by the molding process and was oxygenized for one min at high mode in advance. The system was pressed by hand for five min and then was heated for two hours at 80 C while a 1lb of weight was placed on top of the integrated system. At the end, bonded MNAs on thin layer of PDMS-made base was bonded on top of microchannel layer. Two layers were plasma activated for 1 min at high mode, and immediately stuck to each other, pressed by hand for 1 minute then was left in the oven for one hour at 80 C. For keeping the good flexibility of bandage, the thickness of all two layers are designed to be ~ 1.5 mm. Figure 3.9 shows the flexible bandage including microchannels and MNAs island.
3.3.3 Engineering the Integrated Platform and Preparing Electric Drivers

To form a fully integrated bandage, peristaltic micropumps (Clark Solutions, MA) were utilized to suffuse the drug solutions into the microchannel arrays and then through the MNAs islands (Figure 3.10). To reduce the bandage operating cost and keep it flexible and light, it was designed with two modules, a disposable module with the MNA islands and microchannel arrays, and a reusable module, which housed the drug reservoirs, micropumps, power source, and electrical circuitry. The two modules were connected using two flexible silicon tubes.

For having a temporal delivery of therapeutics in a way to deliver them at the right time and right dosage an automated system was needed for our system. We chose two PRQ1 peristaltic micropumps (Clark Solutions, MA) to facilitate drug administration given its ability to accommodate a range of medication quantities while avoiding contamination. In order to run two pumps bidirectionally, we selected the L293D half H-bridge (LCSC, USA) as a regulator driver.
To accommodate the input voltage, drop from the L293D, an Arduino Nano Clone (Elegoo, USA) was chosen as the microcontroller for its 5V logic output. To provide wireless communication, the HC-05 Bluetooth module (Hiletgo, China) was selected. The circuit was then assembled on a breadboard and attached to an Arduino Uno clone for testing. Once the confirmed to be operational, a schematic and PCB layout was designed with the web software EasyEDA (easyeda.com). This was then sent to JLC PCB (jlcpcb.com) printing. Upon receipt of the circuit boards were assembled and tested. Once function had been confirmed, a cover box was designed in Solidworks and then was 3D printed. Figure 3.11 shows the electric driver box including the internal equipment. The system could be contained within a commercially available smartphone armband.
3.3.4 Wireless Software

The micropumps were controlled by applying a pulse width modulated digital signal from a microcontroller. The platform could be interfaced with smartphones through Bluetooth. An app was designed to regulate the remote programming of the bandage.

For maintaining proper function during administration; first, a simple code was written to apply a pulse width modulated signal from the Arduino pins. The code allowed the PWM value to be adjusted from 0 to 255 to simulate a variable voltage input to the pumps. This code was used to correlate the effective voltage input level to the volume of fluid dispensed. Next, an Android smartphone application was programmed using the drag and drop programming of MIT App Inventor (appinventor.mit.edu). This app allows the user to set pump dosing volume, either manually or on a timer, with the two pumps able to deliver different doses at different times. This creates the opportunity to provide two different drugs to the wound. To complete the software system, an Arduino program was written to read the input transmitted from the app via Bluetooth.
As it showed in figure 3.12, the smart bandage including the electric driver can communicate wirelessly with smartphone for controlling the true drug delivery.

![Image of integrated system operation on the human body.](image)

**Figure 3.12:** Image of integrated system operation on the human body.

The program then takes the user input and converts it to milliseconds. For the interval this is straightforward; however, the volume input was converted to the time which the pump must run at full speed to deliver the desired dose, the calculation was done based on the data from the correlation between pump’s Voltages vs. flow rate (will be discussed in coming chapters) showing that 0.47816 mL was pumped in 1 minute.
Chapter 4- Results and Discussions

At this chapter, it is tried to cover the details of the different experiments for testing the correct operation of the designed drug delivery system. These experiments were performed for characterizing the different aspects of the system including mechanical properties of the miniaturized needles, biocompatibility of the MNAs, strong bonding between MNAs island and flexible bandage, true operation of electric drivers and wireless system and release characterization of the MNAs in vitro. In addition, results of all these experiments were discusses.

