2015

Multi-platform arabinoxylan scaffolds as potential wound dressing materials

Donald C. Aduba Jr
Virginia Commonwealth University

MULTI-PLATFORM ARABINOXYLAN SCAFFOLDS AS POTENTIAL WOUND DRESSING MATERIALS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biomedical Engineering at Virginia Commonwealth University

By

Donald C. Aduba, Jr.

Bachelor of Science, University of Virginia, 2008

Master of Science, Virginia Commonwealth University, 2012

Director: Hu Yang, Ph.D., Associate Professor, Biomedical Engineering

Virginia Commonwealth University
Richmond, Virginia
August 2015

Acknowledgement

I would like to thank the Lord for all the blessings you have provided for me, my family and friends to get to this point, encountering all the life's obstacles but still standing strong. To still be here enjoying life with my support system is a great blessing and without you, I wouldn't be here today. I want to thank Dr. Gary Bowlin for giving me the chance to attend VCU. He had enough faith in me that despite my mediocre undergraduate grades saw I had enough potential and drive to succeed at VCU. I would like to thank Dr. Rosalyn Hargraves for her instrumental support, particularly in my early years of VCU. She always looked out for me and is someone of color I can look up to as a source of inspiration and hope. I want to thank Dr. Ansley Abraham and Tammy Wright from the Southern Regional Education Board for funding my doctoral program in a time I needed financial support. I want to thank my advisor Dr. Hu Yang for his unwavering support and guidance during my six years at VCU. He allows me to explore and do my work freely while expecting quality in return. I appreciate his positive demeanor which allowed me to push through the difficult periods of research and any personal issues. It was a great six years and look forward keeping in touch. I appreciate Dr. Andrew Yeudall for providing his lab space, selfless support and expertise in cell biology. Thanks to Dr. Rebecca Heise, Dr. Bowlin and Dr. Chris Lemmon for their expert feedback and suggestions to help me grow as a doctoral student. I thank Dr. Todd Kitten, Dr. Seon-Sook An and Dr. Joseph Turner for their assistance in my drug release and microbiology studies. Thanks to all my lab mates the last three years: Larry (Dr. Xu), Olga (Dr. Zolotarskaya), Mike, Jingfei, Jeff, Chad and Natasha. You all are great and will definitely be missed. Lastly, I want to thank my friends and family for making me who I am today in this life: Mom, Dad, Naza, Ike and my girlfriend Shara. It's been a very long road but we did this together and will enjoy this achievement together. I love you all.

Table of Contents Page

List of Figures

Page

List of Tables

Page

List of Abbreviations

AX	Arabinoxylan
AXF	Arabinoxylan ferulate
CFU	Colony forming unit
BHI	Brain heart infusion medium broth
ECM	Extracellular matrix
FBS	Fetal bovine serum
FFT	Fast fourier transform
G3.0 PAMAM	Generation 3.0 poly(amido amine) dendrimer
GAG	Glycosaminogylcans
GEL	Gelatin
GEL-AXF	Gelatin-arabinoxylan ferulate blend
HA	Hyaluronic acid
HFP	Hexafluoro-2-propanol
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
ICP-OES	Inductively coupled plasma optical emission spectroscopy
LAL	Limulus amebocyte lysate
LB	Lysogeny broth
mPEG	Methoxypolythylene glycol
MMP	Matrixmetalloprotease
MTS	Mechanical testing system
NIH3T3	Mouse embryonic fibroblast cell line
NS	Nanofiber scaffolds
PBS	Phosphate buffer saline
PCL	Polycaprolactone
PEG	Polyethylene glycol
PEG-DA	Polyethyene glycol di-acrylate

PEO	Polyethylene oxide
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic acid)
PGs	Proteoglycans
PVA	Polyvinyl alcohol
SEM	Scanning electron microscopy
TGF-α	Transforming growth factor
UV	Ultraviolet light
VEGF	Vascular endothelial growth factor

Abstract

MULTI-PLATFORM ARABINOXYLAN SCAFFOLDS AS POTENTIAL WOUND DRESSING MATERIALS

By Donald Chukwuemeka Aduba, Jr., Ph.D.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biomedical Engineering at Virginia Commonwealth University.

Virginia Commonwealth University, 2015

Research Director: Dr. Hu Yang
Associate Professor, Biomedical Engineering

Biopolymers are becoming more attractive as advanced wound dressings because of their naturally derived origin, abundance, low cost and high compatibility with the wound environment. Arabinoxylan (AX) is a class of polysaccharide polymers derived from cereal grains that are primarily used in food products and cosmetic additives. Its application as a wound dressing material has yet to be realized. In this two-pronged project, arabinoxylan ferulate (AXF) was fabricated into electrospun fibers and gel foams to be evaluated as platforms for wound dressing materials. In the first study, AXF was electrospun with varying amounts of gelatin. In the second study, AXF was dissolved in water, enzymatically crosslinked and lyophilized to form gel foams. The morphology, mechanical properties, porosity, drug release kinetics, fibroblast cell response and anti-microbial properties were examined for both platforms. Carbohydrate assay was conducted to validate the presence of arabinoxylan ferulate in the electrospun GEL-AXF fibers. Swelling and endotoxin quantification studies were done to evaluate the absorptive capacity and sterilization agent efficacy respectively in AXF foams. The results indicated successful fabrication of both platforms which validated the porous, absorptive, biocompatibility and drug release properties. The results also exhibited that silver impregnated

AXF scaffolds inhibited growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* bacteria species, anti-microbial properties necessary to function as advanced wound dressing materials. Future work will be done to improve the stability of both platforms as well as evaluate its applications in vivo.

Keywords: *Absorptive, arabinoxylan, biocompatibility, non-toxic, advanced wound dressing, foam, nanofiber, biopolymer*

CHAPTER 1: INTRODUCTION

Fabrication of a functional wound dressing material is critical in managing wound healing. Wound dressing materials have been developed to help treat acute wounds by creating a moist microenvironment conducive to regeneration of new tissue while preventing infection at the injury site. An ideal wound healing material scaffold must possess the following properties to help aid wound management and healing. It is expected to have good biocompatibility. It is expected to have appropriate mechanical strength for insulation, wound protection and exudate removal from the injury site. A wound dressing material should be absorptive, impermeable to bacteria and inexpensive.

Many current wound dressing materials on the market possess the aforementioned properties but none have used arabinoxylan (AX) as a base material. AX has the ability to absorb exudate at the injury site. Thus, more research needs to be done to examine whether AX can serve as a wound dressing material that facilitates efficient healing of acute, moderately exudating wounds. AX possesses appropriate properties of an acute wound dressing material with potential to improve exudate distribution and wound biological environment. AX is very hydrophilic and possesses the ability to absorb large amounts of water – up to 100 grams of water per gram of polymer. Also, it is bio-inert and not vulnerable to physical changes by surrounding ionic environments.[1] There is little research studying the biocompatibility of pure AX scaffolds in vitro or in vivo. In this study, the physical and biocompatible properties of two arabinoxylan ferulate (AXF) based formulations are evaluated:

1) Electrospun Arabinoxylan ferulate-Gelatin (GEL-AXF) nanofiber composites
2) Lyophilized Arabinoxylan ferulate (AXF) foams

These formulations studied highlight the versatility yet simple fabrication design for a polymer that has an untapped niche in the wound dressing market. The goal of this study is to investigate in vitro whether arabinoxylan ferulate based formulations are compatible wound dressing materials aimed to treat acute, moderately exudating wounds.

CHAPTER 2: Review of polysaccharides as wound dressing materials

Donald C. Aduba, Jr.[1], & Hu Yang[1,2]

[1]Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284

[2]Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298.

Abstract:

Longer life expectancies, an increased elderly population and a higher prevalence of dietary disorders such as diabetes have led to an increased global demand for wound care. Wound dressing materials are critical for wound care because they provide a physical barrier between the injury site and outside environment, preventing the wound from further damage or infection. Wound dressings also manage and even encourage the wound healing process. There are many different types of wound dressings that exist in the market, applied specifically against particular types of wound classified by their condition, shape and other pathologies to encourage more efficient healing. Polysaccharide biopolymers are slowly becoming popular as modern wound dressings because they are naturally derived, highly abundant, inexpensive, non-toxic and non-immunogenic. However, there is no thorough review of this class of polymers and their applications as wound dressing materials. This review primarily focuses on polysaccharide platforms such as nanofibers and hydrogels designed and tested by research groups in vitro and in vivo to evaluate their potential as wound dressing materials. In addition, a brief background of the anatomy and physiology of skin, their function and relevance in wound healing is discussed. Other important discussion points such as acute and chronic wound healing wound management and wound dressing types are included. This comprehensive review will aim to focus on the properties of polysaccharide materials which will hopefully be the impetus towards further investigation of this class of polymers in wound dressing development.

Keywords: *wound healing, wound dressing, foam, nanofiber, hydrogel, wound management, skin*

2.1 Introduction

Wound care due to traumatic injury, surgery, burns and disease is an overlooked but growing problem in the United States. According to the National Center for Health Statistics, 40 million inpatient surgical procedures were done in the United States in 2000.[2] These procedures are implicated in emergency care, natural disasters and in war battlefields. Unfortunately, over 6.5 million Americans suffer from chronic wounds post-surgery which leads to an estimated $6 to $15 billion spent annually in health care costs.[3] In 2010, the market for wound care products was estimated at $15.3 billion.[2] Acute wounds often arise from incisions of skin created from trauma, surgery and superficial burns. Acute wounds typically heal quickly and tissue affected by the incision is fully restored. However, chronic wounds which are more difficult to heal stem from systemic diseases such as diabetes and obesity.[2] Aging also plays a role in limiting wound healing due to the skin losing elasticity with time. These wound pathologies will put a further strain on hospitals globally with projected populations of individuals with diabetes, obesity and being over 65 more than doubling by 2050.[4, 5]

Fabrication of a functional wound dressing material is critical in managing wound healing. Wound dressing materials have been developed to help treat acute wounds by creating a moist microenvironment conducive to regeneration of new tissue while preventing infection at the injury site. A wound dressing material is expected to possess the following properties: It must have good biocompatibility. It should have appropriate mechanical strength for insulation, wound protection and exudate removal from the injury site. A wound dressing material is also expected to be absorptive, impermeable to bacteria and inexpensive.

2.2 Anatomy & physiology of skin

The skin is the largest organ of the body but also requires a lot of maintenance. This is required for the skin to function in all the conditions faced by the body. The skin protects the rest of the body from trauma, regulates the body temperature and is the first barrier of the immune response. The skin also serves as a unit of metabolism and communication.[6] The skin is the largest organ in the body due to its large surface area. The skin of an adult covers 3000 square inches and weighs approximately seven pounds because of the large volume. The skin also demands over one-third of the body's blood circulatory system. The skin's large barrier allows the internal organs to enjoy a homeostatic environment. Skin has an inherent ability to self-regenerate and can recover from mild injuries due to physical trauma or chemical damage.[6] The ability of the skin to recover may be dependent on where on the body the injury took place. The skin thickness varies in different parts of the body for organ specific protection.[6]

The skin is broken down into two layers which provide different structural properties and functions; the outer and inner layers are the epidermis and dermis respectively. Each layer has sub-anatomic regions which have different functions. The epidermis is the outer most skin layer which is avascular and has relatively uniform thickness throughout the body. The average thickness of the epidermis is 75-150 μm although the palms and feet exhibit thicknesses between 400-600 μm.[7]

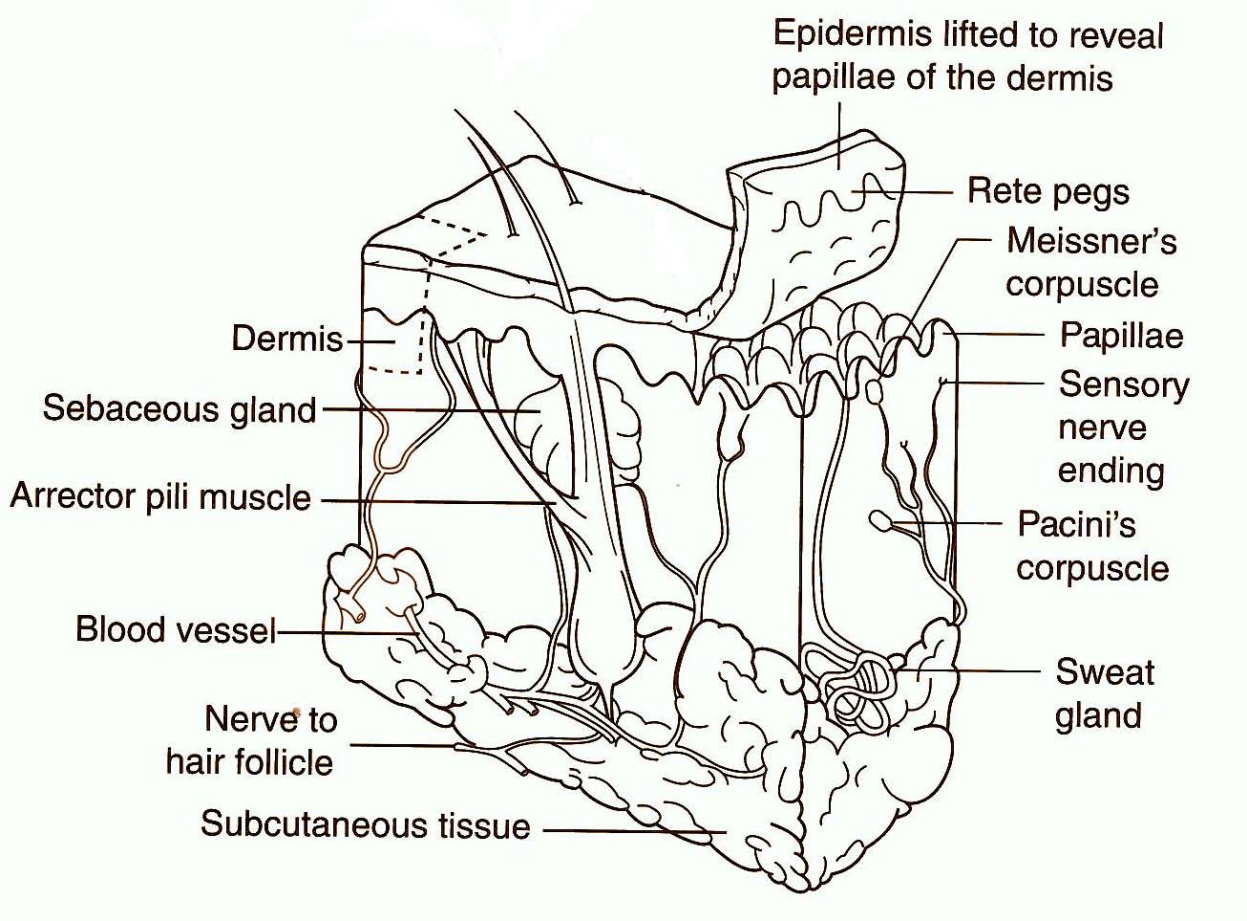


Figure 2.1: Schema of anatomy of skin adapted from Hooper et al. [8]

2.2.1. Epidermis

The epidermal layer is comprised of keratinocyte epithelial cells which are further broken down into the following five layers: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. (**Figure 2.2**) The stratum corneum is the top layer of the epidermis and is made up of terminally differentiated keratinocytes. They are highly fibrous due to keratin protein making up 80% of the cell. They are flat, pancake shaped, anucleated cells that constantly slough off the skin during everyday activities such as handwashing, changing clothes, bathing and exercising. Keratin's highly fibrous nature allows the cells in this layer to withstand

environmental changes in pH and temperature. The stratum lucidum is a very thin layer beneath the stratum corneum. It is only 1-5 cells thick and is typically absent from parts of the body where the epidermis is not thick. In this layer, active lysosomes are present to degrade the keratinocyte nucleus before they move up to the stratum corneum.[9] The layer beneath the stratum lucidum is the stratum granulosum. It is the granular layer in the epidermis whose cells are diamond shaped in morphology. The cell's nucleus is still active and has protein components such as profilaggrin, intermediate keratin filaments and lorcrin that are organized by the keratohyalin granules. This layer is only 1-5 cells thick. The stratum spinosum is beneath the stratum granulosum and is a layer above the stratum basale. The stratum spinosum contains three dimensional oblong shaped cells that has new batches of keratin filaments.[10] It is the thickest of the epidermal sublayers.

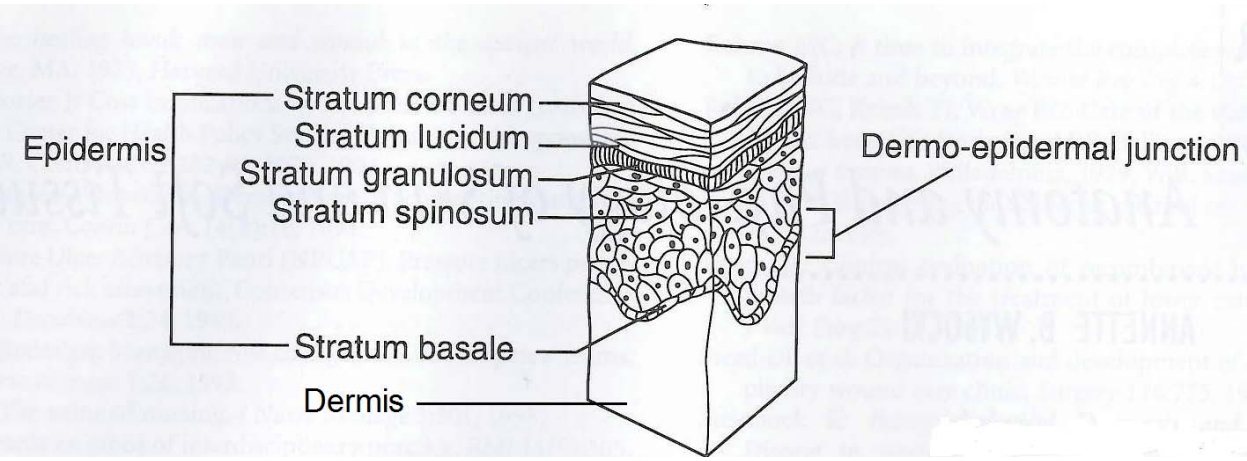


Figure 2.2: Schema of anatomy of subcutaneous tissue adapted from Hooper et al. [8]

Last, the basal layer of the epidermis, the stratum basale is home to keratinocytes that actively respond to extracellular matrix, growth factors and other biological cues. Glucose metabolism takes place in this layer as the metabolite permeates less to the upper layers in a gradient fashion. The basal keratinocytes are very active by traveling through the epidermal

layers in approximately 28 days.[10] During this migration process, the basal cells differentiate. Also in the basal layer, exists the dermo-epidermal junction, which contains valleys called rete ridges that help anchor the dermis in place and provide structural integrity. In addition, it is a source of basal stem cells. These stem cells are slow growing but produce daughter transient amplifying cells that make up 50% of the entire basal cell population. These cells have much shorter mitotic cycles (36 hours) during wound healing compared normal keratinocyte cells (300 hours).[11]

2.2.2. Dermis

The dermis is the thickest layer of the skin and is highly vascularized and innervated. It is modestly populated with fibroblasts. It has an average thickness of 2 mm and varies in different parts of the body. The dermis' vasculature allows the skin to receive nutrients and signals for important functions in homeostasis, wound healing, immune response and inflammation. The vasculature of the dermis also frequently involves angiogenesis due to the presence of important growth factors such as vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF). These growth factors are modulated by hypoxic conditions during the inflammatory stage of wound healing or in tumor growth.[6]

The major proteins present in the dermis are: elastin and collagen. Within these protein fibrils are ground substance which are matrix components comprised of proteoglycans (PGs) and glycosaminoglycans (GAGs). PGs and GAGs provide flexibility to the dermal matrix because of its high water absorption capability. Hyaluron is another matrix component which provides binding sites for important growth factors and cells relevant to skin regeneration.[12] Elastin is a

secondary structural protein in the dermis that provides elastic properties to the skin. Elastin has a coil configuration which winds and unwinds as it undergoes cyclic tension.[13-15]

All of the ECM components that build up the dermis are classified into two layers: the papillary and reticular dermis. The papillary dermis is located at the dermo-epidermal junction just below the epidermis. This area is made of interlocking papillary loops that provide blood circulation and nutrients to the area. The collagen fibers are much smaller in this layer than the reticular dermis but have more ground substance than the reticular layer. Meanwhile, the reticular layer is the most basal layer in the dermis and contains larger collagen fibers with less ground substance than the papillary layer.[6]

Collagen is the major structural protein and source of nourishment in the dermis.[16] It is produced by fibroblasts and its construction determines if the dermis is fully healed after wounding. It makes up 25% of dry protein weight in mammals and is best known for its great tensile strength. [17] Collagen is critical in wound healing because it serves as a reservoir for cell attachment, proliferation and differentiation.[18] It also is a guide for cell migration and helps catalyze angiogenesis. Collagen is ubiquitous in the skin and native in organs throughout living mammalian systems.[18] There are two types of collagen primarily present in the body: type I and III. Type I and III collagen composes of 77-85% and 15-23% of total collagen respectively. There are other types of collagen such as type V and type VII that are present in trace amounts.[19] Collagen in healthy dermis is a network of fibers intersecting perpendicularly into a woven mesh. In compromised dermis tissue, collagen is poorly organized which leads to excessive scarring.[16]

2.2.3. Skin function

The skin provides protection from mechanical trauma, injury from chemicals, pathogenic infiltration and UV radiation. Skin is also key for water retention. A study in literature highlighted individuals who had skin burns lose six times as much fluid as normal individuals.[20] The skin is able to conserve the body's general homeostasis because of its barrier like properties that typically inhibits high molecular weight drug permeability. The tensile and elastic properties of collagen and elastin give the skin strength to withstand forces.[6]

The stratum corneum is the primary line of defense against pathogenic organisms. This layer eliminates microbes either through shedding or by function inhibition.[21] During stratum corneum shedding, microbes that would have otherwise been attached to the skin fall out. On the other hand, the sebaceous glands in this layer produce a lipid substance called sebum which has a slightly acidic pH range from 4 to 6.8.[22] This acidic consistency with natural anti-bacterial components in the substance prevent microbe growth. [22]

Flora is present in the skin which also assists in reducing bacterial growth by inhibiting its function.[23] The key bacterial species inhibited by skin flora include: *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*.[24] These bacterial species are pathogenic and create chronic wounds because of producing high levels of toxins that lead overexpression of inflammatory cytokines.[25] Biofilm formation results from these bacteria after they become embedded in a polysaccharide matrix. The biofilms can colonize and express resistance against antibiotics and host immune response.[26] Thus, it is important that skin flora can regulate levels of pathogenic bacteria to prevent systemic inflammation and shock.

Several cell types within the skin are key players in the skin's immune response. They are Langerhans cells, mast cells, macrophages and dendrocytes. Langerhans cells are antigen

presenting cells that recognize and bind to T-cell receptors that destroy the invading microbe. Tissue macrophages come from bone marrow derived monocytes and have a variety of functions. First, they have a receptor which binds antigens on its surface that later is recognized by T-cells in the immune response. They engulf and phagocytose microbes while producing important growth factors and cytokines for wound healing and tissue remodeling.[12] Mast cells are located in the papillary dermis and at the junction between organ tissues and their surrounding environments. They are called upon during inflammation to send chemical cues such as histamine and vasodilators to induce phagocytosis of microbes, parasites and other injury debris as wound healing transitions from the inflammation to proliferation stages.[12] Dendrocytes are located in the dermal layers and function primarily as phagocytic cells that are highly expressed in diseased skin states.[27]

The skin thermally protects the body because it is a physical barrier between the internal organs and outside environment. The skin also has more sophisticated physiological mechanisms in place to control the body's temperature. These mechanisms are through blood circulation and sweating.[6] Blood circulation in the skin can vasoconstrict to help the body retain heat in cold environments. In hot environments, blood vessels vasodilate to release heat out of the skin to help the body stay cool.[9] In more pathological body states like a fever, the body needs to increase its caloric and fluid uptake by 13% for every 1° C the body core temperature increases. During rest, over 70% of heat production comes from the brain, trunk and visceral organs. In exercise, the skin produces 90% of the heat output.[28] Sweating is the other skin thermoregulation mechanism dictated by the function of eccrine sweat glands. They are coils invaginated from the epidermis to dermis layer. They are located mostly on the palms and soles of hands and shoes respectively. There are 2 to 5 million of these glands with sizes of 0.05 to 0.1 millimeters.

Eccrine glands are stimulated by temperature changes and nervous system effects. The sweat glands produce fluids that are 99-99.5% water but also contain phosphate, urea, sulfate and sodium chloride ions.[22, 29] Sweating controls temperature either through the retention, release and evaporation of sweat that maintains the body's core temperature.