4.1 Compression Test on MNAs

Miniaturized needles should be robust enough to reach to the wound bed without causing an extra problem for the wound. For instance, one of this problem could be breaking the miniaturized needle inside the tissue because of an unexpected force on miniaturized needle and it may worsen the situation for the wound. There the mechanical strength of MNAs island was evaluated at this study. For running the compression test a mechanical (CellScale Univert) was applied to measure the strength of miniaturized needles. First MNAs island was glued on lower jaw. Figure 4.1 shows the position of jaws and MNAs before running the force. Then Upper jaw was set to apply 200 N compression force. Device was set to apply force at different speeds (0.092 mm/s, 0.275 mm/s, 0.916 mm/s). The logical result was obtained when MNA was targeted by the speed of 0.092 mm/s. Then, this speed was set and repeated 4 times.
Figure 4.1: Image of MNA under compression by a mechanical tester machine.

Applied force at different time points and displacement of upper jaw were recorded and machine was set to keep the experiment until it reaches to 200 N force. As shown in Figure 4.2 and 4.3, the MNAs did not break and were only bent under a compressive force of ~ 78 N.

Figure 4.2: Microscopic view of MNA before and after compression test.
4.2 Penetration and Pulling-out of MNAs on Pig Skin

The characterization of MNAs penetration into pig skin was evaluated using a mechanical tester. For this reason, MNAs were glued to the upper jaw of the mechanical tester and a 3cm×3cm fresh pig skin was placed on the lower jaw. The jaws were moved until the MNAs were touching the skin and the device was run under the compression mode at the rate of 0.27 mm/s. After penetration, the samples were left at rest for 1 min and then they were retracted by running the mechanical tester under the tensile loading mode (at the same rate of insertion) to measure the pull-out force (Figure 4.4). No deformation and breakage were observed upon penetration and removal from the pig skin. (Figure 4.5).

![Figure 4.4: Penetrating MNAs into pig skin by mechanical tester device.](image)
Figure 4.5: Microscopic view of MNAs (i) before and (ii) after pig skin penetration test.

Results showed that the majority of the MNA penetrations on fresh pig skins occur with less than 2 N force and a full penetration was achieved with about 7 N. The pull-out force for the MNAs were measured to be about 2 N. The trace of full penetration of colored MNAs can be seen on the pig skin by red dots (Figure 4.6).

Figure 4.6: Characterization of the insertion and pull out force of the MNA islands applied to pig skin and image of pig skin targeted with painted MNAs.
4.3 Biocompatibility of MNAs

The biocompatibility of the resin-based MNAs was assessed by seeding the MNAs with endothelial cells and performing a live/dead assay. For running live/dead assay HUVECs cells were cultured in the endothelial growth media (Sigma-Aldrich) and were used up to passage 6. To assess the biocompatibility of the MNAs, they were coated with Geltrex diluted 1:20 in media at 37 °C. The MNAs were seeded with about 20,000 cells and after 1 day their viability was assessed using a Live/Dead™ Viability/Cytotoxicity Kit (Invitrogen, state) as per the protocol recommended by the manufacturer. In brief, samples were incubated with a mixture of 2μl/mL ethidium homodimer and 0.5 μl/mL calcein-AM in PBS for 10 minutes at 37°C. The samples were imaged using a Zeiss Observer fluorescence microscope, where live cells appeared as green while the dead cells appeared in red (Figure 4.7). As the result shows, cells were able to be alive during the experiment time.

Figure 4. 7: Live-Dead assay of 3D printed hollow miniaturized needles demonstrates good cell viability.
In addition, the effect of MNAs on cellular proliferation was assessed indirectly by measuring the metabolic activity of cells using a PrestoBlue assay (Invitrogen, state) as per the manufacturer recommended protocol. For this reason, 15,000 of cells were cultured in 24-well plates and MNAs of 7mm×7mm were interfaced with them. On days 1 and 3, the PrestoBlue reagent mixed with media (1:10 ratio) was added and the cultured cells were incubated for 1 hr. After that, the fluorescence intensity of the solution was measured using a Cytation 5 UV-Vis spectrophotometer (Biotek, State). Four samples were used, and the growth rate was compared to the cell cultured in multiwell plates without being interfaced with MNAs.