2.3 Wound healing process

The wound healing process is highly complex and requires extensive communication among extracellular matrix components, cells and signaling factors that fluctuate during each of the wound healing stages.[16, 30] The acute wound healing process involves a progression of connected stages where cellular and tissue matrix changes are made to help remodel damaged tissue with new tissue. There are four stages in normal wound healing: hemostasis, inflammation, proliferation and remodeling.[31, 32] (**Figure 2.3**) Hemostasis begins immediately after the skin undergoes an incision or trauma. Bleeding is initiated to wash out bacteria and other pathogenic agents from the wound. Next, platelets are recruited to the injury site to minimize the bleeding, protect the wound and recruit other cells involved in the wound healing response. Platelets create a temporary extracellular matrix which become the destination of the recruited cells. [16] They also activate the clotting cascade to produce tissue factor seven that leads to fibrin formation and a scab at the injury site.[31,33] The inflammation phase begins about one day after injury where cytokines and chemokines produced by platelets activate vasodilation and angiogenesis of the injured area. [16] Subsequently, leukocytes are recruited to destroy bacteria and later convert into macrophages that engulf dead cells and debris, produce pro-inflammatory cytokines to recruit fibroblasts and keratinocytes.[16, 34] Recruited fibroblasts and keratinocytes secrete fibroblast growth factor (FGF) and transforming growth factor (TGF-α) among other cell signals produced.

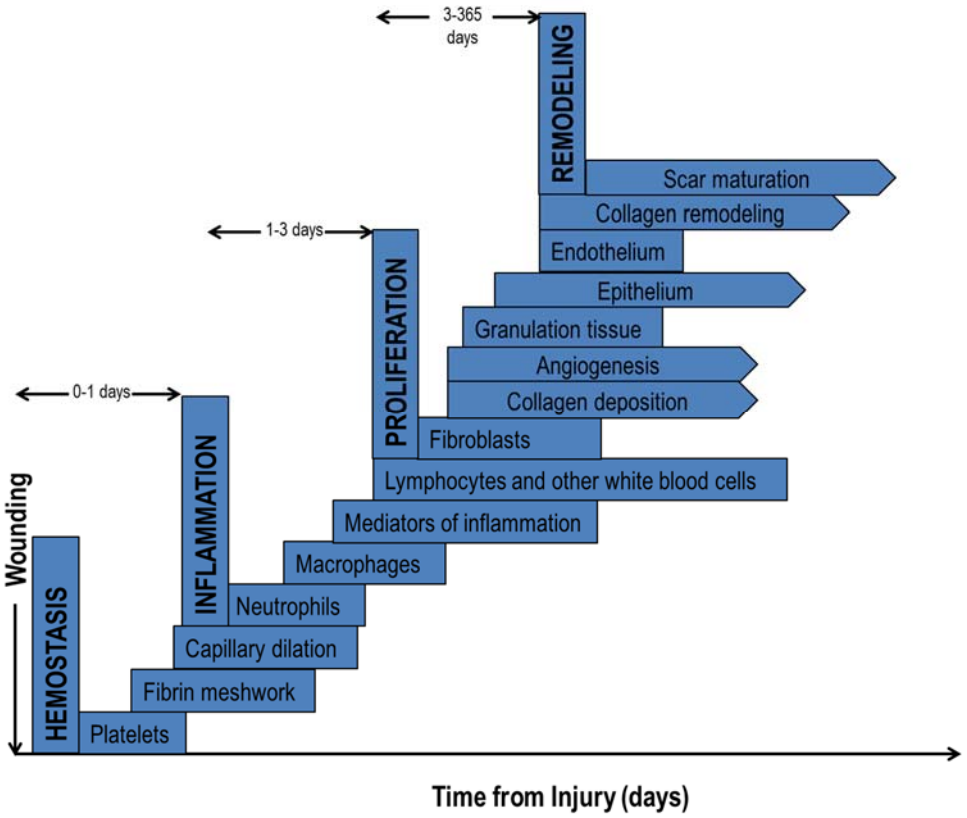


Figure 2.3: The four phases of normal wound healing: 1) homeostasis, 2) inflammation, 3) proliferation and 4) remodeling. Each step has many components. The pointed edge depicts an ongoing process. Adapted from [35, 36]

In the proliferation stage one to three days post-injury, fibroblasts up-regulate receptor expression to allow migration and anchoring of these cells to fibrin, fibronectin and vitronectin present in the clot.[37] Matrix metalloproteases (MMPs) are upregulated at the wound edge to degrade ECM components at the clot. This enables keratinocytes to unbind from the clot and migrate to the wound edge for wound epithelialization.[38] Once the fibroblasts fully integrate in the clot, they change into myofibroblasts and synthesize new extracellular matrix, made mostly from collagen to rebuild the tissue. New blood vessels from granulation and increased expression of vascular endothelial growth factor (VEGF) also take place to re-establish and strengthen the injured wound closer to its original state before injury.[16] A new layer of epithelial cells cover the wound site on top of the granulating tissue to protect the wound. Last, in the remodeling phase,

which can be up to a year after the original injury, fibroblast activity declines and collagen, fibronectin and proteoglycans are remodeled and formed around the scar site.[31] The remodeled extracellular matrix is recovered but not perfectly back to its original form. In chronic wound healing, macrophages are continuously recruited to the injured site, inducing production of excess proteases and reactive oxygen species which constantly degrades the surrounding extracellular matrix. The imbalance between matrix degradation and production prevents the wound from progressing through the healing stages.[39] As a result, tissue is repeatedly injured and cannot heal over time. **Figure 2.4** summarizes the differences between acute and chronic wound healing.

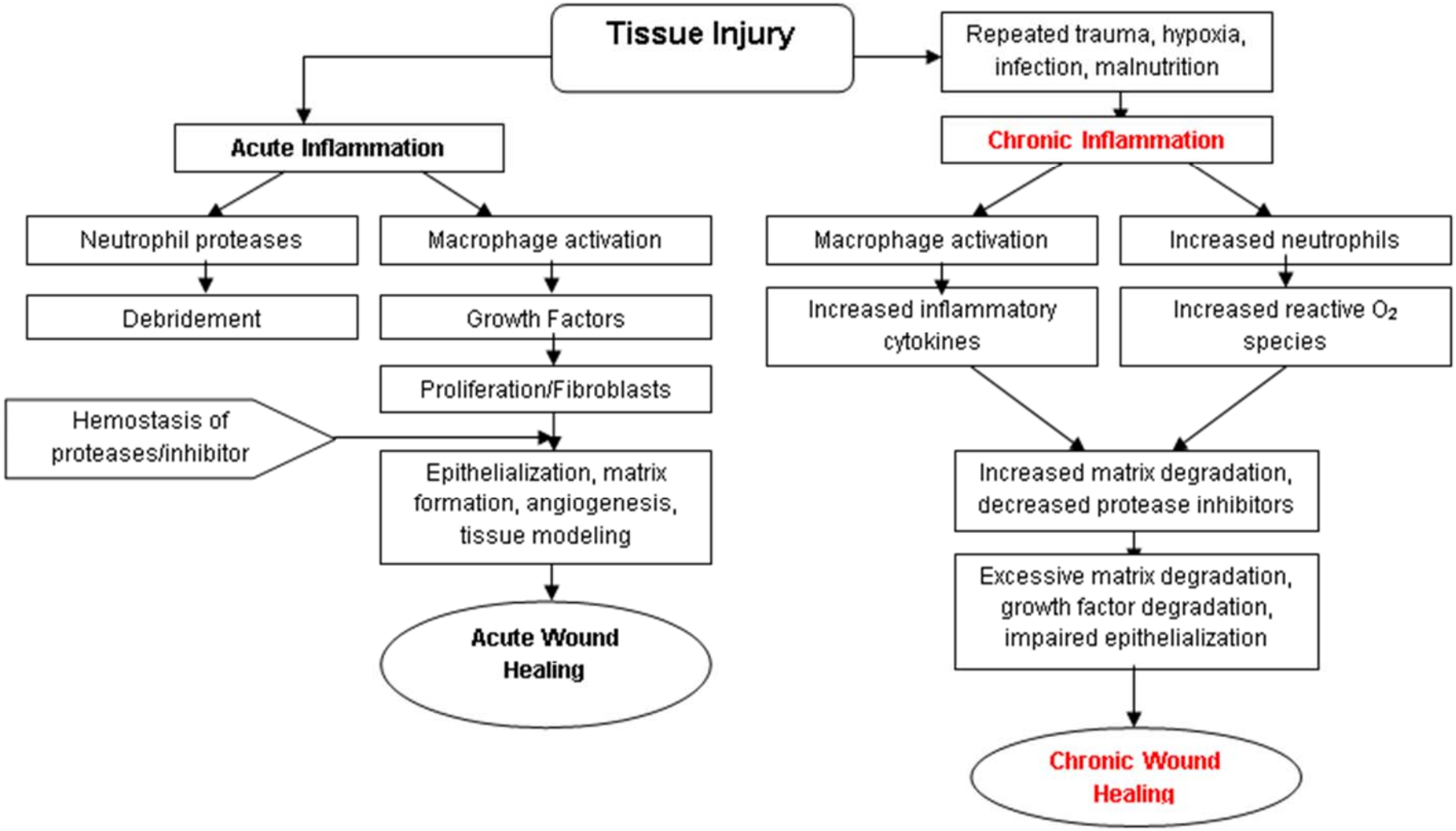


Figure 2.4: Normal versus chronic wound healing adapted from Nwomeh et al. [40]

2.4 Wound management

Wound management is an important aspect of the wound healing process. Many different types of wound dressings are available for particular wound conditions or etiologies. Wound dressings are classified to help determine which type of wound dressing is most appropriate for a specific application. Debridement is one characteristic that is very important for washing out necrotic tissue and foreign material which would otherwise prolong the inflammatory phase.[31] There are several debridement methods for treating necrotic wounds including: surgical removal, wound irrigation and enzymes for material breakdown.[31] Another approach described by acronym TIME, **T**issue assessment and management of tissue deficits, **I**nflammation and **I**nfection control, **M**oisture balance and **E**nhancing epithelial advancement around the wound edges.[41] The objective in choosing a wound dressing is to provide an environment at the wound surface where it can completely heal at its maximum rate while maintaining a cosmetically acceptable appearance.[42] Usually a combination of dressings is needed to achieve this objective – whether they are primary dressings that interface directly with the wound or secondary dressings that cover and compress the primary dressing.

2.5 Modern wound dressings in the market classified by function

Many wound dressings are in the market to cover wounds specific to its size, shape, moisture, adherence, material and antimicrobial properties.[31] Wound dressings can also be classified by the era of its development. Traditional wound dressings such as gauze and bandages are the first type to be used for wound healing while more modern wound dressings create a moist environment.[31] Each type of dressing has strengths and weaknesses as well as a wound environment where it is most effective. Traditional dressings are dry and are effective for

insulation and protection from bacteria. However, they do not provide a moist environment and have to be changed frequently to prevent the wound bed from drying out. They can also adhere to the wound tissue which makes it painful to remove.[31]

Table 2.1 Classifications and examples of commercialized wound dressings by function

Adapted from References [43, 44]

Dressing Classification	Dressing Type	Dressing Name	Manufacturer
Traditional	Gauze	First Aid Gauze Pads	Johnson & Johnson Healthcare
		Sterile Gauze Pads	Medi-First
Modern	Alginate	Sorbsan	Bertek Pharmaceuticals
		Curasorb Calcium Alginate Dressing	Tyco Healthcare/Kendall
		Tegaderm	3M Healthcare
	Hydrogel	Purilon Gel	Coloplast Corp
		Skintegrity Hydrogel	Medline Industries
		Nu Gel Wound Dressing	Johnson & Johnson Wound Management
		FlexiGel Hydrogel Sheet Dressing	Smith & Nephew
	Foams	Aquacel Hydrofiber Wound Dressing	ConvaTec
		Optifoam Nonadhesive Dressing	Medline Industries
		Tielle Hydropolymer Adhesive Dressing	Johnson & Johnson Wound Management
		Allevyn Adhesive	Smith & Nephew

Bioactive	Biostep Collagen Matrix Dressing	Smith & Nephew
	Puracol Plus Collagen Dressings	Medline
	Hyalomatrix	Anika Therapeutics
Hydrocolloids	Duoderm CGF Sterile Dressing	Convatec
	Replicare Thin Hydrocolloid Dressing	Smith & Nephew
Semi-permeable	Bioclusive	Systagenix
	Mefilm	AliMed
	OpSite Plus	Smith & Nephew
	Tegaderm	Johnson & Johnson Wound Management
Anti-microbial	Acticoat absorbent	Smith & Nephew
	Actisorb Silver 220	Johnson & Johnson Wound Management
	Aquacel AG	Convatec
	Contreet H	Coloplast Corp
	Contreet F	Coloplast Corp
	Iodosorb	HealthPoint Ltd
	Silvasorb Antimicrobial Silver Dressing	Medline Industries
	Kerlix and Gauze	Tyco Healthcare/Kendall

2.5.1. Alginates

Alginates is a type of polysaccharide dressing which has excellent absorbance properties for wounds that create a high volume of exudate. They are salts of alginic acid polysaccharides that form gels upon exudate absorption and can swell 15 to 20 times their weight which can be

applied to wounds.[44] Alginate also has had implications in wound healing where it induced fibroblast proliferation and macrophage signaling of tumor necrosis factor (TNF-α).[45, 46] Alginates are biocompatible and generally ideal for all stages of wound healing. However, they require secondary dressings, need to be changed daily and are not ideal for dry wounds with little exudate.[31, 44]

2.5.2. Hydrogels & foams

Hydrogels are non-toxic, inert cross-linked amorphous or elastic polymeric dressings that are insoluble in aqueous media. They can be made of synthetic polymers such as poly(methacrylates), polyvinylpyrrolidine or alginate based composites.[31] Hydrogels provide moisture to the wound site and can absorb a certain amount of exudate depending on its composition. They also provide a non-adherent and cool surface that encourages wound debridement and provides comfort to patients.[31] Hydrogels are indicated for necrotic and sloughy wounds.[31, 44] Its drawback is it cannot absorb high amounts of exudate, otherwise leading to infection.[44] Additionally, they are mechanically weak, leading to constant dressing changes. Hydrogels also typically require a secondary dressing to be held in place. Foam dressings are like semipermeable films in that they are made of polyurethane plastics and have an occlusive backing to prevent water loss and bacterial absorption; however, they are more absorbant.[31] It is more absorbent due to the foam's pore size that allows a high moisture vapor transmission rate.[47, 48] Foam dressings provide great insulation and have hydrophilic-hydrophobic layers which allow optimal distribution of exudate within the dressing in addition to preventing leakage. Foam dressings also come in thicker conformations to fill cavity like wounds.[44] Foams, however do not work well with dry wounds because these dressings require wound exudate to provide the necessary moisture at the injury site.

2.5.3. Bioactive dressings

Bioactive dressings are the most compatible of wound dressings because of their origin in the human body, particularly extracellular matrix components in addition to actively playing a role during the wound healing process. In literature, collagen, elastin, hyaluronic acid and chitosan are natural biomaterials that have been explored as wound dressing materials.[49-51] They are biodegradable, do not illicit an immune response and directly assist in the wound healing process. Collagen, for example induces clotting in the homeostasis phase while encouraging fibroblast proliferation and endothelial cell recruitment to the wound site during the proliferation stage.[52, 53] Collagen sponges were reported to absorb exudate, debris and inflammatory cells from the wound site.[54] Hyaluronic acid, whose origin as a joint lubricant has been used as a carrier for growth factor delivery in acute wound treatment.[55] In more recent years, chitosan has received attention as a potential wound dressing biomaterial as a study helped validate its ability to accelerate granulation during the wound healing process.[56]

2.5.4. Hydrocolloids & semipermeable films

Hydrocolloid dressings are occlusive films or sheets made of materials such as carboxymethylcellulose, gelatin and pectin which form a gel-like consistency upon adherence to either dry or wet surfaces.[44] They are water impermeable and usually have adhesive layers on the perimeter of the dressing to maintain a moist wound environment.[44] These dressings are indicated for low and moderately exudating wounds and have been used to treat diabetic ulcers.[31, 44] One disadvantage of hydrocolloid dressings is that their adhesive layer can prevent oxygen exchange between the outside environment and the wound. Another problem is fiber residues that remain in the wound bed have to be removed upon dressing change.[31]

Semipermeable films are transparent layers made of polyurethane with an acrylic adhesive backing which are highly flexible for application at joints, body contours and other hard to reach areas.[31, 44] They are impermeable to fluids but actively exchanges gases around the wound site. Semipermeable films can only be used for low to moderately exudating wounds and are not thick enough to fill cavity like wounds. [31, 44]

2.6 Wound dressing therapeutics

Therapeutics have been added to wound dressings over the years to enhance patient comfort and to help accelerate the wound healing process. Doxycycline is an antibiotic therapeutic used to inhibit tissue degrading MMPs during chronic wound healing.[57] It is a tetracycline class of antibiotics that combats bacterial infections. It works by deactivating the 30S ribosomal subunit that would otherwise initiate translation of the destructive MMPs. As a result, doxycycline prevents biofilm formation common in chronic wounds. In addition, this antibiotic is active against E. coli, methicillin-resistant Staphylococcus aureus (MRSA), respiratory and urinary tract infections.[58]

Antimicrobial dressings are important during the wound healing process because they contain therapeutic agents which inhibit potential bacterial infections caused by acute tissue injury, post-operative surgery or from more chronic, pathological states such as diabetes.[31] Many anti-microbial dressings are impregnated with silver, which broadly act against infections caused by skin burns and wounds. Antimicrobial dressings have been used to help fight looming infections after tissue injury, particularly in diabetic ulcers. Silver has been the traditional antimicrobial agent to treat bacterial colonies such as *Staphylococcus aureus and P. aeruginosa.*[44] Its mechanism of action involves the influx of silver ions to the bacterial cytoplasm, where they shut down enzyme activity and as a result, potassium ions leak out the

cell.[59] The released ions cause the cytoplasm to burst and destroy the cell wall, leading to apoptosis. [59] Silver can only be applied locally but has been effective inhibiting bacterial growth and its resistance.[60] Commercially, silver sulfadiazine is widely popular as a topical antimicrobial agent for skin wounds and burns. However, its spectrum of use should be limited because of its cytotoxicity. Iodine complexes, povidone-iodine and cadexomer iodine have been integrated into fabrics to improve wound healing due to its ability to inhibit microbe growth and encouraging debridement of the wound site.[44] Gentamycin and oflaxcin have been impregnated into collagen and silicone substrates respectively to form antimicrobial dressings. [31] Antimicrobial dressings are excellent broad-spectrum acting materials due to its ability to prevent systemic infections that could otherwise cause acute wounds to become necrotic and evolve into a chronic state. Nonetheless, the amount of anti-microbials should be moderated and treated locally to prevent systemic toxicity or bacterial resistance.

2.7 Electrospun polysaccharides: material properties & applications

Electrospinning was first patented by Anton Formhals in 1934 as a technique to create non-woven fibers using a voltage gradient between the syringe tip and collecting mandrel.[61] Specifically, the polymer solution ejected from the syringe tip has an applied charge given by the high voltage power supply. The applied charge in the solution overcomes its surface tension to create a jet which dries into fibers as it propels to the collecting mandrel to create a non-woven fiber sheet. (**Figure 2.5**)

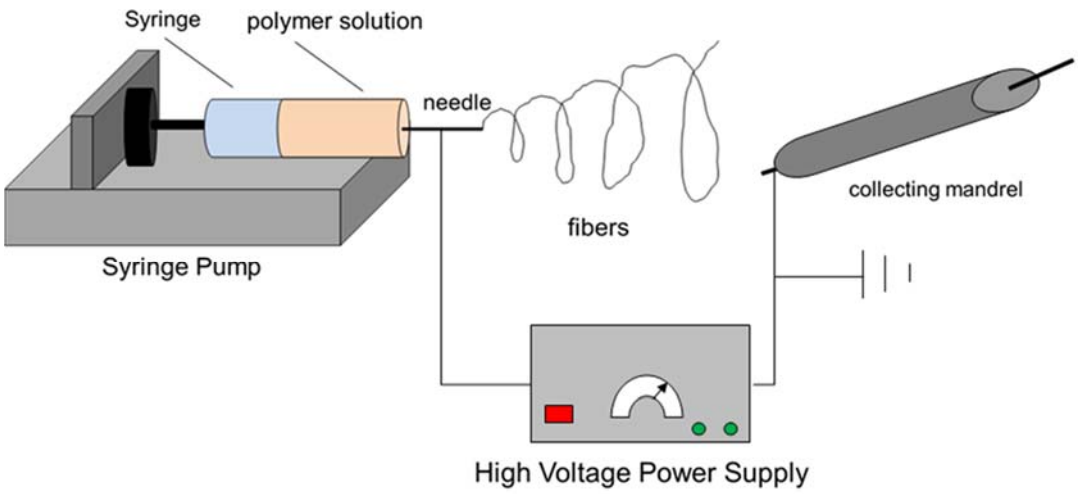


Figure 2.5: Electrospinning scheme

Electrospun nanofibers are an attractive platform as a wound dressing material because of its high surface to volume ratio and porosity to allow moisture and exudate transport between the dressing and injury site.[62] The high porosity of nanofiber dressings have shown to absorb more wound exudate than film dressing formulations.[63] In addition, the high porosity of nanofibers provides an environment where cells can exchange oxygen and inhibit bacterial permeation at the wound-nanofiber interface.[62] Nanofiber wound dressings are highly flexible and can conform to the shape of the wound because of the very fine fiber diameter. This provides better patient compliance and comfort.[62] Beyond the physical characteristics, nanofibers can express or maintain biological functionality after integrating bioactive components such as therapeutics, growth factors and antifungals to enhance the wound healing process.[62] These bioactive agents can be homogenously distributed within the nanofiber scaffold, unlike other wound dressing formulations which compartmentalize the agents in separate layers. The nano-scale morphology also can encourage cell attachment and proliferation at the fibers for extracellular matrix

production.[64, 65] The physical properties of electrospun fibers can be easily modulated by its polymer, solvent and electrospinning setup parameters.[64] This is summarized in **Table 2.2.**

Table 2.2 Parameters affecting physical properties of electrospun fibers. [66]

Polymer Properties	**Solution Properties**	**Other properties**
Molecular weight	Viscosity	Substrate properties
Molecular-weight distribution	Viscoelasticity	Solution Feed Rate
Glass-transition temperature	Concentration	Field strength
Solubility	Surface tension	Geometry of electrode(s)
	Electrical conductivity	Vapor pressure of the solvent
		Relative humidity

Polysaccharide biopolymers have become widely popular in wound dressing development recently because of their natural abundance, biodegradability, non-toxicity, biocompatibility, and anti-microbial properties.[65, 67, 68] They are versatile polymers because their material properties can be manipulated by molecular weight, charge and chemical composition. They are found in plants, animals and microbial organisms.[69] Polysaccharides such as glycosaminogylcans (GAGs) or proteoglycans are also a component of the extracellular matrix whose integrity is important during wound healing. Cellulose, alginate, heparin and hyaluronic acid with additives or in multiple solvent systems have been successfully electrospun into nanofibers.[69] Meanwhile, chitosan and chitin have been blended with other biopolymers such as collagen and gelatin into nanofibers to study their potential use as a wound dressing material.

2.7.1. Alginate

Alginate is an anionically charged, water soluble polysaccharide derived from brown seaweed. It is a linear copolymer made up of M (mannuronic acid) and G-block (guluronic acid) monomers. The proportion of M and G-blocks can influence the physical properties of the nanofiber.[70] Alginate fibers with high M-block content exhibit fibers with high absorption capacity but low mechanical strength. Meanwhile, alginate fibers with high G-block content are much stronger but has lower fluid absorption capacity.[70] Alginate is attractive as a wound dressing because it is non-toxic, non-immunogenic and biocompatible.[69] Shalumon et al. created Alginate/PVA blended nanofibers with zinc oxide as an anti-bacterial wound dressing.[71] However, its potential in electrospinning has not been fully realized because of existing challenges to fabricate uniform, continuous fibers. This is due to low chain entanglement created by negative charge repulsions and length of polymer chains within the alginate network.[69, 72, 73] As a result, groups introduced other polymers to assist in electrospinning such as polyethylene oxide (PEO), polyvinyl alcohol (PVA) and glycerol to neutralize the electrostatic repulsions which help promote greater fiber entanglement.[69]

2.7.2. Chitosan

Chitosan is a positively charged polysaccharide derived from chitin present in crustaceans' sea shells.[69] Chitosan is a deacetylated form of chitin due to the loss of an acetyl group (-$COCH_3$). It is soluble in acidic solutions (pH < 6) whose properties are dependent on the polymer's molecular weight, degree of acetylation and distribution of acetylation on the polymer backbone.[74, 75] Chitosan is a strong candidate as a wound dressing material because it is: biocompatible, non-toxic, anti-microbial, biodegradable, hemostatic, and can be a substrate for

cell attachment.[76] Chitosan nanofibers can be a substrate for cell attachment because their polymer structure is similar to glycosaminoglycans (GAGs) which are a major component of the extracellular matrix.[69] Unfortunately chitosan, like alginate is difficult to spin because of its highly charged nature but there are a handful of solvents that can dissolve it into a solution that can be successfully electrospun. Acetic acid (90 wt. %), trifluoroacetic acid (TFA) and TFA/dichloromethane (DCM) are solvent systems typically used to electrospin chitosan.[77] However, chitosan fibers lack stability in aqueous solutions and have limited electrospinning conditions that can successfully form fibers so additional polymers are introduced to improve spinnability such as PEO, PVA, collagen and silk.[65, 68, 77, 78]

2.7.3. Hyaluronic acid

Hyaluronic acid is a linear polysaccharide that is a major part of the extracellular matrix in connective tissues.[79] Hyaluronic acid is a quality wound dressing material for its biocompatible and biodegradable properties. Hyaluronic acid (HA) has been reported to be successfully electrospun in dimethylformamide and water.[79] However, there has been limited success electrospinning HA on its own due to its high charge density and surface tension. As a result, blended co-polymers are needed for it to be consistently electrospun successfully. Gelatin, PEO and zein, a corn protein have been blended with HA to form fibers. Ji et al. electrospun hyaluronic acid derivatives into fibers as an ECM mimicking substrate favorable for NIH3T3 cell attachment and spreading which is ideal for tissue regeneration.[80]

2.8 Polysaccharide hydrogels: material properties & applications

Hydrogels are crosslinked networks that are very hydrophilic and absorb large amounts of water without dissolving because of irreversible chemical or physical bonds that stabilize the

gel.[81] They are excellent platforms for wound dressing applications because they swell significantly which helps with wound exudate absorption. Also, their hydrophilic properties enable the hydrogel to keep the wound bed moist. Hydrogels have good bioadhesive properties that assist with maintaining moisture at the wound site while potentially delivering antimicrobials that may be integrated in the hydrogel for sustained action against infected wounds. Hydrogels are very flexible and conform in a variety of conditions.[82-85]

The hydrogel's material properties are dependent on: i) amount of fluid absorption and ii) the nature of bonding within the crosslinked polymer chains.[81] The more hydrophilic groups in the hydrogel the greater swelling properties and vice versa. The bonding within crosslinked hydrogels is between functional groups within the polymer chains that stabilize the gel from dissolving.[81] Hydrogel crosslinking can take place by either non-covalent crosslinks through physical entanglement and secondary bonding or by covalent crosslinks.[86, 87] Non-covalent hydrogel crosslinking can reversibly take place while hydrogel crosslinking via covalent bonds is permanent. As a result, the reversibility of the crosslinking reaction has a major influence on the elasticity of the hydrogel's swelling in solution. This swelling takes place through the expansion of the polymer chains as aqueous solution rushes into the hydrogel. In reversible hydrogel systems, its polymer chains constantly fluctuates in expansion and retraction while irreversible systems undergo fluctuation until reaching a swelling equilibrium.[88] The hydrogel's surface wettability, solute diffusion coefficient and mechanical properties are influenced by its swelling equilibrium.[89-91]

Polymer properties such as molecular weight, charge and crosslinking density all play a role in modulating the degree of swelling in aqueous solutions. Typically, hydrogels with high molecular weights and crosslinking densities are stiff and rigid with high modulus values.[92, 93]

Meanwhile, solute diffusion out of hydrogels are controlled by their crosslinking density, mesh and pore sizes.[94, 95] Polysaccharides in hydrogel formulations have been used in many applications because they are highly versatile, complex polymers that are readily available and can be easily manipulated into gels. They are natural, non-toxic while exhibiting immunomodulatory properties.[96] Polysaccharide hydrogels have been predominantly used as drug delivery carriers but are gaining traction as tissue engineering scaffolds because of the similar structure it has to the extracellular matrix. A variety of polysaccharides have been used for tissue engineering and drug delivery such as alginate, gellan, dextran, hyaluronic acid and etc.[97] There are also countless therapeutics that have been integrated into polysaccharide hydrogels to enhance application. Although there has been an explosion of papers in literature that have reported on the uses of polysaccharide hydrogels in drug delivery and tissue engineering, there is a gap in research regarding their potential applications as wound dressing materials.

2.8.1. Alginate

Alginate is an anionically charged, water soluble polysaccharide derived from brown seaweed. [70] It is widely used because of its fast ability to form gels. This is due to the carboxylic acid moieties on the alginate chain interacting with di-valently charged ions such as calcium, lead and copper which initiate crosslinking.[97] The material properties of alginate can be modified by changing the ratio of its M-block (mannuronic acid) and G-block (guluronic acid) monomers. Alginate hydrogels with high M-block content have high fluid absorption capacity but low mechanical strength. Conversely, hydrogels with high G-block content have high strength but low water absorption capacity. [70] Alginate hydrogels have potential as wound dressing materials because they form stable hydrogels while exhibiting biocompatibility, non-immunogenicity and

porosity for fluid absorption. [97] Alginate hydrogels are considered candidates of wound dressing materials because of its ability to form an in-situ hydrogel in calcium ions present in wound exudate.[98] Balakrishan et al. completed a series of studies where alginate, gelatin and anti-septic borax were incorporated into an in-situ forming hydrogel for wound healing applications.[99-101]

2.8.2. Chitosan

Chitosan is a linear polysaccharide β 1-4 linked D-glucosamine and N-acetyl-D-glucosamine units. It is produced by the deacetylation of its parent polymer chitin and is often found in shells of shrimp and crab. Chitosan is different from other polysaccharides by the presence of nitrogen atoms, its cationic charge and ability to form polyelectrolyte complexes. [81] They are good candidates as wound dressing materials because they are biocompatible, non-toxic and biodegradable. They are highly conformable and come in many shapes and sizes.[85] These hydrogels are fabricated via physical mixture or crosslinks to create a structured network. Chitosan hydrogels can be physically mixed into stable networks by introducing anionic ions or macromolecules to neutralize the positively charged chitosan and induce electrostatic attraction within the gelatinized network. Secondary bonding, hydrophobic-hydrophilic interactions and thermoresponsive gelation can also take place in chitosan hydrogels depending on what monomers or catalysts are added to it.[81] While this method is non-toxic, the issue with physical crosslinking is the lack of long term stability and should only be used for short-term applications. Chemical crosslinking of chitosan hydrogels is straightforward using either small molecules, light, enzyme catalysts or polymers to create more stable networks. However, this crosslinking method is more toxic because of the by-products created from the reaction. [81]

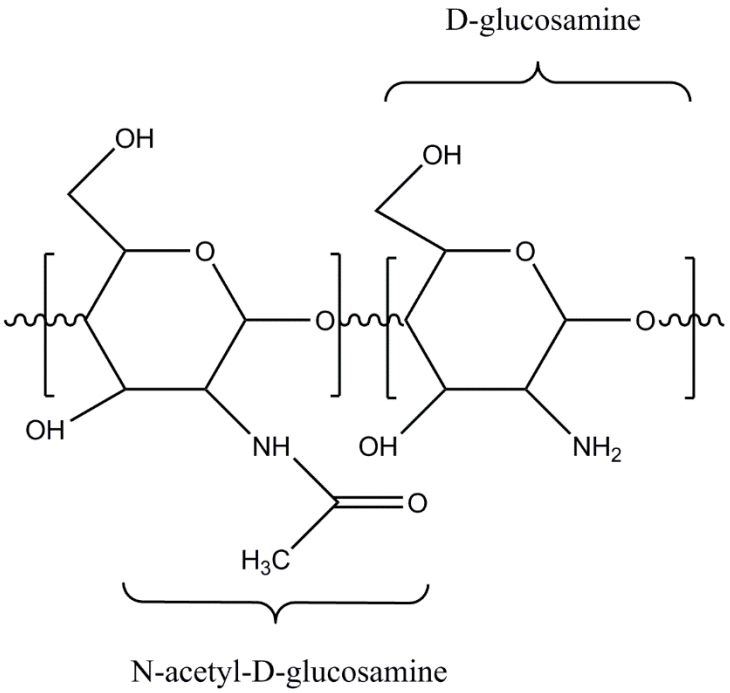


Figure 2.6: Chemical structure of chitosan

Chitosan has been used as a wound dressing material because of its ability to protect the wound while being biocompatible and providing moisture to the wound environment. It has been shown that chitosan accelerates wound healing and promotes smooth scarring due to enhanced vascularization and a high supply of chittooligomers that incorporate collagen fibrils more efficiently at the extracellular matrix.[102, 103] Important wound healing mediator fibroblast growth factor (FGF-2) has been successfully integrated into chitosan hydrogels. It maintained its bioactivity after impregnation into the chitosan hydrogel.[51] Park et al. developed bFGF-loaded chitosan hydrogels to accelerate wound repair in chronic ulcers.[104]

2.8.3. Dextran

Dextran is a glucose homopolysaccharide that is primarily used as a protein drug delivery system because their sustained release kinetics improve the protein's bioavailability compared to being free in circulation.[97] Dextran can be crosslinked physically by gamma irradiation to provide greater stability in the network. The stability of the dextran hydrogel is controlled by the length of irradiation and dextranase levels in the system which hydrolytically degrades the scaffold. The proteins trapped inside the dextran hydrogel have Fickian diffusion release kinetics in aqueous solution because they are much smaller than the pore sizes in the network that allow proteins to travel from an area of high concentration to low concentration. [97] Dextran hydrogels are relevant as potential wound dressing materials because anti-microbials can be introduced whose release is controlled by matrix metalloproteases active at the wound site. They have also exhibited angiogenesis and promotion of complete skin healing in animal burn wound models.[105] Sun et al. created excisions to full-thickness wounds before dextran hydrogel scaffolds were implanted with a secondary dressing layer for up to 21 days. [105] The study showed complete dermal regeneration after implantation of dextran hydrogels compared to non-treated and treated control groups. (**Figure 2.7**)

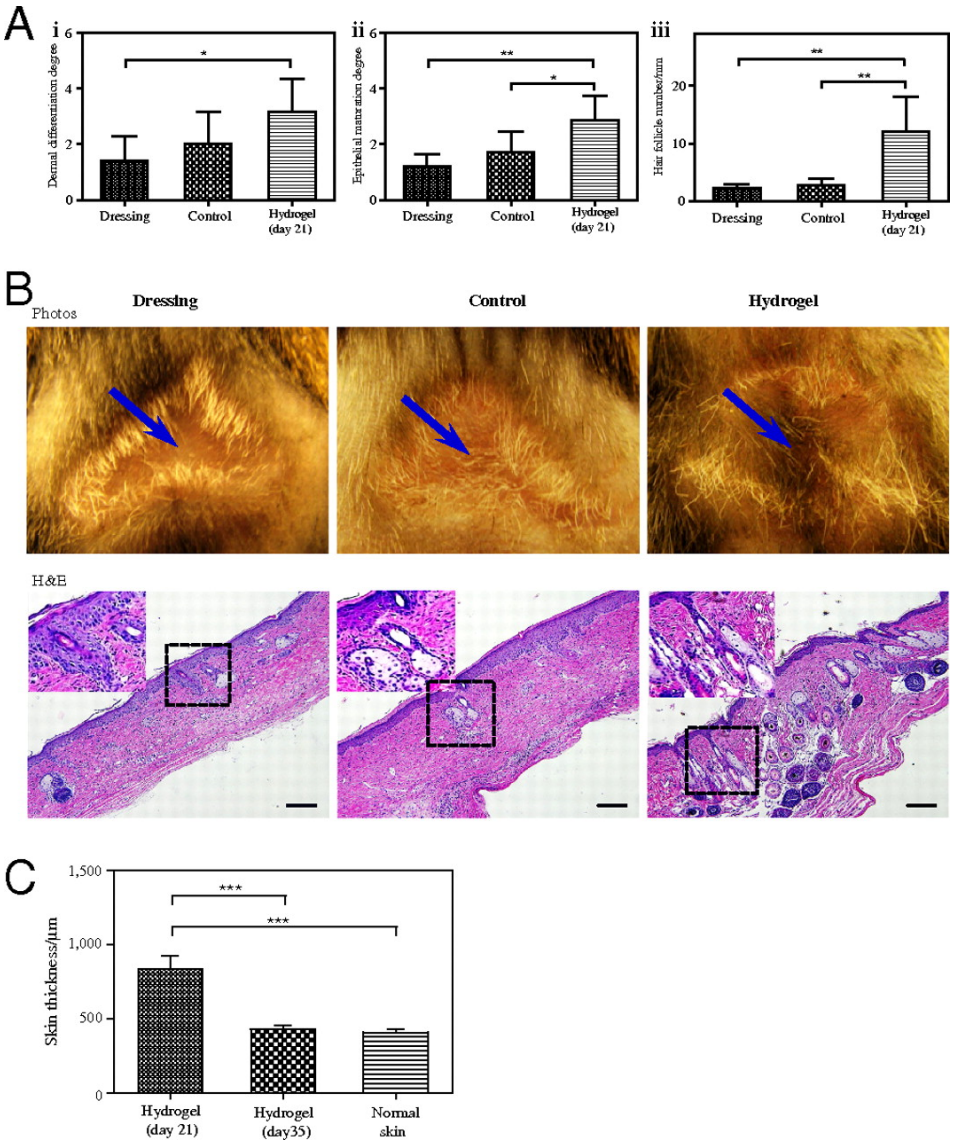


Figure 2.7: Evaluation of regenerated skin structures. (*A*) Quantification of skin structures in terms of dermal differentiation (*i*), epithelial maturation (*ii*), and the number of hair follicles (*iii*). (*B*) A 5-week-long study further demonstrated that dextran hydrogels promote complete skin regeneration with new hair growth, as shown by photos (arrows indicate the center of the original wound; *Upper*) and H&E-stained histologic sections. High magnification corresponds to boxed area in the low-magnification images. (*C*) Quantification of skin thickness after 3-week and 5-week-long treatment compared to normal mouse skin. Significance levels were set at: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. Values shown are means ± SD. Scale bars, 100 μm. (from ref[105] with permission)

2.8.4. Gellan

Gellan gum is a polysaccharide derived from *Sphingomonas elodea* bacterium fermentation. Gellan, like alginate forms gels when mixed with divalent ions. Its mechanical strength is dependent on the degree of acylated groups. [97] The more acylated groups, the more soft and elastic the gel is while deacylated gellan hydrogels are stiff and non-elastic. Although gellan has primarily been explored as ocular, nasal and drug delivery carriers, more recent work has been done to evaluate gellan as a silver impregnated wound dressing.[106-109] Nonetheless, gellan gum is still in an early stage of being evaluated solely as a wound dressing.

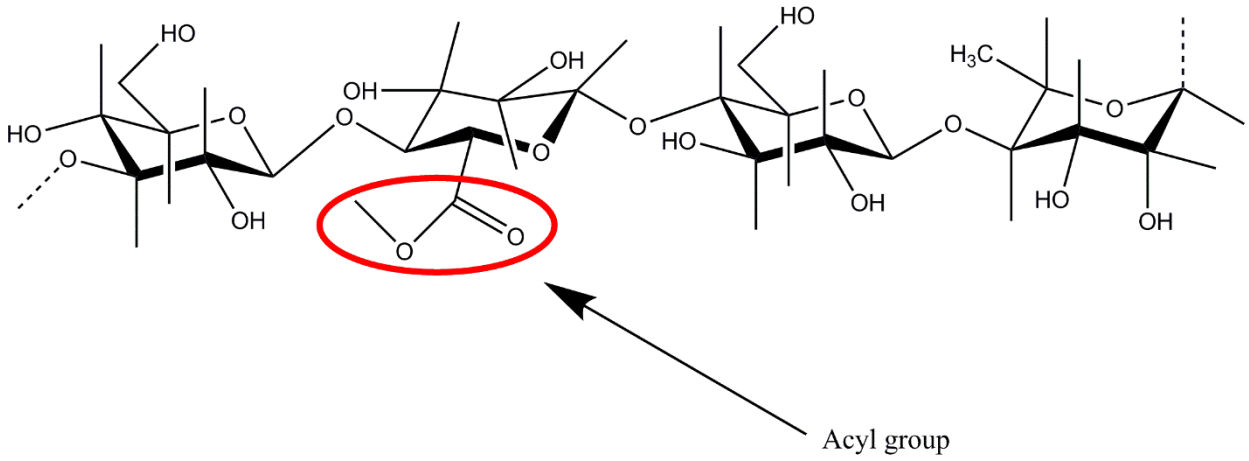


Figure 2.8: Average structures and/or repeating units of gellan

2.8.5. Hyaluronic acid

Hyaluronic acid is a glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine.[110] It is a large component of the extracellular matrix, particularly in connective tissues. Also, it is viscoelastic, highly biocompatible, non-immunogenic and biodegradable which makes it appealing as a hydrogel formulated wound dressing. [97] Hyaluronic acid forms hydrogels upon crosslinking with use of glutaraldehyde and 1-

Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) reagents.[111] One group used hyaluronic acid functionalized with adipic dihydrazide and crosslinked with poly-ethylene glycol (PEG) propriondialdehyde to create a film which successfully delivered anti-microbial and anti-inflammatory agents in vitro, showing promise as a wound dressing.[112] Others have combined hyaluronic acid with agarose or fibronectin to create hydrogels which also could potentially be used as a wound dressing material.[113, 114]

2.8.6. Arabinoxylan

Arabinoxylan (AX) is a neutral non-starch polysaccharide derived from cereal grains such as wheat.[1] They are water extractable and are comprised of a xylose backbone substituted onto arabinose units. Arabinoxylan ferulate (AXF) is arabinoxylan with ferulic acid substituted onto its arabinose monomer. (**Figure 2.9**) AXF can be readily cross-linked with oxidative reagents peroxidase and hydrogen peroxide. They function by creating an ester bond between ferulic acid and arabinose units to form a dimer which crosslinks the arabinoxylan chains together.[115] Cross-linked arabinoxylan gels have high water absorption capacity which allows for potential drug delivery applications of therapeutics such as albumin and ibuprofen.[1, 116] Experiments have shown arabinoxylan hydrogels to have a two and a half fold increase in swelling when introduced into water.[115] This phenomenon was attributed to its honeycomb shaped pores and hydrophilic properties as a lyophilized hydrogel. Arabinoxylan also does not require the use of toxic organic solvents but uses water instead for solubilization.

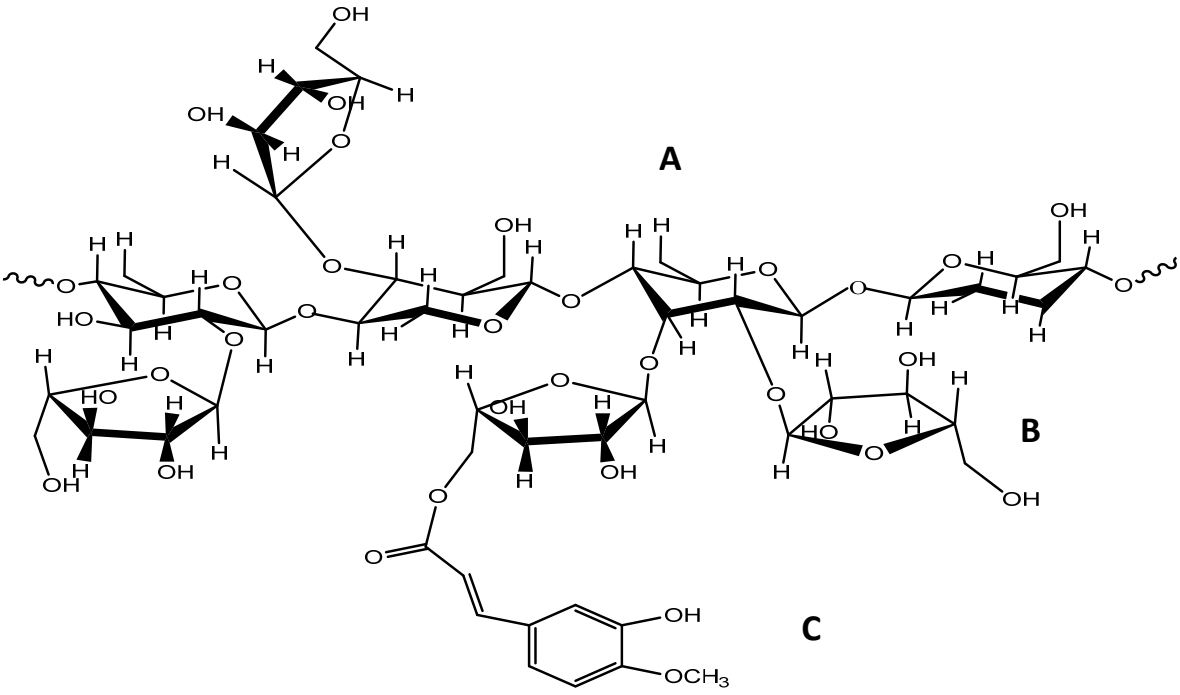


Figure 2.9: Arabinoxylan ferulate structure. Arabinoxylan ferulate (AXF) is composed of three components: A) xylose backbone substituted to B) arabinose sugar units, one of which is substituted to C) ferulic acid.

Arabinoxylan polysaccharides have many benefits outside of its material properties. Arabinoxylan has shown potential as a drug delivery system as a gel by exhibiting high protein release. The rate of release can be modulated by the initial amount of protein loaded into the gel.[1]As a lyophilized hydrogel, it has relatively large pore sizes ranging from 200 to 400 nanometers which allow the proteins to be introduced into without any significant damage to the protein.[1] In addition, the flexibility of arabinoxylan chains enables fluid and solute movement in and out of the delivery system based on the gel's degree of crosslinking.[1] Studies have also shown arabinoxylan to regulate the lining of the gastrointestinal system.[1, 117] Clinical studies with diabetes implications have shown arabinoxylan's ability to modulate gut metabolism affecting glucose levels downstream.[118, 119] Last, arabinoxylan has been integrated with other natural polymers such as alginate and gelatin to form fiber meshed wound dressings.[70]

2.9 Conclusions

Polysaccharides have been primarily used in food, textile or cosmetic products but their potential as wound dressings is vast because of their abundant, non-toxic, biocompatibility and non-immunogenic properties in physiological systems. They also are excellent candidates because they are structurally diverse in their molecular weight and chemical structure which in turn influences the overall material properties. Many of the polysaccharides described in this review have demonstrated their applicability in the laboratory in vitro and in vivo. However, these class of polymers are still considered niche bioactive wound dressings commercially. We hope this review will encourage research groups to further explore polysaccharides and appreciate its value within the wound dressing market.

CHAPTER 3: Electrospinning of arabinoxylan as a novel fiber scaffold

Donald C. Aduba, Jr.[1], Seon-Sook An[2], W. Andrew Yeudall[3], Gary L. Bowlin[4], Todd O. Kitten[2], & Hu Yang[1,5]

[1]Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284.

[2]Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298.

[3]College of Dental Medicine, Georgia Regents University, Augusta, GA 30912.

[4]Department of Biomedical Engineering, University of Memphis, Memphis, TN 38152.

[5]Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298.

Abstract:

Research strategies in developing polysaccharide carbohydrate biomaterials for wound healing have steadily grown over the last decade. Arabinoxylan (AX) is a carbohydrate polymer derived from cereal grains. However, its potential for clinical applications has yet to be fully realized. Arabinoxylan ferulate (AXF), a type of arabinoxylan is hydrophilic and possesses tunable swelling properties for wound fluid absorption. This study aims to demonstrate the feasibility of electrospinning AXF to fibers and investigate the physical and biocompatible properties of the resulting nanofiber constructs. Gelatin (GEL) was blended with AXF to facilitate fiber formation and provide a natural polymer that host tissues can readily accept after injury. Blends of GEL and AXF at 1:1, 2:1 and 4:1 ratios were successfully electrospun and characterized in terms of morphology, tensile properties, pore size and molecular composition. Fiber diameter increased with respect to polymer concentration (0.425, 0.586 and 1.09 μm for 1:1, 2:1 and 4:1 GEL-AXF blends respectively). Moduli values for 1:1, 2:1 and 4:1 GEL-AXF blends were 6.3, 22.9 and 46.0 MPa respectively, indicative of the greater strength with respect to polymer concentration. In addition, the scaffold possessed excellent cytocompatibility (fibroblast cell viability > 95%). Silver was impregnated into GEL-AXF nanofibers at 5% w/w concentration to enhance its anti-microbial properties against wound pathogens. After integration of silver, the drug exhibited near zero-order release kinetics with 20.9, 17.0 and 10.6% cumulative drug release for 1:1, 2:1 and 4:1 GEL-AXF blends respectively after 48 hours. Silver release from the scaffolds also inhibited bacterial growth as confirmed by disk-diffusion assay. This work shows electrospun GEL-AXF fibers are biocompatible and have tunable material properties. However, its stability in water needs to be improved with co-electrospinning with

more stable polymers to achieve the potential of this novel scaffold for wound dressing development.