The assay showed that over the course of a 3-day culture, no statistically significant difference in cell proliferation was observed between the HUVECs cultured on MNAs and those cultured in multiwell plates as positive control (Figure 4.8). The duration of the experiments was selected based on the intended lifespan of the fabricated bandage.

![Figure 4.8: PrestoBlue assay of HUVECs cells on 3D printed needles demonstrates continued proliferation.](image)
One important factor in the use of MNAs is understanding their degradation rate in wound conditions. To assess that, MNAs were placed in exudate-mimicking solutions and their mass was measured over a period of 3 days. The solution contained 0.368g CaCl₂ and 8.29g NaCl (pH 8.2), which were dissolved into 1000 mL of distilled water and stirred for 10 min. Twelve MNAs were prepared as previously described, rinsed with distilled water, and fully submerged in 3 mL of exudate mimicking solution at 37°C. On days 1, 3, 5, and 7, the weight of the dry weight of MNAs was measured to determine the rate of mass loss. No significant change in mass was observed in the MNAs (Figure 4.9), suggesting an erosion resistivity of the selected materials.

**Figure 4.9:** Graph of erosion of hollow MNA in wound-mimic solution showing no change in mass of MNAs over time.

### 4.4 Strength of Bonding

The bonding strength between the 3D printed resin and PDMS substrate was measured using a peel-off test. Sample was designed to be fixed at the mechanical tester. This sample was made out of two cubes which one of them was printed out of same resin which was used for MNAs.
Following the method which was discussed previously, this cube was bonded to a PDMS-made cube (Figure 4.10).

![Resin-made cube bonded to PDMS-made cube.](image)

**Figure 4.10:** Resin-made cube bonded to PDMS-made cube.

As it is shown at figure 4.11, samples (n=6) were fixed between the grips of mechanical tester and was set in tension mode to apply the maximum force of 200 N.

![PDMS-Resin bonded sample under tension force.](image)

**Figure 4.11:** PDMS-Resin bonded sample under tension force.
Applied stress and strain on sample were determined based on recorded data including the displacement of grips and applied force at different time points. The results suggested an average bonding strength of 237 kPa (Figure 4.12) for the partial detachment of resin from PDMS substrate (Figure 4.13).

**Figure 4.12:** A representative stress-strain curve of peel-off test for assessment of the bonding strength resin to PDMS (left) And right plot shows the bonding strength of resin and PDMS substrates (n=6).

**Figure 4.13:** Separated resin sample from PDMS sample after tension test.
4.5 Correlation between Flow Rate and Voltage at Micropump

Correlation between micro pump voltage and its flow rate was measured. This data was essential for prospective programming for true wireless operation of the system. Micropump was ran in different voltages (0, 0.5, 1, 1.5, 2, 2.5, 3) and delivered amount of DI water was weighted every 30 s and up to 5 minutes by accurate scale. Considering the density of DI water at room temperature (997 kg/m³), the volume of delivered DI water was measured. this experiment was operated considering 3 replicates (Figure 4.14).

![Calibration plot of the micropump flow rate as a function of applied voltage.](image)

**Figure 4.14:** Calibration plot of the micropump flow rate as a function of applied voltage.

The minimum threshold of the pumps was determined to be 0.5V, which resulted in the flow rate of 43.6 µL/min. The data in Figure 4.14 was used to generate calibration curves to program the app and precisely control the flow rate.
4.6 Wireless Operation of System

To test the micropumps response to dynamic actuation, the drivers were programmed to periodically apply a constant maximum voltage of 3.0V. The response of the micropumps to dynamic actuation for 1 hr is assessed. Pump 1 was set to deliver 200 µL every 6 minutes, and pump 2 was set to deliver 400 µL every 10 minutes (Figure 4.15).