Keywords: *gelatin, electrospinning, nanofiber, arabinoxylan, wound dressing*

3.1 Introduction

Electrospun fibers are an attractive platform as wound dressing materials because of its high surface to volume ratio, porosity, conformity at the wound site and ease of fabrication. [120] The properties of electrospun fibers can be easily manipulated by the polymer concentration, flow rate, voltage and air gap distance. Electrospun polysaccharide polymers have become widely popular in wound dressing development recently because of their natural abundance, biodegradability, non-toxicity, biocompatibility, and anti-microbial properties.[65, 67, 68] They are versatile polymers because their material properties can be manipulated by molecular weight, charge and chemical composition. Polysaccharides such as glycosaminogylcans (GAGs) or proteoglycans are play a role in the extracellular matrix as a substrate for cell migration and attachment during the proliferative phase of wound healing. They are found in plants, animals and microbial organisms.[69] Polysaccharides such as cellulose, alginate, heparin, chitosan and hyaluronic acid combined with additives or in multiple solvent systems have been successfully electrospun into nanofibers.[69]

Arabinoxylan (AX) is a polysaccharide biopolymer containing a xylose backbone with arabinose substituted in the O-2 or O-3 positions of the backbone.[1] The xylose and arabinose units are linked together by ester linkages which makes AX hydrophilic. The applications of arabinoxylan range from packaging materials, as prebiotics to regulate gut metabolism to fiber meshes.[70, 119, 121]AX's hydrophilic and absorbent properties allows for moisture exchange between the wound dressing and wound.[115] However, little work has been done to explore AX as

an electrospun wound dressing material despite many of the aforementioned strengths. Like other polysaccharides, AX cannot be electrospun solely due to the polymeric backbone's repulsive charges reducing chain entanglement. In this study, arabinoxylan ferulate (AXF) blended with gelatin produced novel, co-electrospun fiber mats that are highly porous, exhibit tensile strength, are biocompatible and can exhibit anti-microbial properties after integration with silver sulfadiazine. The results suggest there is potential of this biomaterial in advanced wound dressing development.

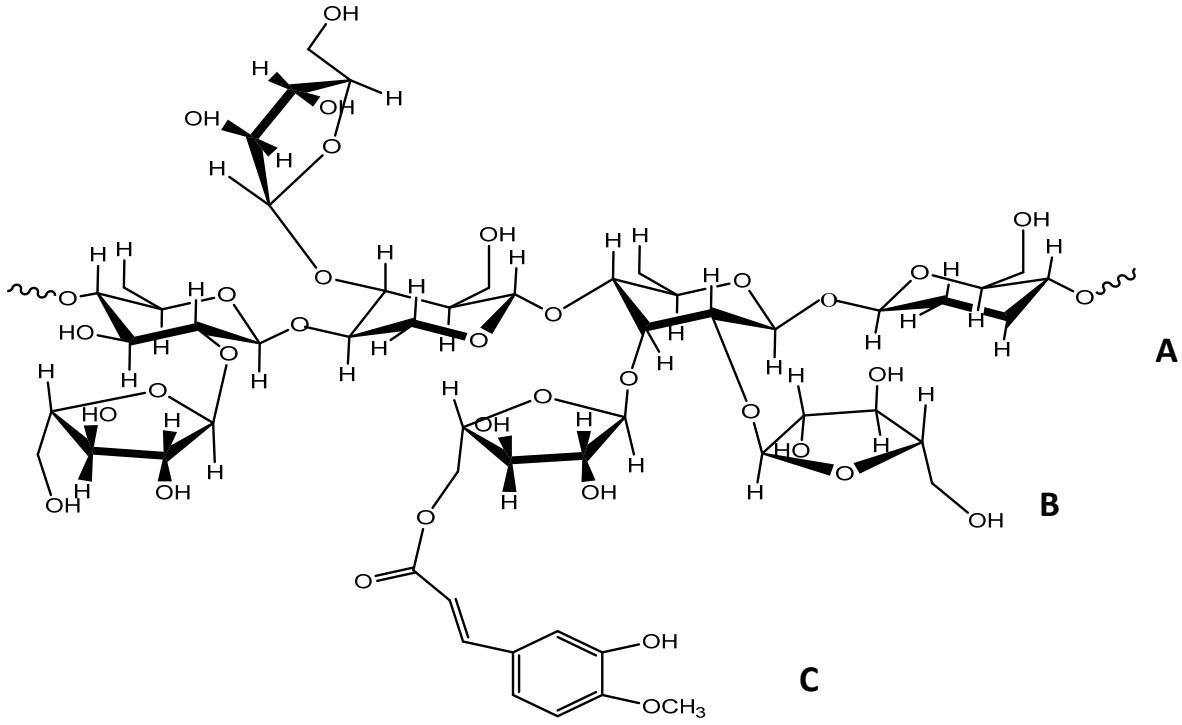


Figure 3.1: Arabinoxylan ferulate structure. AXF is composed of three components: A) xylose backbone substituted to B) arabinose sugar units, one of which is substituted to C) ferulic acid.

3.2 Materials and methods

3.2.1. Materials

Arabinoxylan ferulate (AXF) was purchased from Cambridge Biopolymers (Cambridge, UK). Porcine type-A gelatin, Total Carbohydrate Assay Kit, 70% Nitric Acid, Silver (I) Sulfadiazine were purchased from Sigma-Aldrich (St. Louis, MO). 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was purchased from TCI America (Portland, OR). Phosphate buffered saline (PBS) was purchased from EMD Chemicals (Gibbstown, NJ). Dulbecco's modification of eagle's medium (DMEM) was purchased from Invitrogen (Carlsbad, CA). Fetal calf serum was purchased from Lonza (Walkersville, MD). Hyclone 0.05% Trypsin was purchased from Thermo Scientific. (Logan, UT) Penicillin Streptomycin was purchased from Life Technologies. (Grand Island, NY). Sulfuric Acid was purchased from Fisher Scientific (Pittsburgh, PA).

3.2.2. Scaffold preparation

Preparation of GEL-AXF nanofiber blends involved two steps: homogenization and electrospinning.

3.2.2.1. Preparation of electrospinning solution

A mass of 250 milligrams of AXF was added to 10 mL of HFP. The mixture was homogenized for five minutes to break down particles in the solvent. Next, 250, 500 and 1000 mg of gelatin were quickly added to the resulting mixture. The resulting mixtures led to 1:1, 2:1 and 4:1 GEL-AXF mixtures respectively. The vials were placed on a shaker plate and shaken continuously overnight.

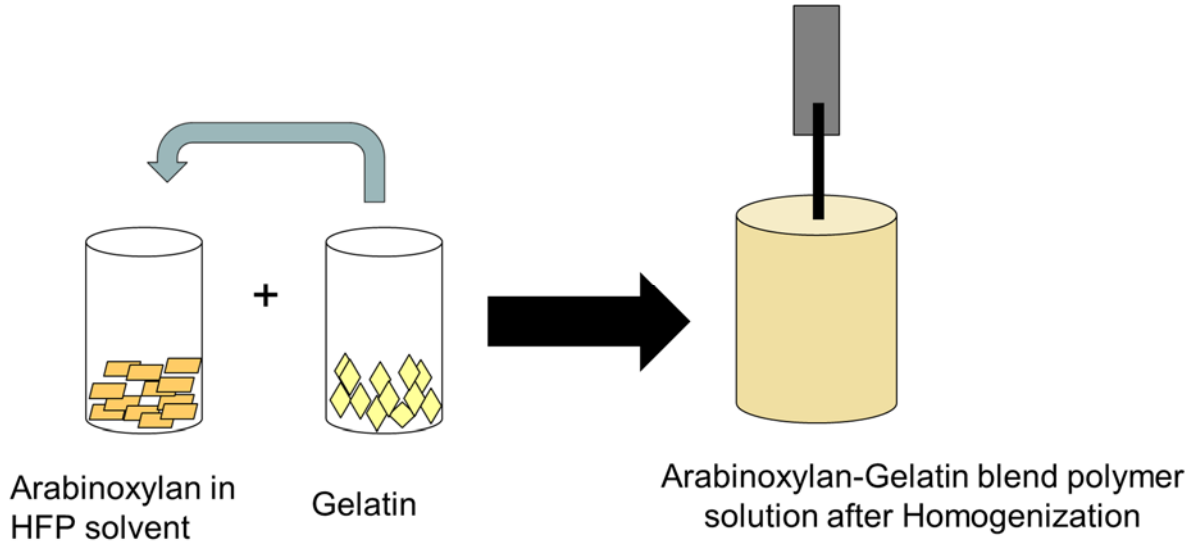


Figure 3.2: Schematic of GEL-AXF electrospinning solution preparation

3.2.2.2. Preparation of silver-loaded GEL-AXF electrospinning solution

To create 1:1, 2:1 and 4:1 GEL-AXF blends with 5% w/w silver sulfadiazine, 30, 40 and 70 milligrams of silver sulfadiazine was added with 250 milligrams of arabinoxylan in HFP before homogenization. (**Table 3.1**) This concentration of silver in the GEL-AXF blends was used to be equivalent to the amount in the 3M Alginate commercialized wound dressing containing an ionic silver complex made of silver, sodium, hydrogen, zirconium and phosphate.[122, 123] After homogenization, 250, 500 and 1000 milligrams of gelatin was added to the resulting mixture and placed on a shaker plate overnight.

Table 3.1 Preparation of silver loaded GEL-AXF scaffolds

Formulation	GEL (mg)	AXF (mg)	Silver sulfadiazine (mg)
1:1 GEL-AXF	250	250	30
2:1 GEL-AXF	500	250	40
4:1 GEL-AXF	1000	250	70

3.2.2.3. Preparation of GEL-AXF scaffolds

To fabricate electrospun gelatin fibers, the GEL-AXF solution was drawn up through the blunted needle (18G×1½ in) of a 10 ml syringe. The syringe was loaded into a syringe pump, propelling the gelatin solution out of the needle 150 mm away from the collecting mandrel at a rate of 5 ml/hr for 4:1 and 2:1 blends. The flow rate was decreased to 2 ml/hr for 1:1 blends because the solution would otherwise bead at 5 ml/hr. The needle was connected to a positive electrode of a high voltage power supply (Spellman CZE100R, Spellman High Voltage Electronics Corporation). The positive electrode applied a 20 kV voltage to the needle. This voltage created an electric field opposite to the grounded target to overcome the surface tension at the needle tip. These conditions generated a Taylor cone which allowed a steady stream of gelatin solution to flow from the needle to the grounded collecting plate in a jet-like fashion. As the GEL-AXF solution was being streamed from the needle tip, the HFP solvent evaporated. Randomly aligned nanofibers were collected on a flat, stainless steel mandrel (7.5 cm×2.5 cm×0.5 cm, L× W×T) rotating at 500 rpm. Scaffolds were sterilized by UV light for ten minutes on each side.

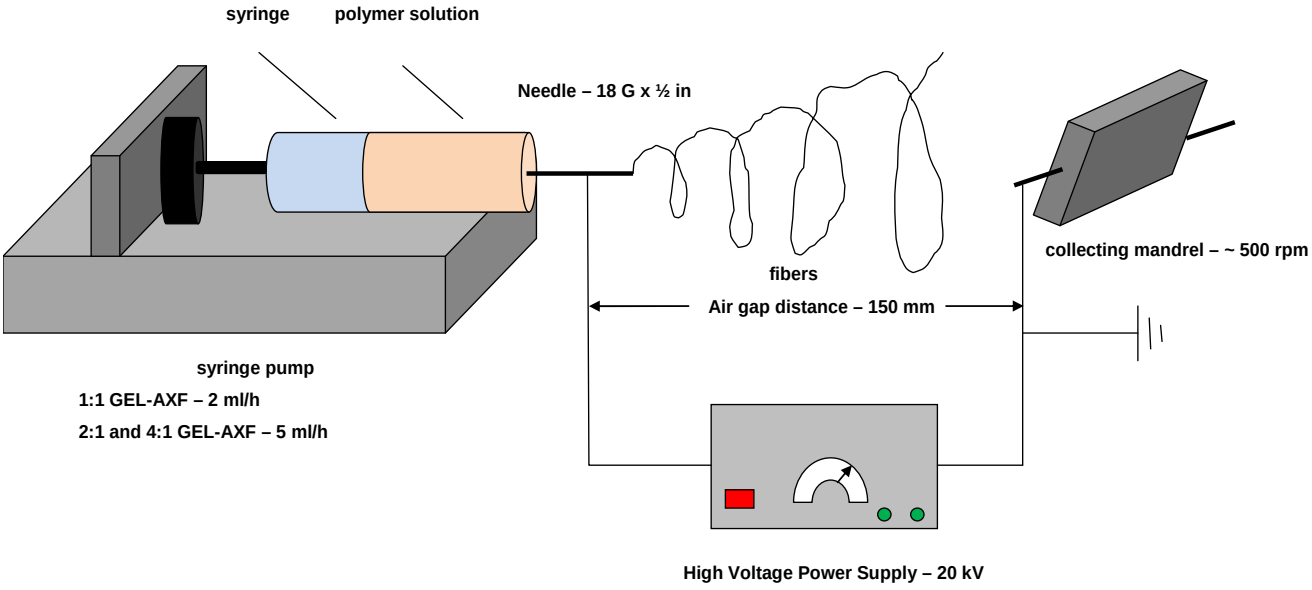


Figure 3.3: Electrospinning setup and conditions.

3.2.2.4. Crosslinking of GEL-AXF scaffolds

GEL-AXF electrospun scaffolds were immersed in approximately 500 µl of 1% w/v PEG-Diacrylate and 10% w/v Eosin-Y photoinitiator solution per 100 mg of scaffold before 10 minute UV light treatment for each side of the scaffold.

3.2.3. Scanning electron microscopy (SEM)

Prior to SEM imaging, scaffolds were placed on a 1 cm diameter stub. The stub was placed on a specimen holder and platinum sputter coated. SEM images were taken and analyzed under JEOL JSM-5610LV Scanning Electron Microscope. One hundred randomly chosen fibers and their pores in each SEM images were analyzed with UTHSCSA ImageTool^{TM} software for fiber diameter and pore size measurement.

3.2.4. Tensile testing

"Dog-bone" shaped samples (n=12) were obtained using a punch die (ODC Testing & Molds) of the dimensions 19.0, 3.2 and 6.1 mm at its length, narrowest point and widest point, respectively.[124] Mechanical properties of the samples, including peak load, peak stress, modulus, strain at break and energy to break, were tested using the MTS Bionix 200 Mechanical Testing System in conjunction with TestWorks 4.0 software.

3.2.5. Carbohydrate assay

The entire electrospun 1:1, 2:1 and 4:1 Gel-AXF blends were weighed with their masses recorded.[124] Then the mats were dissolved in 10 mL of PBS. Based on their corresponding concentrations, the dissolved mats were diluted to 2.5 mg/mL. 2 mg/mL concentration of glucose standards in the carbohydrate assay kit produced by Sigma Aldrich (St. Louis, MO) were used to reference the sample concentrations. A 20 µL microliter aliquot was taken out of 1:1, 2:1 and 4:1 GEL-AXF 2.5 mg/mL stock solutions and added to a 96 well plate in five replicates before being filled to a 50 µL volume with de-ionized water. Next, 150 µL of sulfuric acid was added to each well and incubated in a water bath at 90°C for 15 minutes. After cooling down, 30 µL developer solution from the assay kit is added to each well that is placed on a horizontal shaker plate for 5 minutes. After shaking, the plate is read at 490 nm for quantitative spectrophotometric measurement to confirm the presence of arabinoxylan in the scaffolds after electrospinning.

3.2.6. Drug release kinetics studies

One half of the electrospun 1:1, 2:1 and 4:1 Gel-AXF blends impregnated with silver sulfadiazine was weighed with their masses recorded. The scaffolds were immersed in a conical flask containing 20 mL of 2% nitric acid solution. At 1 hr, 2 hr, 6 hr, 12 hr, 24 hr and 48 hr time-

points, a 5 mL aliquot of release media was taken out and collected into a 15 mL centrifuge tube. The sample aliquots were then analyzed for silver release quantification by ICP-OES. Intensity measurements for silver were calibrated against a standard curve to estimate silver concentration values which were then converted to mass. Triplicate samples were independently measured twice for the study.

3.2.7. WST-1 cell viability assay

The entire electrospun 1:1, 2:1 and 4:1 GEL-AXF blends were weighed with their masses and concentrations recorded. Next, they were sterilized with 1000 ppm peracetic acid for 15 minutes and washed in PBS three times for 10 minutes using the protocol developed by Yoganarasimha et al.[125] The sterilized fibers were centrifuged for five minutes at 3000 rpm. Afterwards the fibers was dissolved in DMEM and 10% PBS for 15 minutes to a stock concentration of 25 mg/mL. The treatment medium was centrifuged again for five minutes at 3000 rpm before collecting the supernatant which was serially diluted to 5, 0.5, 0.05 mg/mL with pure DMEM serving as a control. NIH3T3 fibroblasts were seeded at a density of 10,000 cells per well in 96 well plates then 200 µL of the degradation media was added. The cells were incubated with the degradation media for 24 hours at 37°C and 5% CO_2. After incubation, the seeded cells were given 100 µL of fresh DMEM media before adding 10 µL of WST-1 reagent. The cells were incubated for one hour before the plate is read spectrophotometrically at 450 nm. Relative cell activities from treated groups were measured by taking their absorbance measurements and normalizing them versus absorbance values from untreated control groups. WST-1 assay works through the reagent salt, tetrazolium being cleaved into a dark red formazan

dye whose intensity can be quantified. The salt cleavage is due to mitochondrial enzymes produced by metabolically active cells whose proliferation and viability correlate colormetrically.

3.2.8. Trypan blue cell viability & proliferation assay

The same preparation procedures were done for these electrospun blends as in Section 3.2.7. before treating the cells with the prepared degradation media. After preparation, NIH3T3 fibroblasts were seeded at a density of 50,000 cells per well in a 24 well plate before 500 µL of the degradation media was added. The cells were incubated with the degradation media for 24 hours at 37°C and 5% CO_2. After incubation, the seeded cells were trypsinized with and suspended in 1:1 trypsin-DMEM solution before centrifugation for five minutes at 3000 rpm. After centrifugation, the supernatant was aspirated and the cell pellet was re-suspended in a 1:1 mixture of PBS-trypan blue dye for five minutes before 20 µL aliquots were taken out to measure viability and cell number.

3.2.9. Kirby-Bauer disk diffusion assessment

The Kirby-Bauer Disk Diffusion susceptibility test determines bacterial antibiotic susceptibility or resistance based on the size of its inhibition zone.[126] *Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis* were subject bacteria in this study because of their occurrence being the three most frequent bacteria species in an epidemiology study on patients with post-surgical wound infections.[127] Five microliter frozen aliquots of gram-positive bacterial species *Staphylococcus aureus and Enterococcus faecalis* were cultured in BHI broth media for 18 hours at 37°C. Gram-negative strain *Pseudomonas aeruginosa* was cultured in LB broth media for 18 hours at 37°C. *S. aureus* and *P. aeruginosa* were cultured in a standard incubator shaking at 200 rpm while *E. faecalis* was incubated in a 6% oxygen Anoxomat jar. All

species were cultured overnight to a concentration of approximately 1-2 x 10^9 CFU/mL after incubation.

After incubation, a sterile cotton swab was inoculated in each of the cultures and spread on agar medium in a 100 mm Petri dish and allowed to dry for five minutes. After drying, 6 mm diameter filter paper samples impregnated with a known concentration of antibiotic for the test organism was used as a positive control. Representative 6 mm diameter 4:1 GEL-AXF fibers impregnated with silver sulfadiazine and negative control 4:1 GEL-AXF fibers without silver sulfadiazine were placed in the remaining two quadrants on the agar. (**Table 3.2**) The Petri dishes containing samples inoculated with *S. aureus* and *P. aeruginosa* were stored in a standard incubator for 24 hours. Petri dishes containing test samples of *E. faecalis* were incubated in a 6% oxygen Anoxomat jar for 24 hours. After incubation, top and bottom images of the Petri dishes were acquired using a digital camera and image processing software (Photo/Analyst® PC Image, Fotodyne, Inc.). The growth inhibition zones diameters were measured manually with a caliper. Disk diffusion susceptibility testing was performed twice under the same conditions on different days. The mean of the inhibition zone diameters of each treatment were then determined.

Table 3.2 Kirby-Bauer susceptibility test setup

Bacterial Species (Strain)	**Positive Control (Antibiotic)**	**Treatment**	**Negative Control (no treatment)**
Pseudomonas aeruginosa (PAO1)	Tetracycline; 30 µg	4:1 GEL-AXF fibers w/ 5% silver sulfadiazine	4:1 GEL-AXF fibers w/o silver sulfadiazine
Staphylococcus aureus (RN450)	Erythromycin; 15 µg	4:1 GEL-AXF fibers w/ 5% silver sulfadiazine	4:1 GEL-AXF fibers w/o silver sulfadiazine
Enterococcus faecalis (V583)	Tetracycline; 30 µg	4:1 GEL-AXF fibers w/ 5% silver sulfadiazine	4:1 GEL-AXF fibers w/o silver sulfadiazine

3.2.10. Statistical analysis

Statistical analysis was carried out using an unpaired t-test and one way analysis of variance (ANOVA) with post-hoc analysis for subgroup comparison. *P* values less than 0.05 were considered statistically significant.

3.3 Results and discussion

3.3.1. Fiber morphology, diameter and pore size

Uniform and random aligned fiber morphology were seen in all uncrosslinked GEL-AXF blends. Fiber diameter and pore size increased with respect to gelatin concentration. The 1:1, 2:1 and 4:1 GEL-AXF blends have fiber diameters of 425 nm, 586 nm and 1.09 μm respectively. (**Figure 3.5**) Meanwhile, the pore sizes for 1:1, 2:1 and 4:1 Gel-AXF blended fibers are 0.66, 1.4 and 3.6 μm^2 respectively. (**Figure 3.5**) This data suggest that the electrospun mat's pore sizes increase with respect to higher GEL-AXF blends because their larger fiber diameter reduces the number of intersecting points which creates larger void spaces within the mat. Another possible reason that fiber diameter and pore size correspondingly increase with gelatin concentration is the 2 ml/hr flow rate for 1:1 GEL-AXF blends propel less polymer out of the syringe compared to 5 ml/hr flow rates for 2:1 and 4:1 GEL-AXF blends.

Ethanol and tri-ethanolamine were used to dissolve crosslinking reagents, PEG-DA and Eosin Y before crosslinking the electrospun mat. The scaffolds completely lost their morphology after the crosslinking step. This was attributed to the hydrophilicity of gelatin and arabinoxylan which created fiber fusion in their mats. Scaffold shrinkage also took place after crosslinking. As a result, the fiber diameter and pore size could not be measured quantitatively.

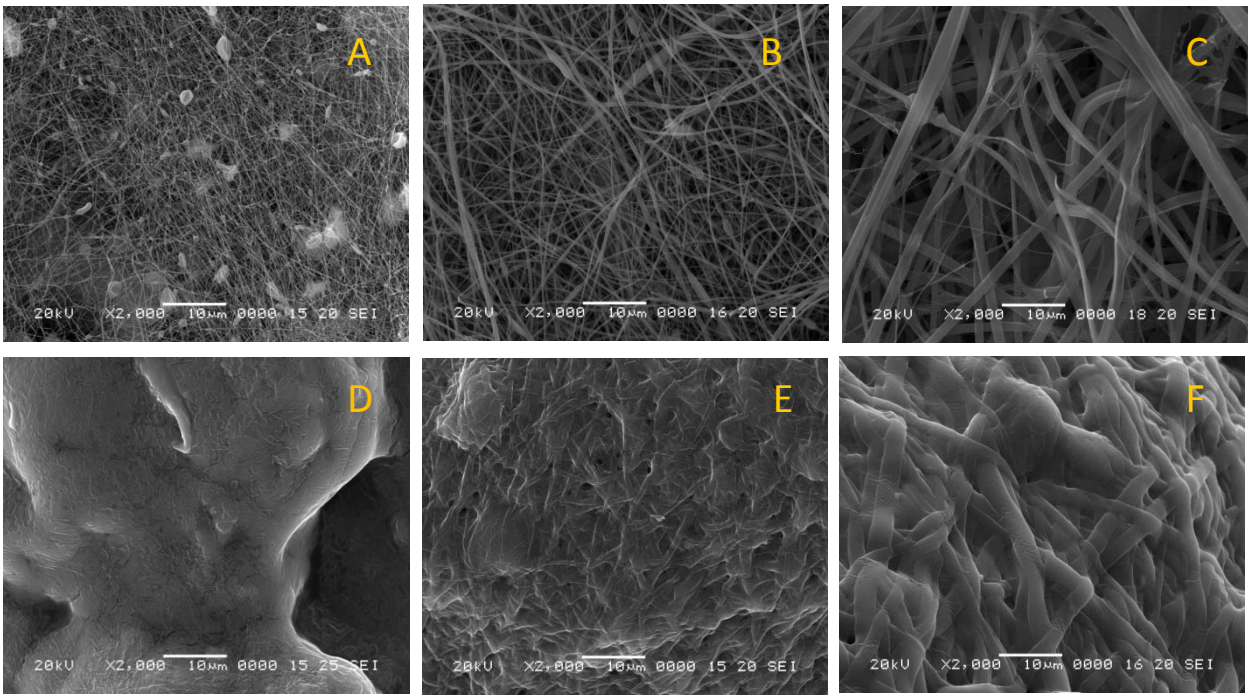
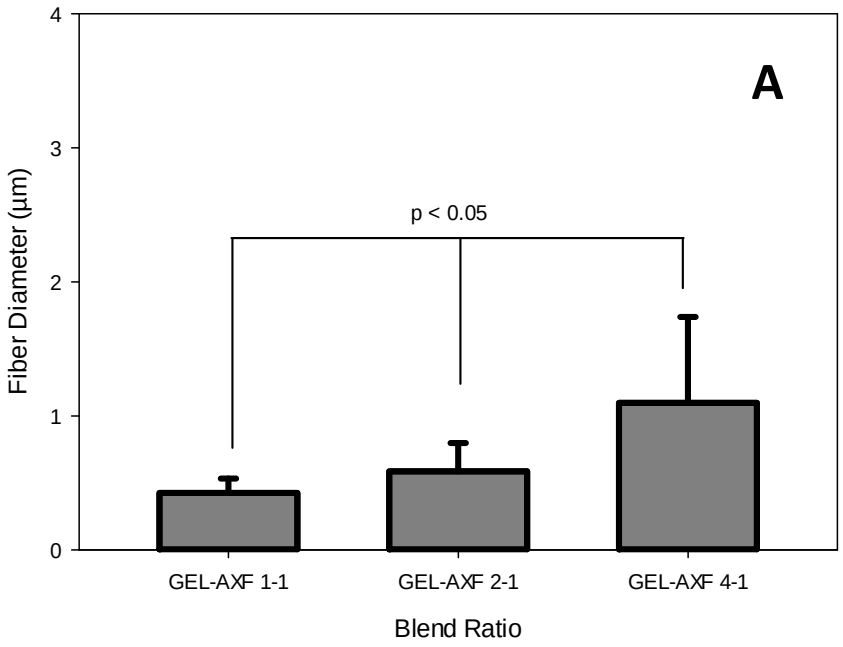


Figure 3.4: SEM of GEL-AXF scaffolds. **A)** 1:1 GEL-AXF blend; **B)** 2:1 GEL-AXF blend; **C)** 4:1 GEL-AXF blend; **D)** 1:1 GEL-AXF blend with crosslinker; **E)** 2:1 GEL-AXF blend with crosslinker; **F)** 4:1 GEL-AXF blend with crosslinker.



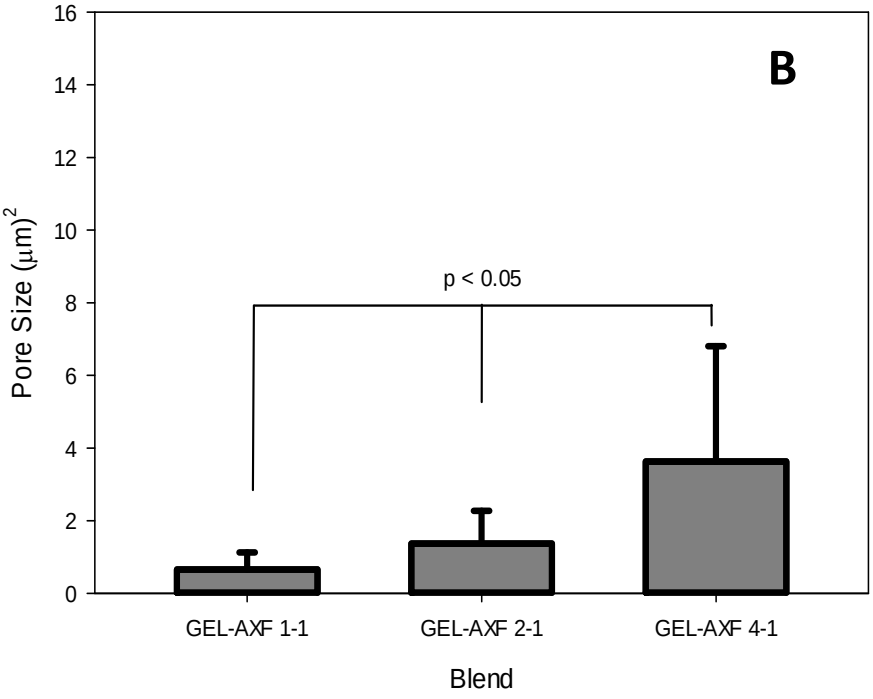


Figure 3.5: Fiber diameter & pore size of GEL-AXF blended scaffolds (n = 100)
A) Fiber Diameter; **B)** Pore Size (*$p < 0.05$; significant differences among each of 1:1, 2:1 and 4:1 GEL-AXF groups tested vs. each other)

3.3.2. Tensile testing

Uncrosslinked GEL-AXF nanofibers had moduli values of 6.3, 22.9 and 46.0 MPa for 1:1, 2:1 and 4:1 GEL-AXF blends respectively. (**Table 3.3**). It was also clear that peak load and peak stress increased in proportion to amount of gelatin blended into the fibers. Meanwhile, strain at break decreased with respect to gelatin blended. Due to strain being inversely proportional to modulus, the higher moduli values for GEL-AXF blends with greater amounts of gelatin possessed lower strain values. Crosslinked 1:1, 2:1 and 4:1 GEL-AXF fibers had moduli values of 1.00, 3.10 and 2.00 MPa respectively. Although peak load and peak stress increased in proportion to gelatin, it was not as pronounced when compared to uncrosslinked GEL-AXF scaffolds. No correlation between GEL-AXF blend ratios and modulus was present in

crosslinked scaffolds. (**Table 3.4**) This is possibly due to the crosslinker changing the original thickness of the scaffold which directly relates to modulus as shown for uncrosslinked scaffolds.

Table 3.3 Mechanical properties of uncrosslinked GEL-AXF scaffolds (n =12)

GEL-AXF Blend Ratio	Thickness (in)	Peak Load (N)	Peak Stress (MPa)	Modulus (MPa)	Strain At Break (mm/mm)	Energy to Break (N*mm)
1:1	0.0084 ± 0.00133	0.29 ± 0.093	0.50 ± 0.16	6.3 ± 2.2	0.14 ± 0.056	0.15 ± 0.071
2:1	0.011 ± 0.0015	0.59 ± 0.16	0.77 ± 0.19	23.0 ± 9.3	0.075 ± 0.040	0.12 ± 0.031
4:1	0.017 ± 0.00077	1.4 ± 0.18	1.2 ± 0.15	46.0 ± 5.7	0.033 ± 0.0069	0.17 ± 0.049

Table 3.4 Mechanical properties of crosslinked GEL-AXF scaffolds (n = 12)

GEL-AXF Blend Ratio	Thickness (in)	Peak Load (N)	Peak Stress (MPa)	Modulus (MPa)	Strain At Break (mm/mm)	Energy to Break (N*mm)
1:1	0.001 ± 0.0023	1.2 ± 0.56	1.80 ± 0.62	1.1 ± 0.28	2.2 ± 0.38	9.8 ± 5.8
2:1	0.015 ± 0.0065	2.2 ± 1.1	2.3 ± 0.63	3.1 ± 2.3	1.9 ± 0.64	16 ± 8.9
4:1	0.013 ± 0.0049	2.8 ± 0.83	3.3 ± 1.19	2.0 ± 0.92	2.2 ± 0.33	23 ± 7.9

3.3.3. Carbohydrate assay

Overall, arabinoxylan was present in all of the electrospun 1:1, 2:1 and 4:1 GEL-AXF blends, with 11%, 4% and 2% of arabinoxylan in the entire scaffold respectively. (**Figure 3.6**) The 1:1 GEL-AXF blend had a greater absolute proportion of arabinoxylan amount remaining compared to other blends. However, the 1:1 GEL-AXF blend lost a significantly greater proportion of arabinoxylan during electrospinning than the 2:1 and 4:1 blends. Meanwhile, the GEL-AXF 4:1 blend lost a significantly smaller proportion of arabinoxylan during electrospinning than the 2:1 and 1:1 blends. (**Figure 3.7**) This is attributed to the ability of gelatin and its ability to be electrospun and capture arabinoxylan particles at higher concentrations such as the 4:1 GEL-AXF blend. A greater proportion of arabinoxylan is left behind during electrospinning for 1:1 and 2:1 GEL-AXF blends where the gelatin concentrations are lower and less suitable to generate fibers on the mandrel.

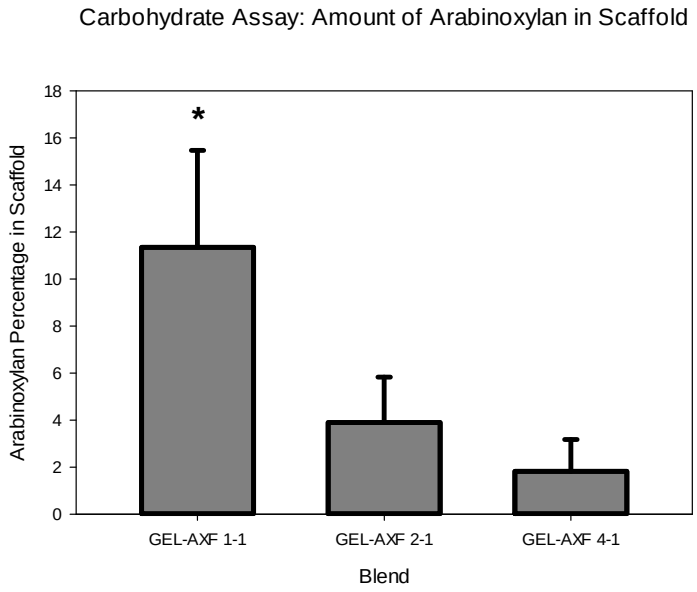


Figure 3.6: AXF percentage in scaffold after electrospinning (n = 10) (*p < 0.05; significant difference for 1:1 GEL-AXF group vs. 2:1 and 4:1 GEL-AXF groups)

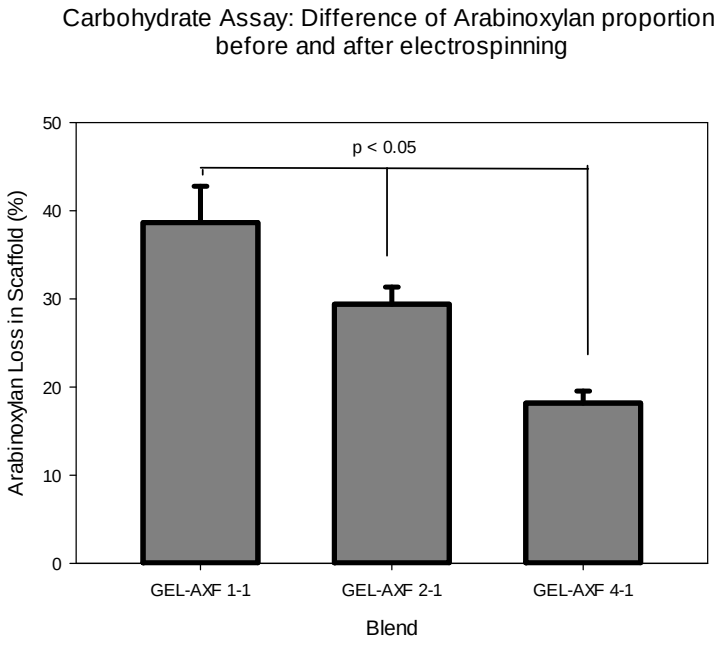


Figure 3.7: AXF loss percentage in GEL-AXF scaffold after electrospinning (n = 10) (*p < 0.05; significant differences among each of 1:1, 2:1 and 4:1 GEL-AXF groups tested vs. each other)

3.3.4. Silver release kinetics

Overall, the release rate of each of the silver loaded GEL-AXF blended nanofiber scaffolds was similar over the 48 hour time course. (**Figure 3.8**) There was an initial burst release within the first two hours before following more controlled kinetics afterwards, characteristic of zero-order kinetics. In the first two hours, the 1:1 and 2:1 GEL-AXF blended nanofiber scaffolds possessed a significantly greater cumulative silver release of 8.49 and 7.29% than the 4:1 GEL-AXF blend which released 4.19% silver. After two hours, only the 1:1 GEL-AXF blend had a significantly greater percent cumulative release than the 4:1 blend. After 48 hours, 1:1, 2:1 and 4:1 GEL-AXF impregnated with silver had 20.9, 17.0 and 10.6% cumulative release respectively. This phenomenon is likely due to the greater density of gelatin nanofibers in

4:1 GEL-AXF blends encapsulating the silver longer before release. Also, the greater density of gelatin reduced bulk degradation within the scaffold which slowed release of the encapsulated silver.

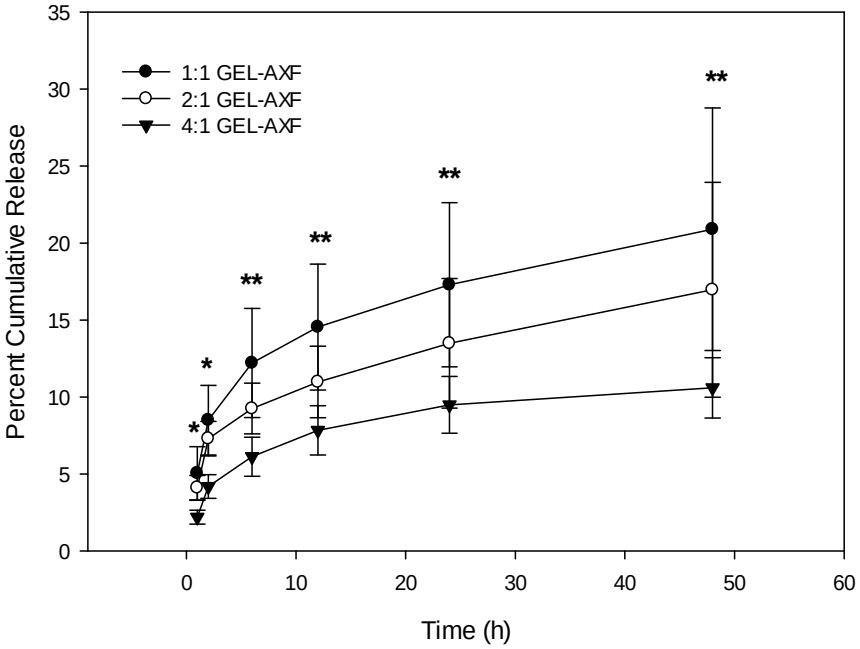


Figure 3.8: Cumulative silver sulfadiazine release from 1:1, 2:1 and 4:1 blended GEL-AXF scaffolds over a 48 hour period. (n = 6) (*$p < 0.05$; significant differences among 1:1 GEL-AXF vs. 2:1 and 4:1 GEL-AXF groups at denoted times) (**$p < 0.05$; significant differences among 1:1 GEL-AXF vs. 4:1 GEL-AXF at denoted times.)

3.3.5. WST-1 assay for cell viability

After 24 hours, all treatment groups containing degradation media produced greater cell activity values than control groups containing plain DMEM. Cell activity steadily increased with respect to concentration of 2:1 and 4:1 GEL-AXF blends up to 1.77 and 1.64 fold respectively

until 25 mg/mL where it slightly declined to 1.67 and 1.38 fold. Meanwhile, 1:1 GEL-AXF blends induced greater cell activity for all concentration values, ranging from 1.08 to 2.25 fold at 0.05 and 25 mg/mL respectively. (**Figure 3.9**) The presence of arabinoxylan may have induced greater cell activity compared to control treatments and with respect to its blend ratio with gelatin. This could be indicative of arabinoxylan's naturally derived origin promoting biocompatibility in the cell microenvironment. Overall, arabinoxylan electrospun in gelatin nanofiber scaffolds encouraged cell activity and was not cytotoxic to fibroblasts which play a key role in tissue matrix regeneration and wound healing.

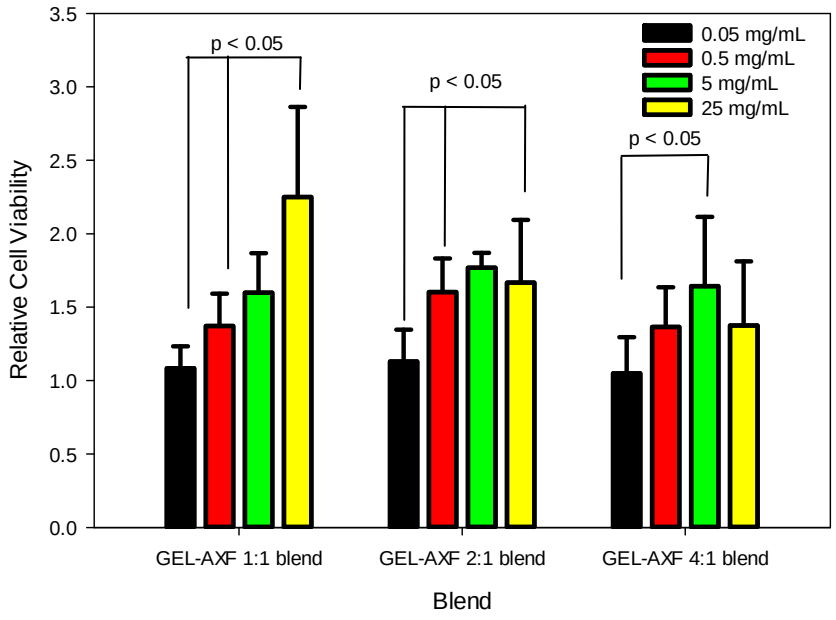


Figure 3.9: Relative cell activity of NIH3T3 cells with respect to GEL-AXF blend ratio and concentration after 24 hour incubation period. (n = 8) (*p < 0.05; significant differences among denoted 1:1, 2:1 and 4:1 GEL-AXF groups)

3.3.6. Trypan blue cell viability & proliferation assay

Introduction of arabinoxylan into electrospun gelatin did not have a cytotoxic effect on NIH3T3 fibroblast cells. Fibroblasts possessed viability percentages of at least 94.7% after treatment with degradation media from 1:1, 2:1 and 4:1 GEL-AXF blended fibers for 24 hours. (**Figure 3.10**) No significant differences were found in percent viability with respect to GEL-AXF blend ratio. Fibroblasts treated with degradation media from 1:1 GEL-AXF, 2:1 and 4:1 fibers had viability percentages ranging from 94.5 to 98.6%, 97.1 to 98.4% and 94.6 to 97.7% respectively. Non-treated fibroblasts were 97.8% viable after 24 hour incubation in DMEM +10% FBS. There were no statistically significant differences among blended fibers with respect to degradation media concentration.

GEL-AXF fibers also had a positive effect on fibroblast proliferation as the cell number doubled from 50,000 to 100,000 after 24 hour incubation for all treatments with exception to 25 mg/ml concentration of 2:1 GEL-AXF blend. The grey dotted line in (**Figure 3.11**) illustrates the doubling mark of 100,000 cells. No significant differences were found in percent viability with respect to GEL-AXF blend ratio. Fibroblasts treated with degradation media from 1:1 GEL-AXF fibers had cell numbers increase with respect to concentration, reaching a peak of 142,000 cells at 5 mg/mL before declining to 125,000 at 25 mg/mL. Fibroblasts treated with degradation media from 2:1 and 4:1 fiber blends also exhibited a similar trend reaching a peak cell number of 155,000 and 151,000 before falling to 73,500 and 110,000 respectively. Non-treated fibroblasts proliferated to 101,000 after 24 hour incubation in DMEM +10% FBS. Despite the high cell viability numbers for fibroblasts treated with 25 mg/mL concentrations of 1:1, 2:1 and 4:1 blend ratios of GEL-AXF, the lower cell proliferation numbers at this concentration may be attributed to a material dosing effect where the cell activity is hindered by the scaffold. Nonetheless, there

were no statistically significant differences among blended fibers with respect to degradation media concentration.