![Screenshot of wireless application with adjustable time interval and dose volume.](image)

*Figure 4.15: Screenshot of wireless application with adjustable time interval and dose volume.*

To confirming the true operation of the system, the electric board was connected to a voltmeter to monitor changing of voltage as a sign for automatic starting and stopping point for running the micropumps at selected time intervals. In addition, it was set to pump DI water and during the delivering process, the fluid was weighted by an accurate scale at selected time intervals. Based on the density of DI water at room temperature and recorded mass of delivered water, the delivered volume was obtained accurately. It can be seen that the pumping process stops as soon
as the applied voltage is zeroed. As expected, pump 1 (Figure 4.16) delivered dosage of 200 µL every 6 minutes, while pump 2 (Figure 4.17) delivered 400 µL every 10 min. It should be noted that the peristaltic pumps could reverse flow direction and generate negative pressure at the MNA islands.

**Figure 4.16:** Wirelessly operation of Pump 1 considering delivery rate of 200 µL every 6 min.

**Figure 4.17:** Wirelessly operation of Pump 2 considering delivery rate of 400 µL every 10 min.
4.7 Quantifying Concentration Pre and Post Delivery

The efficiency of the bandage in drug delivery was evaluated. For this reason, the potential drug adsorption to the engineered bandage was tested. BSA solution having concentration of 54 µg/mL was made by diluting in PBS. Solution was transferred in the drug reservoir and system was run for about 12 minutes continuously considering constant speed for micropumps. The container for collecting the delivered BSA solution was replaced every 2.5 minutes and all container had same volume of delivered solution. At the end, collected data was analyzed based on the standard curve to quantify the concentration at each time interval. This experiment was run considering 3 replicates, and ANOVA analyses was done to plot the graph. Similar method was applied for quantifying concentration of Cefazolin solution (16 µg/mL) into the bandage as model drug. As seen in Figures 4.18 and 4.19 the temporal changes in the drug concentration within the perfused solution was not statistically significant, suggesting low adsorption of proteins and antibiotics.

![Figure 4.18: Concentration of delivered BSA over time.](image)
4.8 Drug Delivery Assessment of MNAs on the Simulated Wound Model

To assess the importance of the MNAs for the treatment of chronic wounds, we developed an *in vitro* model resembling the crust and necrotic tissue covering the viable tissue (Figure 3.20). The *in vitro* model is comprised of a cell culture insert with 3 µm pore size coated by ~2 mm thick 3% (w/v) agarose gel; the interior of the well plate was filled with PBS representing the environment of live cells.

*Figure 4. 20: Schematic of the two-compartment *in vitro* model used for simulating chronic wounds covered by a crust and necrotic tissue used for comparing the topical and MNA-based drug delivery.*
Agarose gel has been used as skin phantom in several studies and offers porosity and texture similar to skin. To simulate topical drug delivery, 100 µL of drug solution was added on top of the agarose gel. To test the MNA-based delivery, a miniaturized bandage with a diameter of 10 mm (Figure 4.21) was fabricated by 3D printing and placed in the agarose gel followed by the delivery of 100 µL of the drug solution using a syringe.

![Figure 4.21: 3D printed MNAs with drug reservoir.](image)

Figures 4.22 and 4.23 represent the protein release over time, revealing that MNAs enabled a rapid delivery of proteins to the lower chamber representing the environment below the necrotic tissue in comparison to the drug delivered topically (**P < 0.0001, ***P < 0.001, ****P < 0.0001). The results suggest that 70% of the protein was delivered into the bottom chamber using MNAs after 180 minutes, while only 1% of the drug was delivered when Bovine serum albumin (BSA) solution was added on top of the agarose gel.
**Figure 4.22:** The concentration of the BSA at the bottom chamber representing the wound bed after the administration of 20 µg/mL solution of BSA through 2 mm thick agarose gel (3% w/v) within a cell culture insert.

**Figure 4.23:** The drug concentration after 5, 60, and 180 min post drug administration (**P<0.001, ****P < 0.0001).

### 4.9 *In vitro* Wound Healing Assessment

A similar methodology was applied for the assessment of the effectiveness of VEGF as an angiogenic factor on the culture of human umbilical vein endothelial cells (HUVECs) *in vitro*. For
the experiments, a 200 µm scratch was made in the confluent monolayer culture of HUVECs and VEGF was delivered. Four groups were studied including: 1) 50 ng/mL of VEGF in the culture medium (positive control); 2) no VEGF (negative control); 3) equivalent to 50 ng/mL of culture media delivered topically; and 4) equivalent to 50 ng/mL of culture media delivered using the MNAs. The data suggest that the group receiving VEGF through MNAs had a migration rate comparable to the positive control group which received VEGF in their culture medium and 100% scratch closure was achieved in 4 hrs. (Figure 4.24).

![Graph](image)

*Figure 4.24: Result of Scratch assay on the culture of HUVECs cells. (**p<0.001, ****p < 0.0001).*

The migration and the scratch closure rates in the group receiving VEGF topically was faster than the negative control. However, the migration rate in this group was significantly slower than the two other groups (Figure 4.25).
Figure 4.25: Micrographs of scratch assay on the culture of HUVECs receiving the following treatments: 1) 50 ng/mL of VEGF in the culture medium (positive control); 2) no VEGF (negative control); 3) equivalent to 50 ng/mL delivered topically; 4) equivalent to 50 ng/mL delivered using the MNAs.
Chapter 5- *In vivo* Study

Results from the *in vitro* study suggested the positive effect of MNAs in increasing the drug bioavailability at the site of healthy cells in the wound bed below the wound crust. To further investigate the potential benefits of MNAs in the treatment of diabetic and chronic wounds, an animal study was conducted on diabetic mice with cutaneous wounds. In addition, tissues were assessed by histological evaluation.

5.1 Animal Study

Homozygous mice for the diabetes spontaneous mutation (*Lepr*<sup>db</sup>) become identifiably obese around 3 to 4 weeks of age. On day 10 to 14 elevations of plasma insulin begin and at four to eight weeks the blood sugar level elevates. With a delayed wound healing and an increased metabolic efficiency, this mouse strain serves as a suitable model for chronic wound research.

5.1.1 Timeline of the Animal Study

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Nebraska, Lincoln. Five weeks old B6.BKS(D)-Lepr<sup>db</sup>/J mice were purchased from the Jackson Laboratory (Bar Harbor, ME) weighted between 34-43 grams and accommodated in University of Nebraska, Life Science Annex, animal facility for one week before the wound injury procedure. The animals were on a special food diet during the entire research period. Sex-matched controlled mice divided into three study groups including: 1) negative control (no treatment) (n=4); 2) test group receiving VEGF topically (n=4); and 3) test group receiving VEGF through MNAs delivery (n=5) and received two sessions of VEGF totaling n=13.
In day 1, all animals were anesthetized by 5% of isoflurane using anesthesia system, (VetFlo, Kent Scientific, Torrington, CT) via a nose cone. The hair on the dorsal region of animals was shaved using an electric razor. The skin was sterilized, and a full thickness skin cut of 1cm×1cm was created. All mice received Buprenorphine SR for pain relief at the time of surgery, and then the wounds were covered with a regular bandage. All mice placed back in separate cages individually after recovering from anesthesia. Animals were monitored for wound condition, vitals and wellbeing, and weight gain/loss trends daily. To simulate the real wound conditions covered by crust and necrotic tissues, treatments start point were on day 5 by which a complete crust was formed on the wounds. Murine VEGF was dissolved in PBS containing 0.1% (w/v) BSA at the concentration of 500 ng/mL used as animal vascular permeability factor. In topical delivery group, 100 ul of the solution was poured topically using a pipette on the top of the wound and the animals were let to sit for about 10 minutes, the wound was then covered by a fresh dressing. In MNAs delivery group, 100 µl of the solution was delivered using the MNAs and after 10 min, the miniaturized needles were removed and the wound was covered by a fresh dressing. Animals of negative control received no treatment. A similar procedure was followed on day 7 to deliver VEGF for a second round. Wounds were inspected every 2 days and the bandage was changed and a picture of the wound was taken using a digital camera. On day 19, all animals were sacrificed using 20% CO2 volume displacement (flow rate cage volume/per minute) anesthetic overdose and wound tissue was harvested and fixed in using 4% paraformaldehyde (PFA).