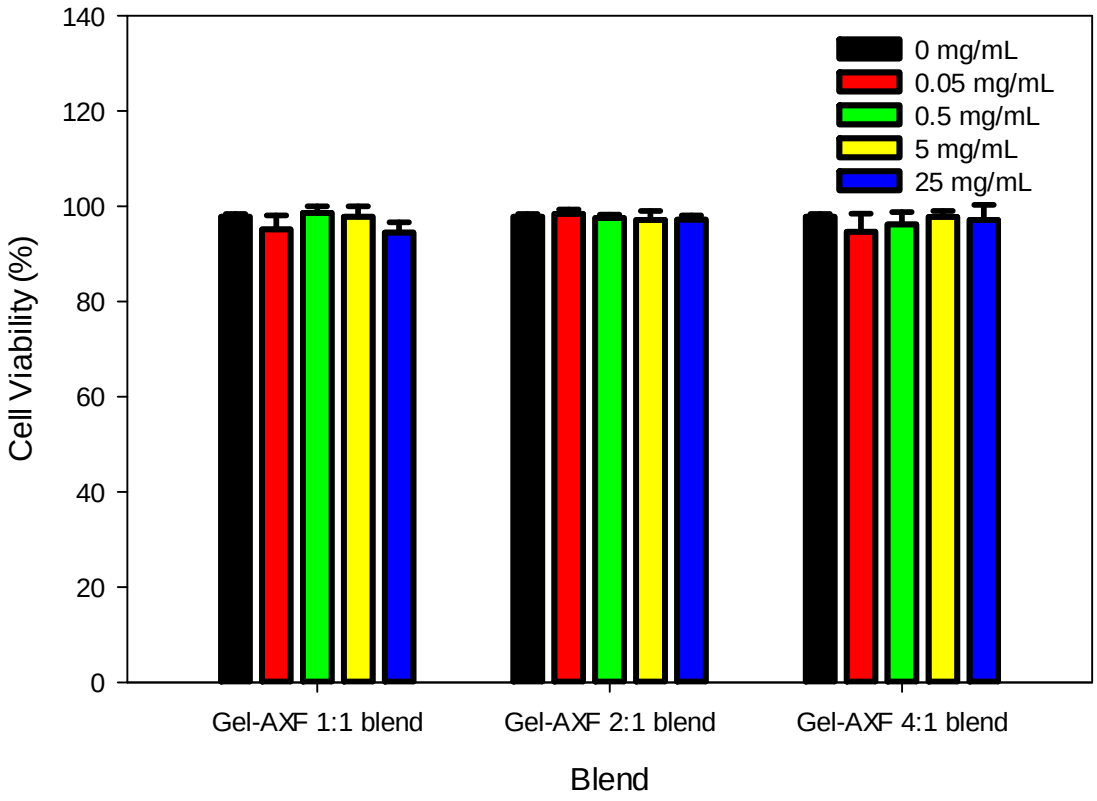


Figure 3.10: Viability of NIH3T3 fibroblast cells with respect to GEL-AXF blend ratio and concentration after 24 hour incubation period. (n = 8)

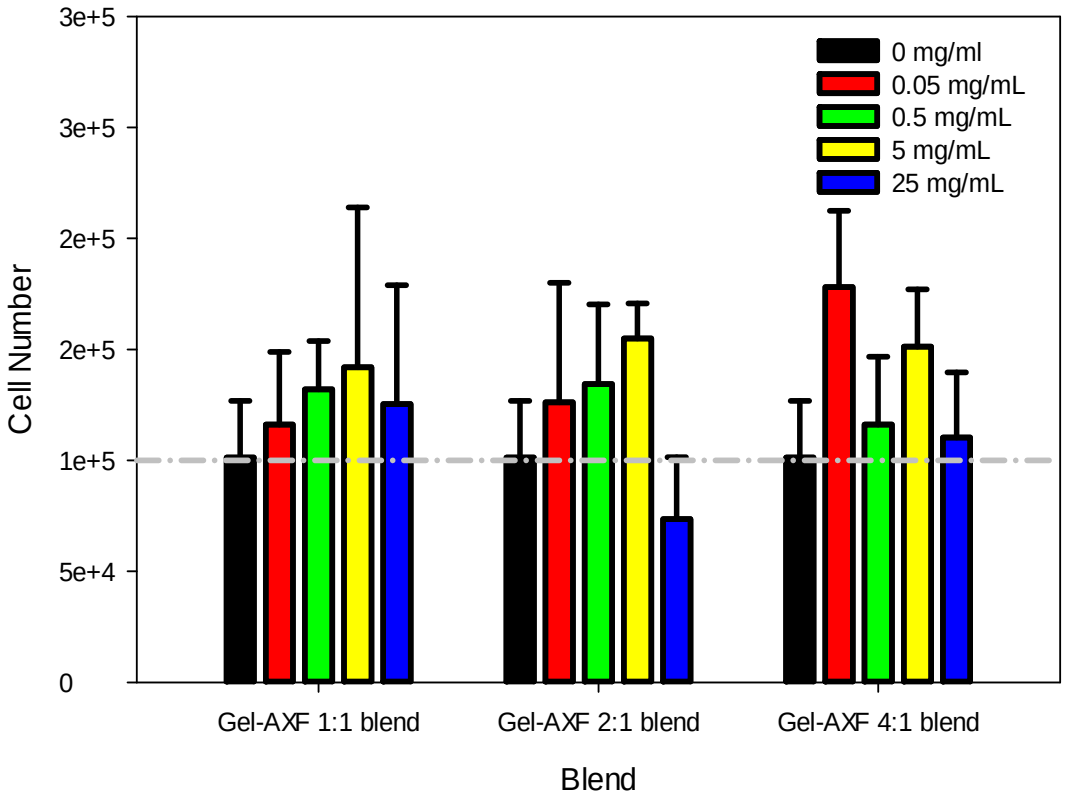


Figure 3.11: Cell number of NIH3T3 fibroblasts with respect to GEL-AXF blend ratio and concentration after 24 hour incubation period. (n = 8)

3.3.7. Kirby-Bauer disk diffusion assessment

GEL-AXF fibers were assessed for their anti-microbial activity by measuring the inhibition zones within each species of bacteria. *P. aeruginosa* was highly susceptible to silver sulfadiazine impregnated 4:1 GEL-AXF fibers, which actively inhibited bacterial growth with an inhibition zone of 16.24±1.43 mm, greater than the inhibition zone of the tetracycline positive control. The effectiveness of silver sulfadiazine was consistent to previous data showing its applicability in a skin wound models.[128] Gram-positive *S. aureus* and *E. faecalis* species were less sensitive to the silver sulfadiazine impregnated 4:1 GEL-AXF fibers with an inhibition zone diameter of 9.51 ± 0.37 and 9.39 ± 0.54 mm respectively. One possible reason that these species showed some resistance compared to gram-negative *P. aeruginosa* is that gram-positive bacteria are more susceptible to antibiotics and have a thicker cell wall that make it more difficult to break down.[129] All three species of bacteria were sensitive to 4:1 GEL-AXF fibers impregnated with silver sulfadiazine. *P. aeruginosa* was most susceptible to the silver impregnated fibers among the three species. All three positive control treatments achieved clear inhibition of each of the bacterial species after 24 hour incubation. (**Figure 3.12**) As expected, all bacterial species grew and showed resistance to 4:1 GEL-AXF fibers with no silver sulfadiazine. The growth inhibition data from two trials are also summarized in **Table 3.5**.

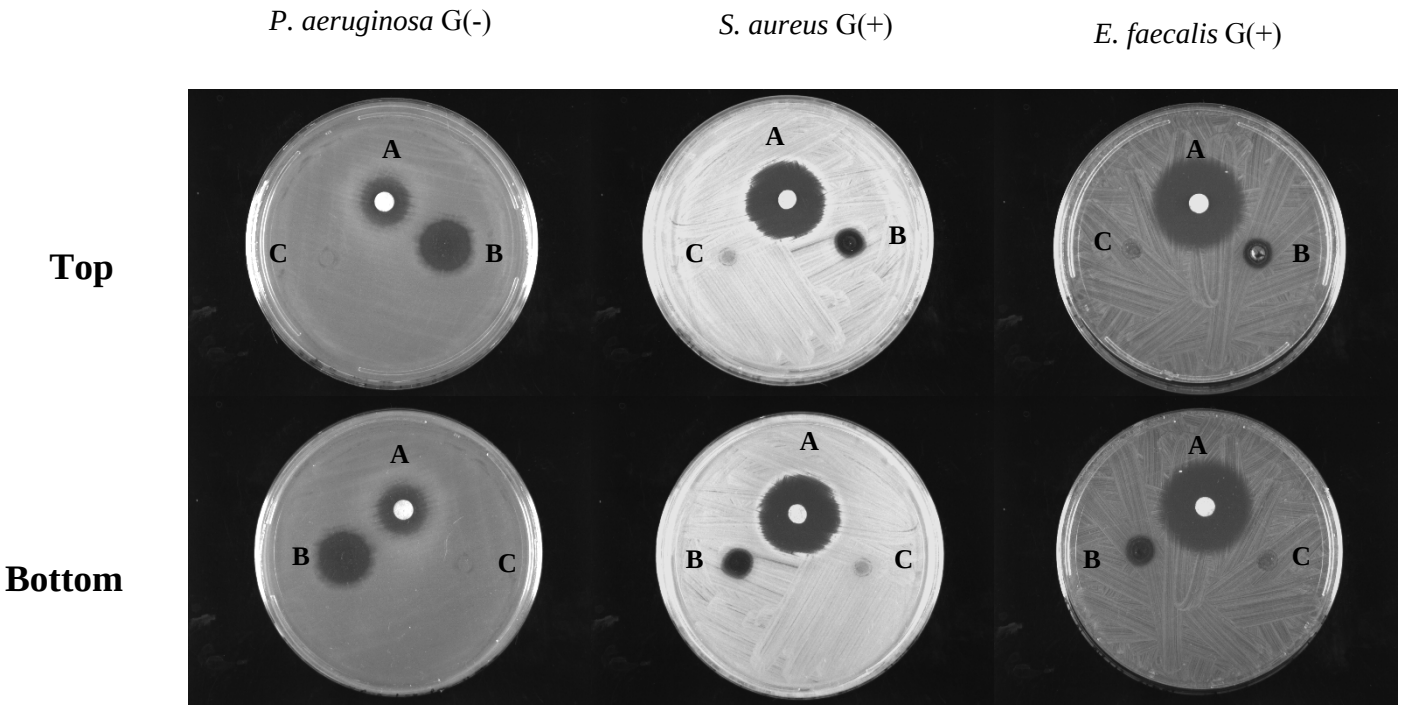


Figure 3.12: Antimicrobial properties of 4:1 GEO-AXF fiber mats using Kirby-Bauer disk diffusion susceptibility test on gram negative G(-) and gram positive G(+) bacteria. Tetracycline (A), Silver sulfadiazine impregnated 4:1 GEL-AXF fibers (B) and 4:1 GEL-AXF fibers (C) were tested on *Pseudomonas aeruginosa and Enterococcus faecalis* bacterial species. Erythromycin (A), Silver sulfadiazine impregnated 4:1 GEL-AXF fibers (B) and GEL-AXF fibers (C) were tested on *Staphylococcus aureus* bacterial strain. (n = 2)

Table 3.5 Sensitivity profiles of bacterial species after treatment

Species	Treatment	Average inhibition zone (mm)
P. aeruginosa		
	Positive Control (30 µg Tetracycline)	15.38±0.53
	4:1 GEL-AXF fibers w/ 5% silver sulfadiazine	16.24 ±1.43
	Negative Control (no treatment)	0
S. aureus		
	Positive Control (15 µg Erythromycin)	23.83 ±0.55
	4:1 GEL-AXF fibers w/ 5% silver sulfadiazine	9.51 ±0.37
	Negative Control (no treatment)	0
E. faecalis		
	Positive Control (30 µg Tetracycline)	28.25 ±1.06
	4:1 GEL-AXF fibers w/ 5% silver sulfadiazine	9.39 ±0.54
	Negative Control (no treatment)	0

3.4 Conclusions

Synthesis of blended GEL-AXF electrospun nanofibers was successful. Addition of gelatin increased fiber diameter and the pore size of the scaffold. In turn, this increased the scaffold's elastic modulus. Crosslinker reagents were formulated to help stabilize the scaffold but the results indicate reduced surface morphology and mechanical strength. Arabinoxylan was successfully integrated into the gelatin nanofibers and has potential to act as a wound dressing. However, the degradation profile indicated the uncrosslinked and crosslinked GEL-AXF scaffold disintegrated within 30 minutes in aqueous media (data not shown). Arabinoxylan should be incorporated with a more stable polymer such as polylactic acid (PLA) or polylactic-co-glycolic acid (PLGA) to reduce scaffold degradation in media.[130] In vitro biocompatibility results were promising as fibroblast cell viability and proliferation were the same or greater than non-treated groups. Silver impregnated GEL-AXF fibers exhibited antimicrobial activity against bacterial pathogens. In future testing, GEL-AXF fibers will be implanted onto an injury site of an animal model where in vivo drug release and tissue viability will assess the drug delivery and biocompatibility properties. The host immune response after foam implantation in vivo will be examined by measuring at neutrophil count or pro-inflammatory cytokines in affected biopsy wounds. Electrospun GEL-AXF fibers are good platforms as wound dressing materials because of their high surface to volume ratio, abundance, their naturally derived origin and their absorptive capacity. This study hopes to exhibit the feasibility and efficacy of fabricating novel electrospun arabinoxylan based fibers in an effort to advance wound dressing development.

CHAPTER 4: Arabinoxylan foams for wound dressing applications

Donald C. Aduba, Jr.[1], Seon-Sook An[2], W. Andrew Yeudall[3], Gary L. Bowlin[4], Todd O. Kitten[2], & Hu Yang[1,5]

[1]*Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284.*

[2]*Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298.*

[3]*College of Dental Medicine, Georgia Regents University, Augusta, GA 30912.*

[4]*Department of Biomedical Engineering, University of Memphis, Memphis, TN 38152.*

[5]*Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298.*

Abstract:

Fabrication of a functional wound dressing material is critical in managing wound healing. Traditional wound dressings such as gauze achieve the minimum by simply protecting and covering the wound. More advanced wound dressings aim to achieve greater functionality by absorbing wound exudate to keep the wound site moist and preventing biofilm formation. Many current advanced wound dressing materials on the market possess the aforementioned properties but none have used arabinoxylan (AX) as a base material. AX should be considered as a wound dressing because of its absorptive properties and naturally derived origin. It is also inexpensive. The goal of this study is to investigate and highlight the material and biocompatible properties of Arabinoxylan ferulate (AXF) foams as a potential wound dressing material. To achieve this goal, the AXF foam's material properties such as morphology, storage modulus, porosity, swelling and cumulative drug release kinetics were examined. Endotoxin quantification, fibroblast activity and microbial growth inhibition studies were completed to test the in vitro biocompatibility of AXF foams. Tegaderm Alginate foam dressings manufactured by 3M were included in many of these studies for comparison. The in vitro results indicate that AXF foams are highly porous, absorptive scaffolds which have swelling ratios over 20 times its original weight. AXF foams are non-toxic to the cell microenvironment, exhibiting cell viabilities over 95% and cell numbers doubling after 72 hours. Silver (5% w/w) was successfully integrated into AXF foams and achieved 45% cumulative release after 48 hours in vitro. Silver impregnated AXF foams also inhibited bacterial lawn growth using a modified Kirby-Bauer disk-diffusion method. Overall, AXF foams possess many appropriate material properties necessary to be a candidate material in wound dressing development.

Keywords: *wound dressing, foam, arabinoxylan, wound management, biopolymer, crosslinking*

4.1 Introduction

Wound dressing development is important in clinical settings because of the emphasis to improve the rate of wound healing while preventing threatening infections after injury. Medical device manufacturers are advancing wound dressing features beyond what is already exhibited in traditional dressings such as gauze. Those types of wound dressings are expected to primarily protect the wound from further injury. Advanced wound dressings are expected to keep the wound site moist, absorb wound exudate, remove the wound odor, prevent biofilm formation and encourage skin regeneration in addition to the features described of traditional wound dressings.[33, 34] It should also have good biocompatibility. It should have appropriate mechanical strength for insulation, wound protection and exudate removal from the injury site. Lastly, it is expected be absorptive, impermeable to bacteria and inexpensive. [31, 43]

Polysaccharide polymers have been used as advanced wound dressings because they are natural, highly abundant, biodegradable and non-toxic. They also have components that are recognized by the body and play a role in rebuilding the extracellular matrix.[131] Polysaccharides can be crosslinked to form gels that are highly absorbent that keep the wound bed moist.[132] They are candidates as wound dressing materials because of their ability to carry and deliver drugs in a controlled fashion. Alginate, chitin, chitosan, hyaluronan and heparin are examples of polysaccharides that have been used as commercially wound dressings.[131]

Arabinoxylan (AX) is a polysaccharide containing a xylose backbone with arabinose substituted in the O-2 or O-3 positions of the backbone.[1] The xylose and arabinose units are linked together by ester linkages which gives the biopolymer its hydrophilic properties. Ferulic acid can be coupled to arabinoxylan via ester linkages to produce the resulting Arabinoxylan ferulate (AXF). AXF in water can be crosslinked to form gels by radical polymerization to create

dimers at the ester bond between ferulic acid and the arabinose unit.[115] The applications of polysaccharides related to AX range from packaging materials to prebiotics that regulate gut metabolism.[70, 119, 121] More specifically, AX gels have been developed by several groups as delivery systems which have proven to deliver therapeutics such as methyl xanthine and albumin in vitro.[1, 115] AX gel fabrication is very simple, does not require use of harsh organic solvents and its mechanical properties can be tuned with respect to the crosslinking density. Arabinoxylan has an ability to swell and absorb liquids because of its hydrophilic properties.[115] In this study, lyophilized AXF foams will be fabricated then characterized to evaluate their material properties, and biocompatibility to evaluate its potential as a wound dressing.

4.2 Materials and methods

4.2.1. Materials

Arabinoxylan ferulate (AXF) was purchased from Cambridge Biopolymers (Cambridge, UK). Horseradish peroxidase and 35% wt. hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO). 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was purchased from TCI America (Portland, OR). Phosphate buffered saline (PBS) was purchased from EMD Chemicals (Gibbstown, NJ). Dulbecco's modification of eagle's medium (DMEM) was purchased from Invitrogen (Carlsbad, CA). Fetal calf serum was purchased from Lonza (Walkersville, MD). Hyclone 0.05% Trypsin was purchased from Thermo Scientific (Logan, UT). Penicillin Streptomycin was purchased from Life Technologies (Grand Island, NY). Tegaderm Alginate and Ag Tegaderm Alginate was purchased from 3M Health Care (St. Paul, MN). LAL Chromogenic Endotoxin Quantitation Kit was purchased from Thermo Scientific (Rockford, IL).

4.2.2. Scaffold preparation

4.2.2.1. Preparation of arabinoxylan foams

Two batches of 600 milligrams of AXF dry powder were added to two 10 milliliter aliquots of deionized water, stirred and shaken until dissolved. Next, 50 µl of 1 mg/mL solution of peroxidase and 60 µl of (3% w/v) hydrogen peroxide was added to each of the reaction mixtures and stirred to initiate enzymatic crosslinking.[133] (**Figure 4.1**) Immediately, the solutions were poured together into a 100 mm Petri-dish before sonication for 30 minutes. After sonication, the crosslinked solutions were set in room temperature for three hours to cure. After curing, the crosslinked gel was freeze-dried overnight to produce the final product. 3M™ Tegaderm Alginate foam dressing served as an established commercialized peer for comparison.

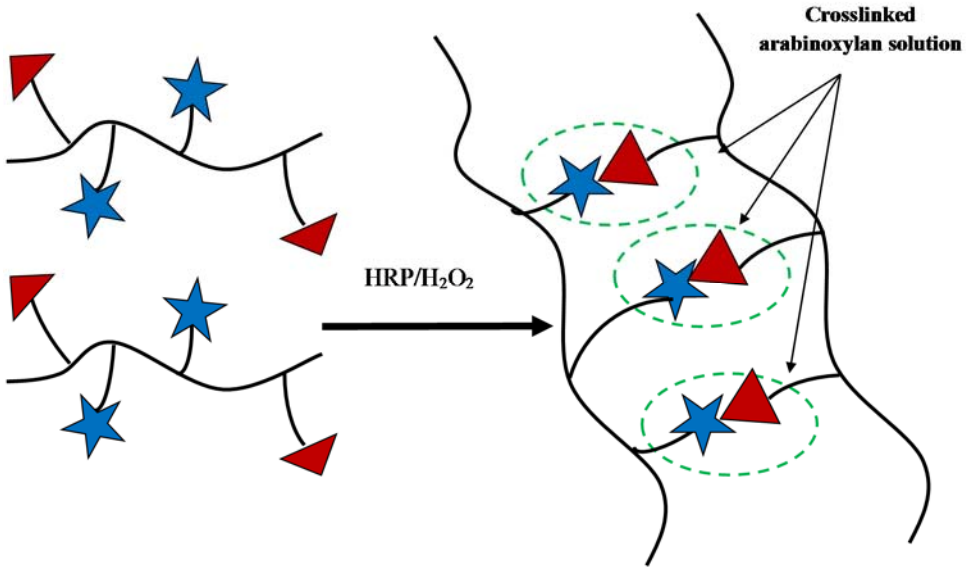


Figure 4.1: Diagram of enzymatic crosslinking of arabinoxylan ferulate (AXF) in solution. The ferulic acid group of AXF (▲) crosslinked with the arabinose sugar of arabinoxylan (★) via horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) to form a hydrogel network on the xylose backbone (∼).

4.2.2.2. Preparation of silver-loaded arabinoxylan foams

To create AXF foams with 5% w/w concentration of silver, approximately 65 milligrams of silver sulfadiazine was added with 600 milligrams of AXF in deionized water before stirring. After stirring, the procedure was followed from Section 4.2.1. Ag 3M^{TM} Tegaderm Alginate foam dressing with 5% silver served as an established commercialized peer for comparison. This concentration of silver in the GEL-AXF blends was equivalent to the amount in the Alginate wound dressing that contained an ionic silver complex made of silver, sodium, hydrogen, zirconium and phosphate.[122, 123]

4.2.3. Morphology

Prior to SEM imaging, scaffolds were placed on a 1 cm diameter stub. The stub was placed on a specimen holder and platinum sputter coated. SEM images were taken and analyzed under JEOL JSM-5610LV Scanning Electron Microscope to see any distinguishing features in the surface morphology of the scaffolds.

4.2.4. Rheology

Rheological testing was performed on samples and cut into 10 mm diameter disks. Testing was performed on dry and wet samples on a temperature controlled plate of a Discovery Hybrid Rheometer. Wet samples were immersed in 1 mL of phosphate buffer saline (PBS) for one hour before blot drying. Each set of samples on the plate at 25 and 37 degrees Celsius were under compression, undergoing shear stress by an 8 mm diameter probe. The parameters for the compression probe were as follows: 500 μm gap size, 0.1-100 Hz frequency and 0.1% strain rate during the measurement. These settings derived from the work done by Zuidema et al.[134] This study was done in duplicate.

4.2.5. Swelling ratio

The sample was cut into 10 mm diameter disks by biopsy punch then weighed to record their masses. Next, they were immersed in 1.5 mL of PBS at room temperature for seven days and taken out at pre-determined time points (6h, 12h, 24h, 48h, 72h and 168h). At each time point, the swollen foam was taken out, blot dried and re-weighed to record its mass. The swelling ratio was calculated by the following formula:

$$\text{Swelling Ratio} = [(W_f/W_a)] \times 100$$

Where W_f = final mass of sample, and W_i = initial mass

This study was done in duplicate.

4.2.6. Drug release kinetics

Samples were cut into 10 mm diameter disks then incubated in 20 mL of 2% Nitric acid solution at 37 degrees. The mass of silver sulfadiazine in the scaffold was estimated by taking 5% of the scaffolds mass. The foams were immersed in a conical flask containing 20 mL of 2% Nitric acid solution. At 1 hr, 2 hr, 6 hr, 12 hr, 24 hr and 48 hr time-points, a 5 mL aliquot of release media was taken out and collected into a 15 mL centrifuge tube. The sample aliquots were then analyzed for silver release quantification using ICP-OES. Intensity measurements for silver were calibrated against a standard curve to estimate silver concentration values which were then converted to mass. Triplicate samples were independently measured twice for the study.

4.2.7. Limulus amebocyte lysate (LAL) endotoxin assay

The AXF foams were weighed then sterilized with 1000 ppm peracetic acid for 15 minutes and washed in PBS three times for 10 minutes using the protocol developed by Yoganarasimha et al.[125] The sterilized AXF foams were air-dried for 10 minutes and along with unsterile foams, were placed into tissue culture inserts for each well. The inserts containing the foam samples were immersed in PBS for four hours. After immersion, a 50 µl aliquot was extracted from the release media and diluted 20-fold with addition of 1 mL of endotoxin free media from the endotoxin assay kit. After dilution, 50 µl aliquots were taken from the diluted samples and added to a 96-well plate. Next, 50 µl of LAL reagent was added to the sample for 10 minute incubation at 37°C. Afterwards, 100 µl of chromogenic substrate was added to the reaction mixture and incubated for six minutes at 37°C to induce colormetric reaction based on protease enzyme activity induced by the endotoxin. A 50 µl aliquot of 25% acetic acid was added to end the reaction before samples were gently shaken before spectrophotometric measurement at 405 nm.

4.2.8. Trypan blue cell viability & proliferation assay

The AXF foams were sterilized with 1000 ppm peracetic acid for 15 minutes and washed in PBS three times for 10 minutes using the protocol developed by Yoganarasimha et al.[125] The sterilized AXF foams were air-dried for 10 minutes and placed into tissue culture inserts which were immersed into individual wells. NIH3T3 fibroblasts were seeded at a density of 50,000 cells per well in a 12 well plate before the inserts were introduced. The cells were incubated with the scaffold containing inserts for 24 and 72 hours at 37°C and 5% CO_2. After incubation, the seeded cells were trypsinized with and suspended in 1:1 trypsin-DMEM mixture before

centrifugation for five minutes at 3000 rpm. After centrifugation, the supernatant was aspirated and the cell pellet was re-suspended in a 1:1 mixture of PBS-trypan blue dye for five minutes before 20 μL aliquots were taken out to measure viability and cell number. 3M Alginate foams were used for commercial scaffold comparison while no scaffold served as a control treatment.

4.2.9. Kirby-Bauer disk diffusion assessment

The Kirby-Bauer Disk Diffusion susceptibility test determined bacterial antibiotic susceptibility or resistance based on the size of its inhibition zone.[126] *Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis* were subject bacteria in this study because of their occurrence being the three most frequent bacteria species in an epidemiology study on patients with post-surgical wound infections.[127] Five microliter frozen aliquots of gram-positive bacterial species *Staphylococcus aureus and Enterococcus faecalis* were cultured in BHI broth media for 18 hours at 37°C. Gram-negative strain *Pseudomonas aeruginosa* was cultured in LB broth media for 18 hours at 37°C. *S. aureus* and *P. aeruginosa* were cultured in a standard incubator shaking at 200 rpm while *E. faecalis* was incubated in a 6% oxygen Anoxomat jar. All species were cultured overnight to a concentration of approximately 1-2 x 10^9 CFU/mL after incubation.

After incubation, a cotton swab was inoculated in each of the cultures and spread on agar medium in a 100 mm Petri dish and allowed to dry for five minutes. After drying, 6 mm diameter samples impregnated with a known concentration of antibiotic for the test organism was used as a positive control. AXF foams impregnated with silver sulfadiazine, Alginate foam impregnated with silver and AXF foams without silver were placed in the remaining three quadrants on the agar. (**Table 4.1**) The Petri dishes containing samples inoculated with *S. aureus* and *P.*

aeruginosa were stored in a standard incubator for 24 hours. Petri dishes containing test samples of *E. faecalis* were incubated in a 6% oxygen Anoxomat jar for 24 hours. After incubation, top and bottom images of the petri dishes were acquired using a digital camera and image processing software (Photo/Analyst^{R} PC Image, Fotodyne, Inc.). The growth inhibition zones diameters were measured manually with a caliper. Disk diffusion susceptibility testing was performed twice under the same conditions on different days. The mean of the inhibition zone diameters of each treatment were then determined.