5.1.2 Wound Healing Result

The statistical analysis showed significant differences in the wound closure rate in the MNA group compared to the topical and control groups from day 13 post-surgery. On day 15, statistical
analysis showed significantly different wound closure rate in animals receiving VEGF by MNAs compared to the negative control group. On day 17 and 19, the wound closure in the MNA group was significantly faster than both topical delivery and negative control groups. At day 19 the average of wound size in the MNAs group decreased to 0.04 cm² with an average of 95% healing rate. At the end of the 19-day study, the animals in the topical delivery group reached about 55% closure, while the negative control group showed about 40% closure. No significant difference was observed in the wound closure rate of animals that received VEGF delivery topically compared to the negative control group (Figure 5.1-5.3)

![Figure 5.1: Assessment of the effectiveness of VEGF delivery MNAs on diabetic wound healing. Full thickness wounds (1 cm × 1 cm) were formed on the dorsum of diabetic mice. (A) Representative images showing wound healing progression in three mice groups (control (no VEGF) (n=4), topically applied VEGF (n=4), and MNA-based VEGF delivery (n=5)) over 19 days.](image)

Another important observation was the significant difference in the quality of wound healing. In all animals receiving VEGF through MNAs, hair growth in the new tissue was observed. Typically, full thickness injuries result in scarring, which results in the lack of hair growth. However, in the MNA group, there was scarring histologically at the edge of the wounds, but it was not visible grossly on the examination of the wounds at the end of the experiments. The observation of hair growth due to VEGF delivery might be due to better wound vascularization
and in growth and differentiation of the new tissue. It should be noted that the positive role of VEGF on new hair growth has been suggested previously. However, more detailed experiments are required to understand the mechanism of stimulating hair growth. Similar to many other studies, no hair growth was observed in the wound area for the animals in both topical delivery and negative control groups.

*Figure 5.2: Wound area of different groups throughout 19 days. Significant increase with an average of 95% wound closure observed in MNA-based VEGF delivery group while no delivery and topical delivery remained with average healing rate of 40% and 50%, respectively over time (*P < 0.05, **P < 0.01).*
5.2 Histological Evaluations

Frozen sections of haired skin were stored in a -80 °C ultralow freezer prior to sectioning on a cryotome. The sections of skin were cut 10 µm thick using a Thermo Scientific CryoStar NX50, routinely stained with hematoxylin and eosin on a Leica ST5020 H&E stainer, and coverslipped with a Leica CV5030 coverslipper. A veterinary anatomic pathologist who is board certified by the American College of Veterinary Pathologists performed all histological evaluations. With histological evaluation, the junction of the surgical wound and adjacent skin of all treatment groups had a healing site, which was mildly to moderately thickened by granulation tissue. This junctional area of granulation tissue bordering the normal haired skin from peripheral to the wound, contained entrapped pilosebaceous units. The appearance of the junctional skin was indistinguishable among all of the mice, indicating that the shrunken wound sizes in the MNA treatment groups had followed orderly wound healing with regrowth of all dermal, follicular, and epidermal elements through nearly the entire wound site. The granulation tissue from within the center of the surgical
wounds in the treatment groups that had not undergone healing was associated with alopecia, deposition of collagen, and neovascularization, compatible with cutaneous scarring (Figure 5.4).

**Figure 5.4:** H&E staining for characterization of granulation tissue and neovascularization as well as hair growth in the skin in all three study groups shown in two different magnifications. The ulcers with an underlying bed of granulation tissue are denoted with black arrowheads and hair growth is denoted with white arrows.
Chapter 6- Conclusions and Future Look

The field of wound healing has been growing rapidly and a large number of groups are investigating various aspect of wound pathogenesis. Chronic wounds remain a major concern of the healthcare system. With a growing number of diabetic patients, the number of diabetic wounds and ulcers will likely increase. Numbers of burns, PUs, and other wounds are also expected to grow, especially in elderly populations. The changes in living standards in developed countries have affected healthcare products and their goals. Currently, tools and devices for cosmetic applications are becoming more important as scarring and some other skin disorders such as keloids can exert psychological discomfort. Thus, there have been substantial efforts in developing more effective therapeutics to treat different types of wounds. These efforts have resulted in the identification of growth factors, proteins, and drugs that can improve or modulate physiological processes that affect wound healing. In addition, various drug delivery strategies have been developed and mastered to more effectively deliver the therapeutics to the wound bed. However, recent developments in the field of flexible and wearable electronics have enabled the generation of new class of dressings that can actively help with understanding the wound condition or the interference of the tissue healing. Although these dressings provide invaluable information about the wound status, they do not target specific markers. One area that can substantially improve the field is the development of wearable biosensors that can detect proteins and antigens in the wound bed. Considering that infection is a key challenge in wound care and can even lead to life-threatening conditions, sensors that can directly measure the bacterial loading would be extremely valuable.

Wearable electronics and devices have also been used to deliver drugs and factors to the wound bed with a more controlled profile. Since the wound environment is dynamic and the rate
of physiological processes varies at different healing stages, the ability to actively control the release of drugs and factors can result in faster healing. However, the majority of these systems suffer from passive release, and this passive release can lead to complications. For example, if bacteria are exposed to an insufficient concentration of antibiotics, they can develop resistance against the antibiotic, which is a major healthcare problem. In addition, chronic wounds exude and are covered with dead or necrotic tissues. The emergence of automated bandages and telemedicine is expected to change clinical practice, especially in remote areas. In the field of wound care, more automated dressings that can sense and deliver therapeutics automatically or semi-automatically would significantly improve a patient’s comfort and reduce the complications associated with these wounds. Here, we developed a programmable platform with the capability of actively controlling the release profile of multiple drugs. The platform benefits from multiple miniaturized pumps wirelessly controlled through an in-house smartphone application. All the electronics were integrated into a smartphone sized module that could be reused. To improve the bioavailability of drugs and active compounds at deeper layer of the tissue, the bandage was equipped with islands of MNAs. The effectiveness of the MNAs in transferring the active compounds through the wound crust and necrotic tissues were successfully demonstrated in vitro. The platform was then utilized for the delivery of VEGF for the treatment of 5-day old full thickness skin injury in diabetic mice. The results showed a significant difference in wound closure and a fundamental difference in healing quality. The animals received VEGF through the MNAs showed signs of complete healing and the lack of scar formation. Our data suggested that in addition to the active compounds and their release profile, the point of delivery should be considered as equally important in treating chronic wounds. Overall, this thesis launches an innovative method for treatment of chronic
wounds, which will provide pathways for future studies to enhance existing wound care techniques.
References


8. Greaves, N. S., Ashcroft, K. J., Baguneid, M. & Bayat, A. Current understanding of molecular and cellular mechanisms in fibroplasia and angiogenesis during acute wound


15 Zhao, R., Liang, H., Clarke, E., Jackson, C. & Xue, M. J. I. j. o. m. s. Inflammation in chronic wounds. **17**, 2085 (2016).


17 Butzelaar, L., Ulrich, M. M. W., Mink van der Molen, A. B., Niessen, F. B. & Beelen, R. H. J. Currently known risk factors for hypertrophic skin scarring: A review. *Journal of*


76 Takada, K. et al. Usefulness of basic fibroblast growth factor (bFGF) loaded dissolving microneedles for local therapy of skin wounds. **4**, 256 (2013).