Table 4.1 Kirby-Bauer susceptibility test setup

Bacterial Species (Strain)	**Positive Control (Antibiotic)**	**Treatment 1**	**Treatment 2**	**Negative Control (no treatment)**
Pseudomonas aeruginosa (PAO1)	Tetracycline; 30 µg	AXF foam w/ 5% silver sulfadiazine	ALG foam w/5% silver	AXF foam w/o silver sulfadiazine
Staphylococcus aureus (RN450)	Erythromycin; 15 µg	AXF foam w/ 5% silver sulfadiazine	ALG foam w/5% silver	AXF foam w/o silver sulfadiazine
Enterococcus faecalis (V583)	Tetracycline; 30 µg	AXF foam w/ 5% silver sulfadiazine	ALG foam w/5% silver	AXF foam w/o silver sulfadiazine

4.2.10. Statistical analysis

Statistical analysis was carried out using an unpaired t-test and one way analysis of variance (ANOVA) with post-hoc analysis for subgroup comparison. *P* values less than 0.05 were considered statistically significant.

4.3 Results and discussion

4.3.1. Morphology

The AXF foams exhibited a porous, smooth morphology which allows high fluid absorption. Also, the pores were in the micro scale and evenly distributed along the surface which encouraged fluid absorption and drug release. Meanwhile, the 3M Alginate foams possessed a smooth surface with a random entanglement of microfibers. The porous structure was less ordered which may affect fluid absorption but not significant enough to dry out the wound bed. (**Figure 4.2**)

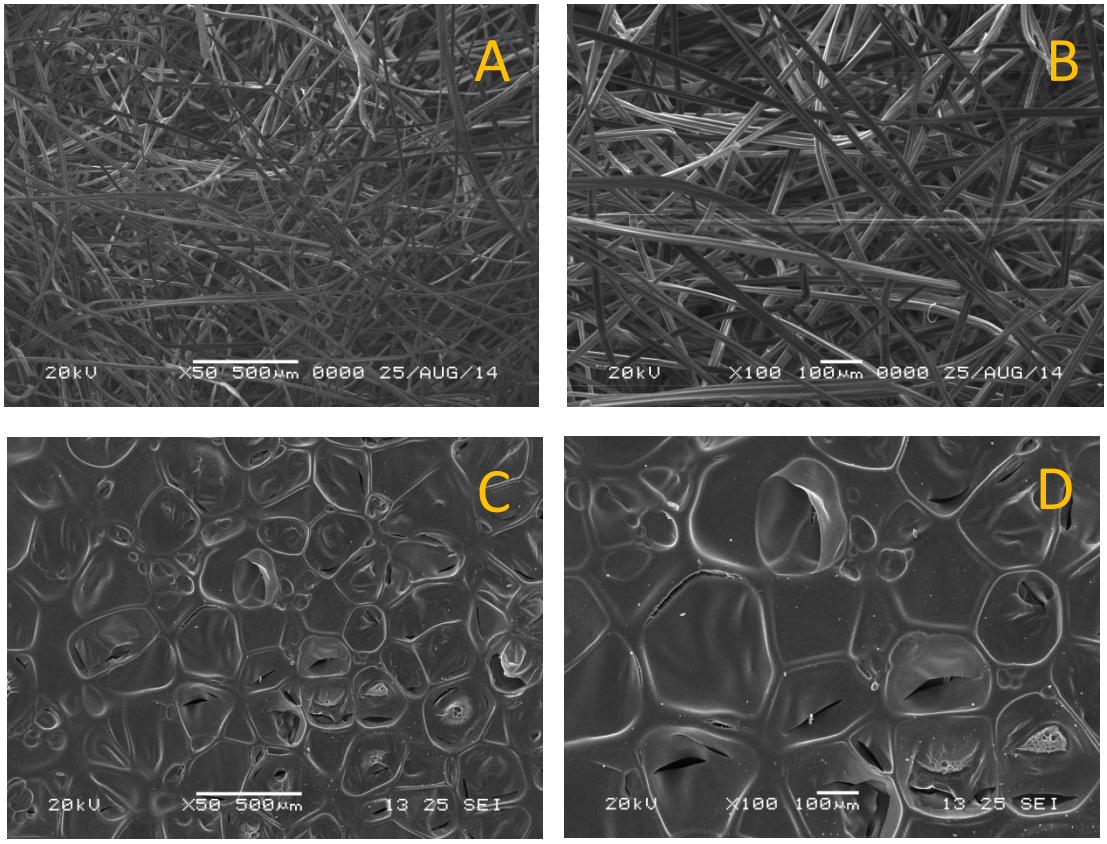


Figure 4.2: SEM images of **A)** 3M Alginate at 500 µm; **B)** 3M Alginate at 100 µm; **C)** AXF foam at 500 µm; **D)** AXF foam at 100 µm.

4.3.2. Rheology

The AXF foams had significantly higher storage modulus values than 3M Alginate foams in the dry state at both 25 and 37 degrees Celsius. (**Figure 4.3**) The storage modulus for dry 3M Alginate foams across 0.1-100 Hz frequencies was 2.58-4.07 MPa. Meanwhile, the storage modulus for AXF foams at 25 degrees Celsius was 30.6-52.2 MPa. At 37 degrees Celsius, 3M Alginate foams and AXF foams had storage moduli of 2.40-3.86 and 24.8-25.9 MPa respectively over the same frequency range. This phenomenon was attributed to the greater stiffness and viscosity in the dry AXF foams. Hydrated 3M Alginate and AXF foams at 25 degrees Celsius exhibited storage moduli of 0.256-1.62 and 0.005-0.792 MPa across 0.1-100 Hz frequency range, respectively. Hydrated 3M Alginate and AXF foams at 37 degrees have storage modulus values of 0.242-1.48 and 0.006-0.822 MPa across the same frequency range respectively. The results suggested hydrated AXF foams to some extent lost some stiffness and were more viscous than elastic. In the hydrated state, 3M Alginate foams had significantly higher storage modulus values than AXF foams because AXF is a hydrophilic polymer that swells significantly when hydrated. As a result, the polymer chains in the foam expanded which compromised its elasticity and strength. Overall, the storage modulus of the AXF and Alginate foams were lowered by three and one order of magnitude respectively after they were hydrated. (**Figure 4.4**)

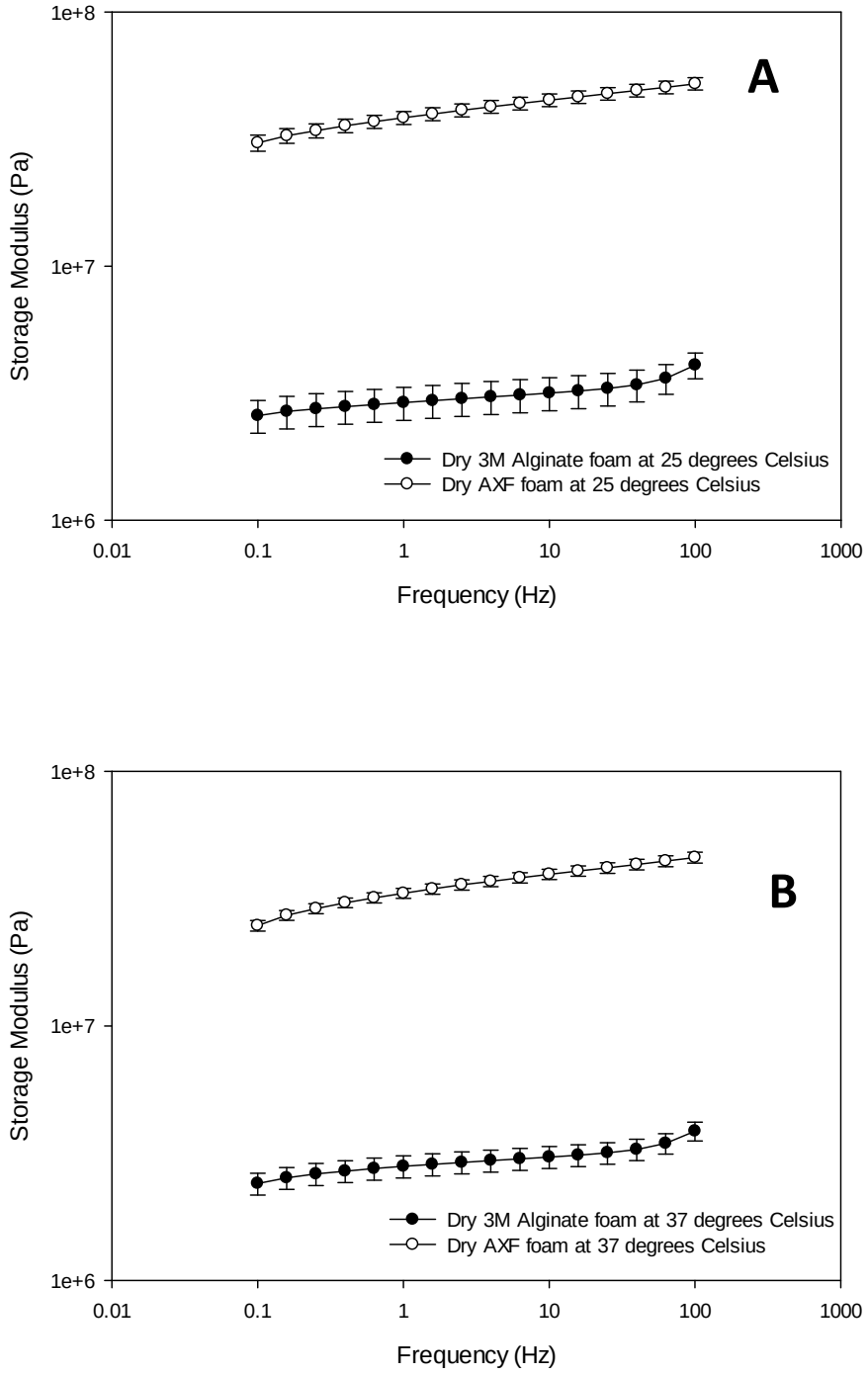


Figure 4.3: Storage modulus of dry AXF and 3M alginate foams under shear stress at **A)** 25 and **B)** 37 degrees Celsius. (n = 16) (*$p < 0.05$; significant differences of AXF foam vs. 3M Alginate foam at both temperatures)

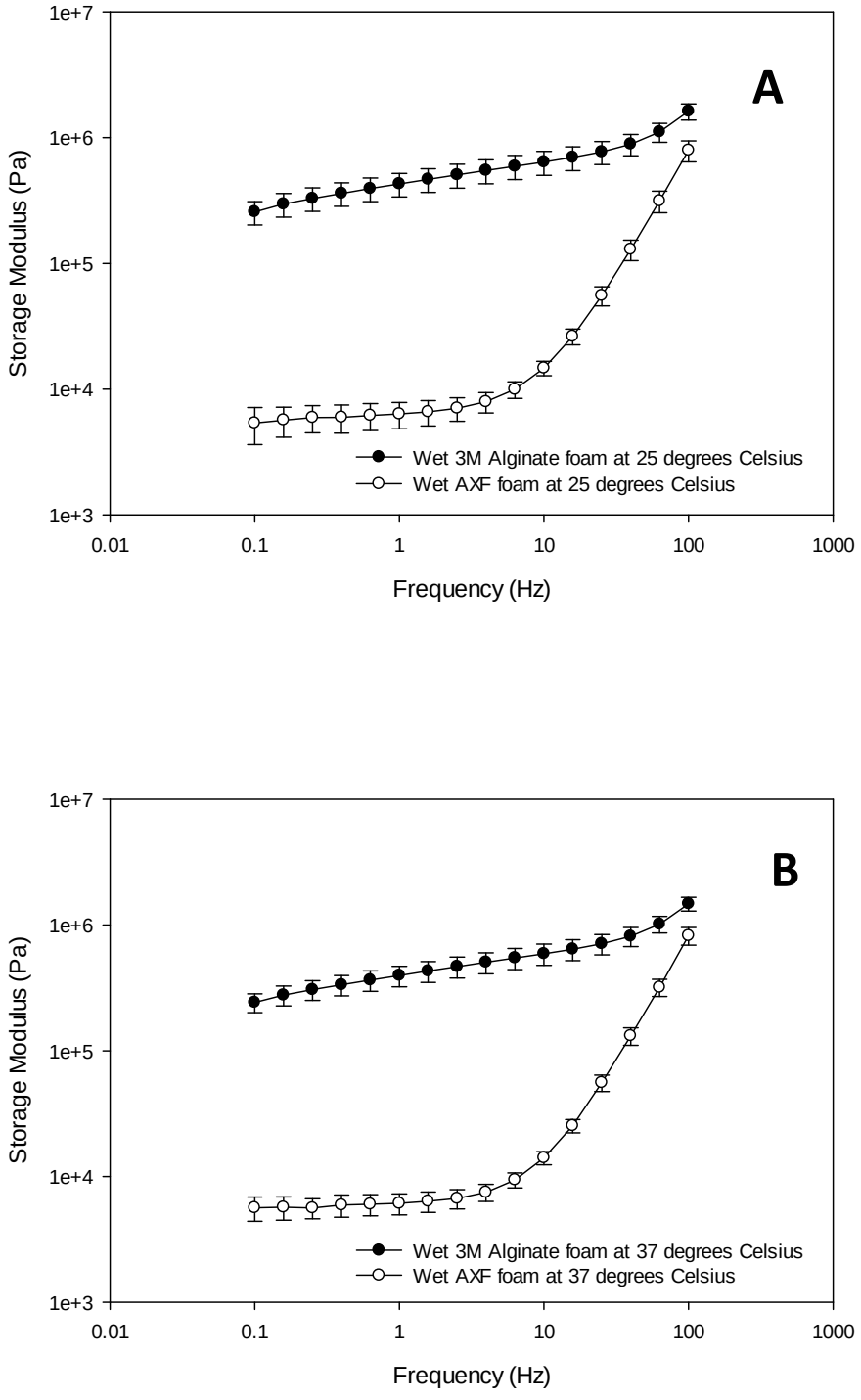
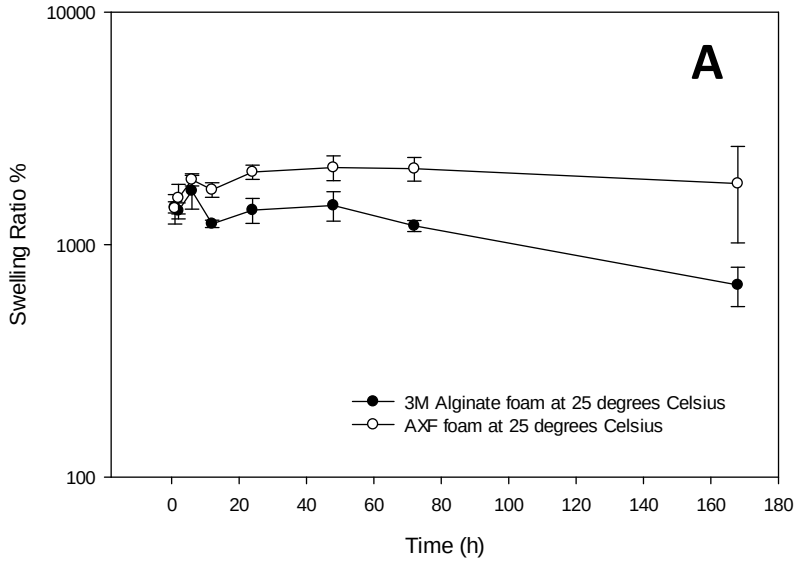


Figure 4.4: Storage modulus of hydrated AXF and 3M alginate foams under shear stress at **A)** 25 and **B)** 37 degrees Celsius. (n = 16) (*$p < 0.05$; significant differences of AXF foam vs. 3M Alginate foam at both temperatures)

4.3.3. Swelling ratio

Overall, both scaffolds had high swelling capacities because of their porous morphology. The swelling ratios of 3M Alginate at 25 degrees Celsius were 670% at six hours to a final swelling ratio of 1703% after 168 hours. AXF foams had a six hour swelling ratio of 1432% at six hours and 2145% after 168 hours. Meanwhile at 37 degrees, 3M Alginate foams possessed a six hour swelling ratio of 930% that swelled to a 1096% after 168 hours. AXF foams at 37 degrees had swollen 1302% then reaching 2025% after 168 hours. Both scaffolds swell rapidly within the first 12 hours before reaching a plateau at 24 hours. (**Figure 4.5**) The AXF foam exhibited a significantly higher swelling ratio than 3M Alginate foams during the 168 hour time period. This is because AXF is highly water absorbent which increases in mass after being immersed in water. AXF foams also have a tendency to retain fluid much more than its 3M Alginate counterparts due to its inelasticity within the polymer network.



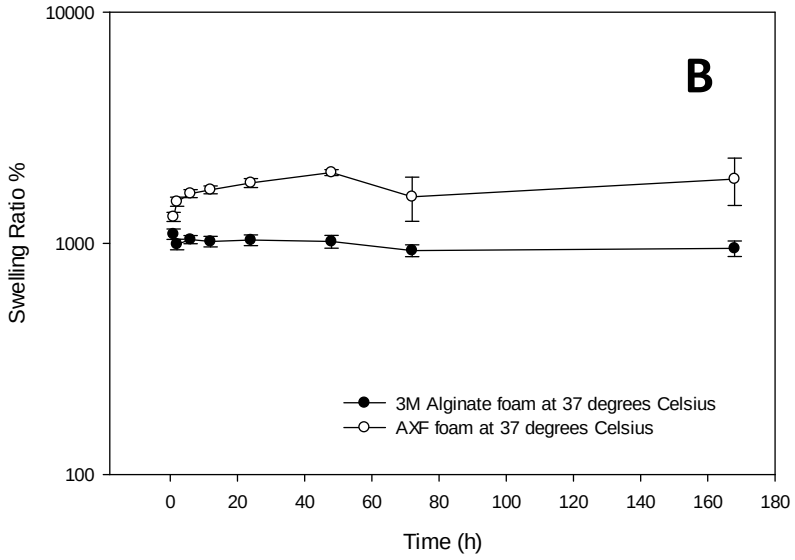


Figure 4.5: Swelling ratio of AXF and 3M alginate foams immersed in PBS at **A)** 25 and **B)** 37°C. (n = 5) (*p < 0.05; significant differences of AXF foam vs. 3M Alginate foam at both temperatures)

4.3.4. Drug release kinetics studies

AXF foams released 12% of silver sulfadiazine by weight in the first hour, and continued to linearly release silver over the study's time course to give a final cumulative silver release of 44% after 72 hours. (**Figure 4.6**) The amount of cumulative silver sulfadiazine release from AXF foams was much higher than the 3M Alginate foam. This was most likely attributed to initial swelling, bulk degradation and erosion of the AXF foam in comparison to Alginate foams. Like many polysaccharide based materials, AXF's hydrophilicity causes aqueous medium to penetrate and cause the foam to swell significantly and allow silver to diffuse out quickly. Subsequently, the foam's polymer chains became loosened, leading to a loss of its integrity and drug solute transport.[135] The amount of silver sulfadiazine released from the 3M Alginate foam was below the limit of quantification threshold during ICP-OES analysis. This may be due to the dressing

being designed for very slow release and being less hydrophilic than AXF foams. The initial burst release of silver sulfadiazine out of AXF foams is expected to inhibit microbes at the wound site which will prevent further inflammation, biofilm formation and the wound from transitioning into the chronic state. However, because the cumulative silver sulfadiazine release becomes linear after the burst effect at 1 hr, it is important to analyze the cytotoxicity of the silver sulfadiazine impregnated AXF foams over an extended period of time. Silver in excessive amounts kill healthy cells in addition to microbes.

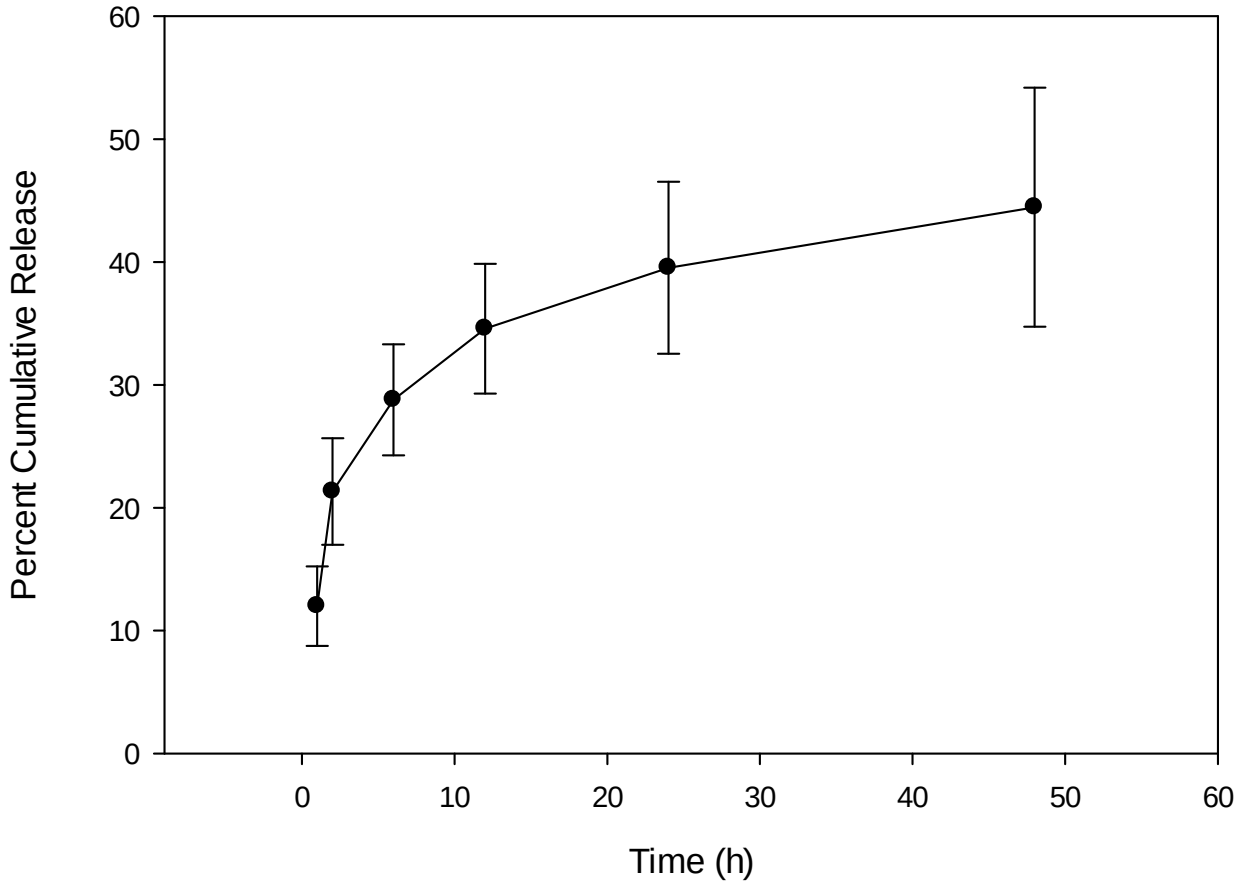


Figure 4.6: Cumulative silver sulfadiazine release from AXF foam. (n = 6)

4.3.6. Limulus amebocyte lysate (LAL) endotoxin assay

LAL Endotoxin levels for unsterilized and sterilized AXF foams were 5.30 ± 1.10 and 3.42 ± 0.87 EU/mg respectively, showing a 35.6% reduction after treatment. (**Figure 4.7**) The Food and Drug Administration does not have a uniform endotoxin level limit for wound dressing materials. However, it is imperative to minimize endotoxins to reduce symptoms such as fever and septic shock. The reduction of endotoxins after peracetic acid sterilization helped contribute to making AXF foams less cytotoxic and maintain baseline cell viability and proliferation in vitro.

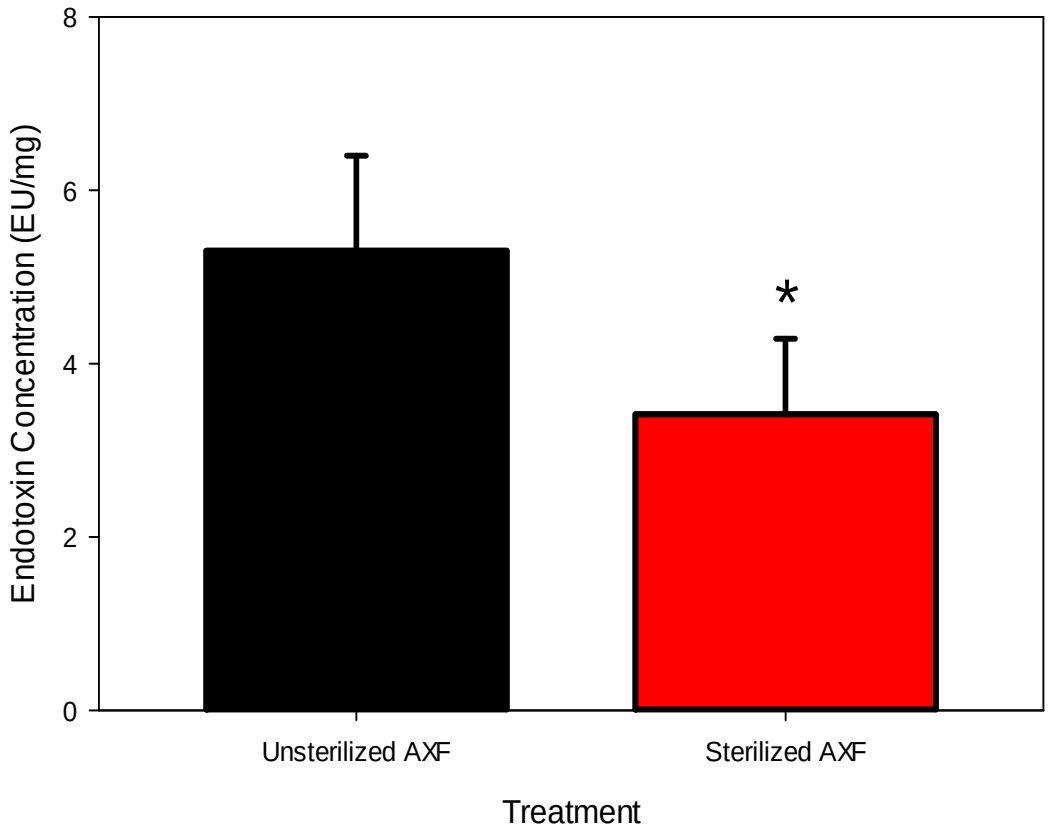


Figure 4.7: LAL endotoxin content in AXF foams before and after sterilization. (n = 8) (*p < 0.05; significant difference of sterilized AXF foam compared to unsterilized AXF foam)

4.3.7. Trypan blue cell viability & proliferation assay

The introduction of AXF foams into the in vitro microenvironment did not have a cytotoxic effect on NIH3T3 fibroblasts. Their viability values were 96.6 and 96.4% over a 24 and 72 hour time period respectively. After exposure to the Alginate foam, fibroblasts were 96.7 and 97.3% viable after 24 and 72 hours. Untreated fibroblasts showed viability values of 95.8 and 96.9% after 24 and 72 hours. (**Figure 4.8**) There were no significant differences in viability between AXF foams, Alginate foams and non-treated test groups. Based on the results, it can be concluded that fibroblasts, which play a prominent role in the proliferative and remodeling stages of wound healing were not negatively affected by exposure to AXF foams as their viability maintained baseline levels.

Fibroblast proliferation after exposure to AXF foams was encouraging partly due to their high viability in vitro. After incubation with an initial cell seeding density of 50,000 cells per well, fibroblast cell number increased to 166,000 and 212,000 after 24 and 72 hours respectively. Fibroblasts cultured with Alginate foams proliferated from 50,000 to 129,000 and 302,000 cells per well after 24 and 72 hours respectively. (**Figure 4.9**) Meanwhile, non-treated test groups had cell numbers of 205,000 and 289,000 cells per well after 24 and 72 hours respectively. There were significant differences in cell number between 0 and 72 hour time-points for all treatment groups.

The results summarized the biocompatibility and bio-inert properties of AXF foams. In fact, they had proliferative inducing effects as they have greater cell numbers than Alginate and non-treated groups after the first 24 hours. However, they were not statistically significant for that time period. Another insight from the data was the lower cell number for AXF foam treated fibroblasts compared to Alginate and non-treated groups after 72 hours. This was most likely

attributed to the highly absorptive, hydrophilic properties of AXF foams as they absorbed important growth factors (i.e. FGF) or intracellular signals necessary for fibroblast proliferation. However, there were no significant differences with respect to treatment group for the 72 hour incubation period.

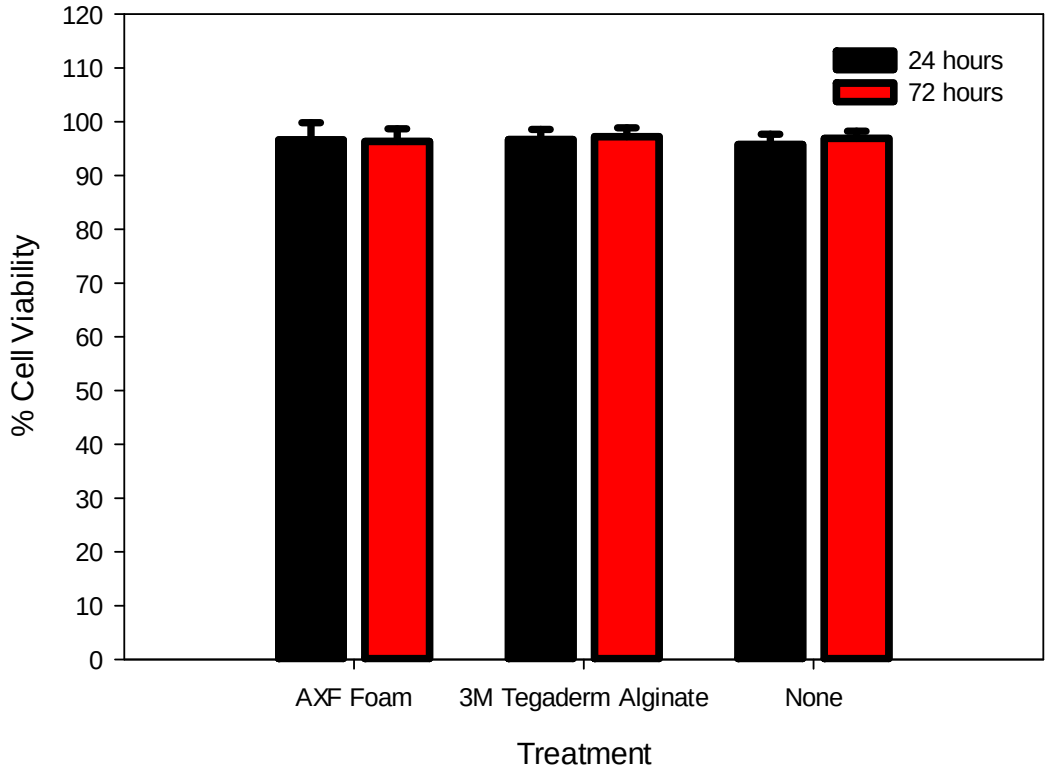


Figure 4.8: Cell viability of NIH3T3 fibroblast cells after exposure to alginate and AXF foams after 24 and 72 hour incubation period. (n = 8)

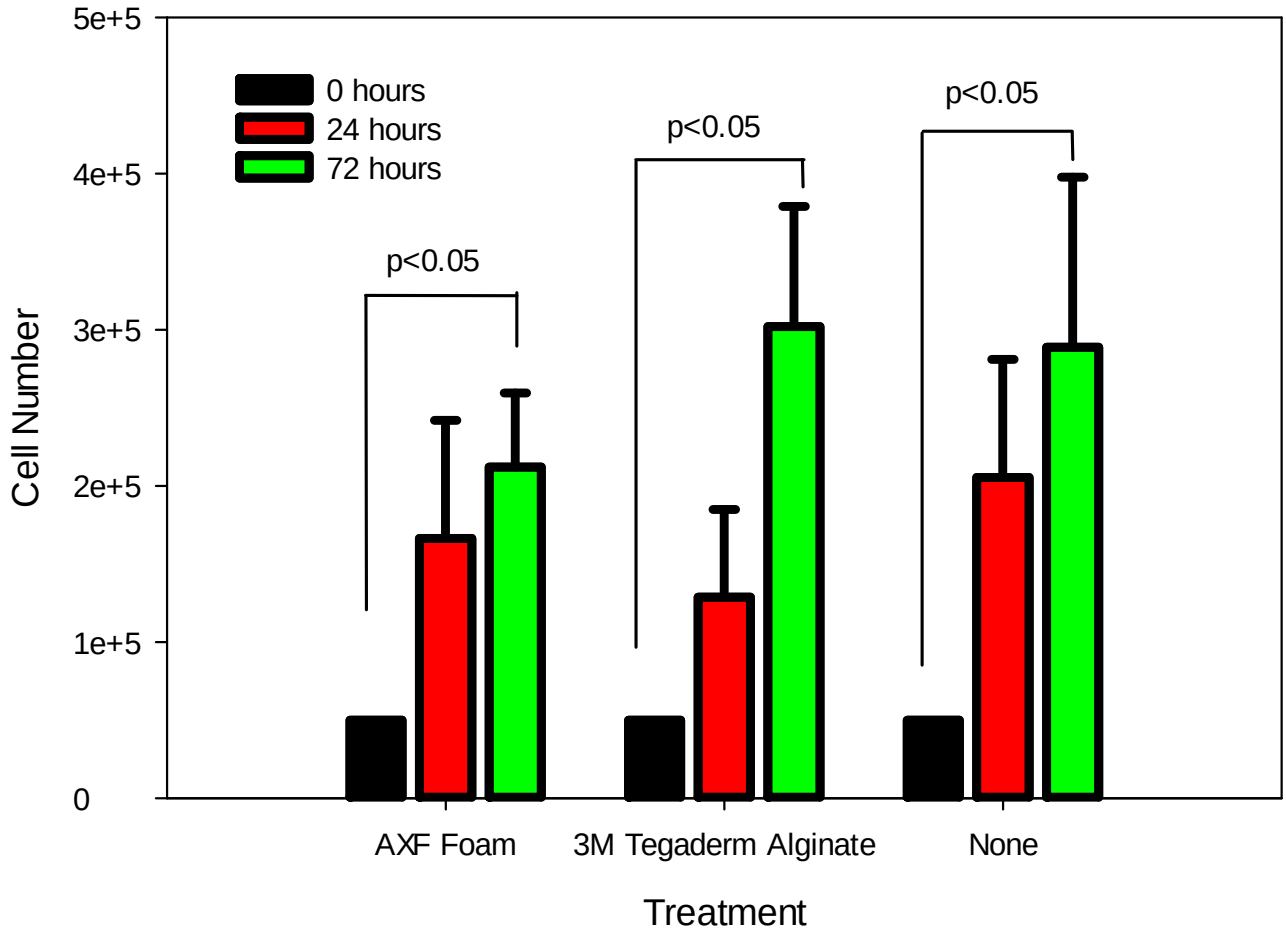


Figure 4.9: Cell proliferation of NIH3T3 fibroblast cells after exposure to 3M Alginate and AXF foams after 24 and 72 hour incubation period. (n = 8) (*p < 0.05; significant differences in cell number at 24 hours compared to cell number at 72 hours for all treatments)

4.3.8. Kirby-Bauer disk diffusion assessment

The four treatment groups were assessed for their anti-microbial activity by measuring the inhibition zones within each species of bacteria. *P. aeruginosa* was highly susceptible to silver sulfadiazine impregnated AXF foams, which actively inhibited bacterial growth with an inhibition zone of 22.17±1.53 mm, greater than the inhibition zone of the tetracycline positive control. The effectiveness of silver sulfadiazine was consistent with previous data in literature showing its applicability in a skin wound model.[128] Gram-positive *S. aureus* and *E. faecalis* species were less sensitive to the silver sulfadiazine impregnated AXF foams with an inhibition zone diameter of 12.39 ± 1.26 and 12.32 ± 0.81 mm respectively. One possible reason that these species showed some resistance compared to gram-negative *P. aeruginosa* is that gram-positive bacteria are more susceptible to antibiotics and have a thick cell wall difficult to break down.[129]

All three species of bacteria were sensitive to AXF foams impregnated with silver sulfadiazine. *P. aeruginosa* was most susceptible to the silver impregnated AXF foams among the three species. For Alginate foams, the bacteria were more resistant with lower inhibition zone diameters than silver impregnated AXF foams. In fact, *E. faecalis* was completely resistant to silver impregnated Alginate foam. Irregularly shaped inhibition zones for silver impregnated AXF foams against *P. aeruginosa* were due to the scaffold melting during incubation. All three positive control treatments achieved clear inhibition of the three species after 24 hour incubation. (**Figure 4.10**) As expected, all bacterial species grew and showed resistance to AXF foams with no silver sulfadiazine. The growth inhibition data from two trials were summarized in **Table 4.2**. The results suggest anti-microbials such as silver integrated into AXF foams can be successfully delivered to the site of infections. In the future, more specific bacteria susceptible antibiotics can be integrated in the AXF foam to improve efficacy. Combinations of antibiotics and silver could

be combined together to create an anti-microbial cocktail to treat against infections. However, the antibiotic selected is important as there is a wide array of bacteria species that is not susceptible to the same antibiotic, possibly developing resistance and other complications at the wound site.

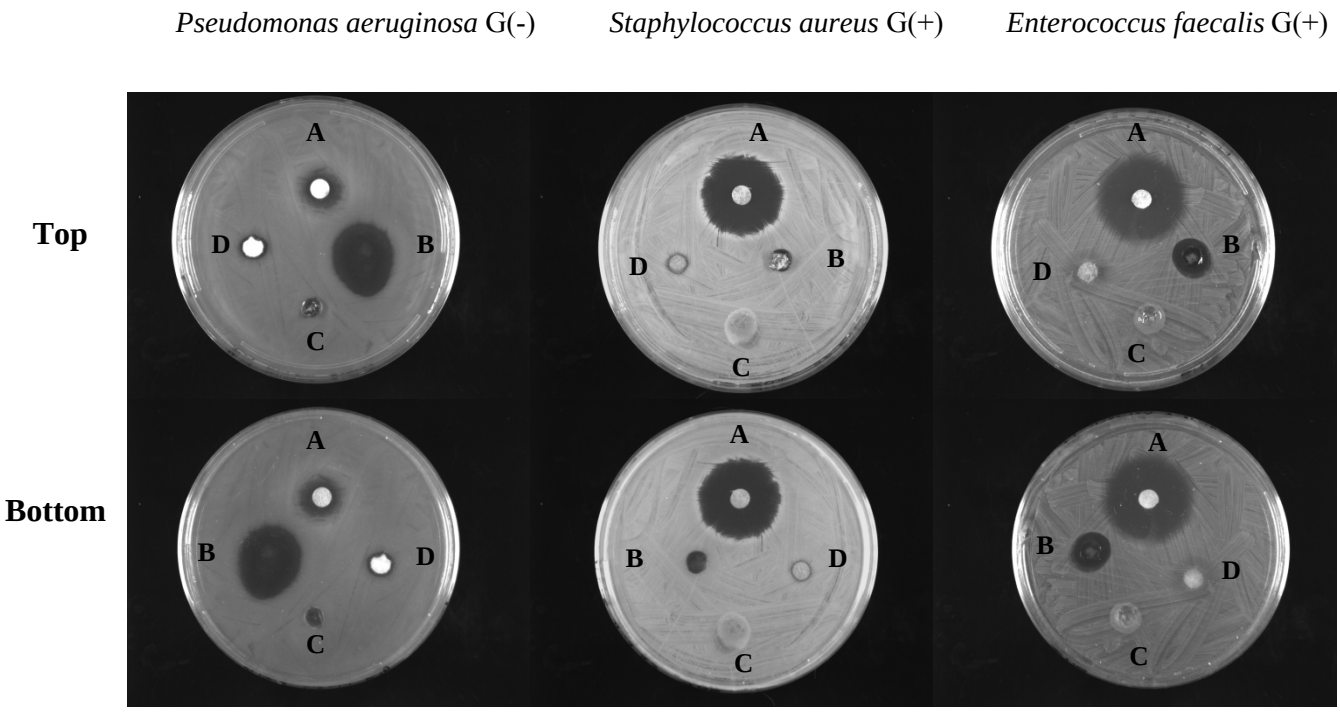


Figure 4.10: Antimicrobial properties of 3M alginate and AXF foams using Kirby-Bauer disk diffusion susceptibility test on gram negative G(-) and gram positive G(+) bacteria. Tetracycline (A), Silver sulfadiazine impregnated AXF foams (B), AXF foams (C) and silver impregnated 3M Alginate foams (D) were tested on *Pseudomonas aeruginosa and Enterococcus faecalis* bacterial species. Erythromycin (A), Silver sulfadiazine impregnated AXF foams (B), AXF foams (C) and silver impregnated 3M Alginate foams (D) were tested on *Staphylococcus aureus* bacterial strain. (n = 2)

Table 4.2 Sensitivity profiles of bacterial species after treatment

Species	Treatment	Average inhibition zone (mm)
P. aeruginosa		
	Positive Control (30 µg Tetracycline)	14.40±5.44
	AXF foam w/ 5% silver sulfadiazine	22.17±1.53
	ALG foam w/ 5% silver	10.04±1.82
	Negative Control (no treatment)	0
S. aureus		
	Positive Control (15 µg Erythromycin)	23.02 ±0.59
	AXF foam w/ 5% silver sulfadiazine	12.39 ±1.26
	ALG foam w/ 5% silver	5.75 ±0
	Negative Control (no treatment)	0
E. faecalis		
	Positive Control (30 µg Tetracycline)	27.62 ±0.16
	AXF foam w/ 5% silver sulfadiazine	12.32 ±0.81
	ALG foam w/ 5% silver	0
	Negative Control (no treatment)	0

4.4. Conclusions

AXF foams are highly porous, biocompatible scaffolds that have a potential niche in the market as a polysaccharide based wound dressing because it is inexpensive, easy to fabricate and has material properties that are beneficial in wound healing. In this pre-clinical study, AXF foams possessed higher swelling capabilities and compared to 3M Alginate foams commercialized in the market. In their dry state, AXF foams also had higher mechanical strength as its storage modulus was greater than for 3M Alginate foams. AXF foams can be impregnated with silver which can be released in moist wound environments. However, more work needs to be done to improve the mechanical stability of the scaffold in aqueous environments. A more stable polysaccharide such as chitosan can be introduced to decrease the scaffold's degradation in solution. Additionally, a greater concentration of crosslinker can be used to strengthen the polymer network. Processing conditions such as AXF gel freezing temperature and lyophilization pressure will need to be explored to examine its effect on its material properties. In the future, in vivo tests such as wound closure rate, in vivo drug release, and host immune response should be done to validate the foam's healing properties in injury and pathogenic wound conditions. This work hopes to bring arabinoxylan polysaccharides to the forefront as a biopolymer wound dressing that can be applied for treatment of wounds.

CHAPTER 5: Summary & future work

5.1 Summary

This dissertation aims to be the first to influence the biomaterials community to investigate arabinoxylan as a wound dressing material. Arabinoxylan should be investigated as a wound dressing material because of its high absorbency and hydrophilicity which absorbs wound exudate. Arabinoxylan is naturally derived and biocompatible to not elicit a cytotoxic or foreign body immune response. Arabinoxylan is also abundant and inexpensive which makes fabrication cost-effective. Lastly, arabinoxylan can be successfully integrated with other polymers or in a variety of formulations. Arabinoxylan can also be impregnated with anti-microbial drugs that can be delivered to prevent wound infections.

5.1.1. GEL-AXF fibers

Arabinoxylan was successfully fabricated and characterized as a nanofiber scaffold after being electrospun with different amounts of gelatin. GEL-AXF electrospun scaffolds possessed a well-defined fiber morphology tunable from the nano and micro scales. GEL-AXF fibers also possessed large pores to allow nutrient and oxygen exchange between the wound bed and scaffold. Increased gelatin concentration in these blended fibers improved the mechanical properties of the scaffold. However, when the scaffold was exposed in aqueous environments, it was prone to degradation and losing mechanical strength which compromised its function as a barrier. Silver sulfadiazine was successfully integrated into GEL-AXF fibers with its release exhibiting near zero-order kinetics and anti-microbial properties against common bacterial species encountered during wound healing. GEL-AXF fibers were biocompatible and did not hinder fibroblast proliferation in vitro.

Overall, these scaffolds show potential as a wound dressing material because of their conformability, high surface to volume ratio and biocompatibility. However, its stability will need to be improved to progress beyond the pre-clinical stage. This would be rectified by blending in more stable polymers such as PCL, PLGA or PLA as additives that can be co-electrospun with arabinoxylan. This would reduce the degradability of the scaffold in aqueous solution while maintaining biocompatibility. In turn, it is important that the amount of these polymers are minimized so the natural component of arabinoxylan can illicit its natural, bio-derived properties within the wound environment.

5.1.2. AXF foams

Arabinoxylan foams were fabricated and tested as a wound dressing material with 3M Tegaderm Alginate foam as a commercial peer to compare against. A detailed summary of properties of AXF foams are shown in **Table 5.1**. AXF foams exhibited a honeycomb morphology with a highly porous structure that enable moisture and fluid exchange at the injury-dressing interface. AXF foams in dry states were much stiffer than 3M Tegaderm Alginate counterparts, illustrating its ability to be a barrier from the external environment. AXF foams can be introduced with silver sulfadiazine to serve as a drug carrier to deliver a sustained dose of anti-microbials to the wound site during a two day period. This delivery was validated by linear release kinetics data and growth inhibition using the disk diffusion method. The in vitro biocompatibility of the foams was also validated with cell viability and proliferation on par with 3M Alginate and non-treated groups. Sterilization of these scaffolds reduced bacterial endotoxin content and ensured contamination was minimized.

In summary, AXF foams possessed many material properties necessary to function as a wound dressing material. Its mechanical stability in highly aqueous media will need to be improved to fully achieve its potential. However, the ultimate application of AXF foams will be to serve as an absorbent layer sandwiched between two polyurethane film layers micro-printed with pores on its surface for wound fluid absorption. (**Figure 5.1**) This design will best optimize the swelling properties of AXF foams while minimizing its weaknesses by surrounding it with a much more stable polymer such as polyurethane. Polyurethane film is non-degradable, a substrate for adhesives and is bio-inert to function as part of the dressing that directly interfaces with the skin.[135, 136] Polyurethane and arabinoxylan would be an ideal combination of polymers whose prototype design would help treat against acute, moderately exudating wounds.

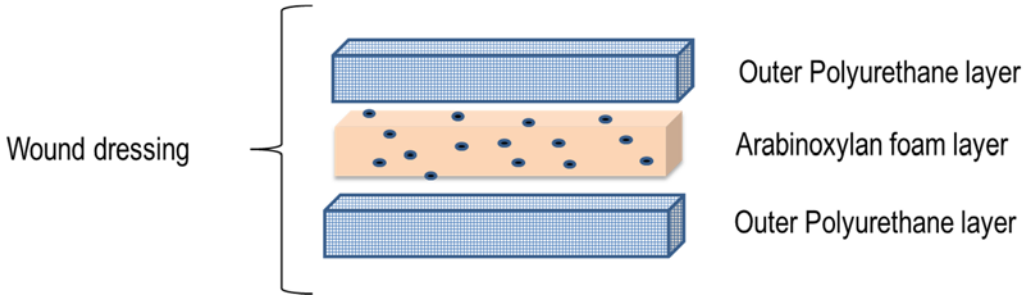


Figure 5.1: Diagram of polyurethane-arabinoxylan wound dressing material prototype.

Table 5.1: Summary of AXF foam material properties.

Properties	*Experimental test*	*Expected Outcomes*	*Results*
Morphology	Scanning Electron Microscopy	Highly porous, smooth surface, close to or better than commercialized control	✓✓✓
Mechanical Properties: Shear Modulus	Rheometry	Close to or better than commercialized control	✓
Porosity	Apparent Volume Method	Achieve porosity values between 70-90%; close to or better than commercialized control	✓✓✓
Swelling	Swelling Ratio	Maximal swelling above 200%; close to or better than commercialized control	✓✓✓
Cumulative Drug Release Kinetics	Inductively Plasma Optical Emission Spectrometry	Burst release kinetics in first six hours before more controlled release; close to or better than commercialized control	✓✓
Bio-inert	LAL Endotoxin Assay	Reduction of bacterial endotoxins after sterilization	✓✓
Biocompatibility	Cell Viability and Proliferation Assays;	Above 80% Cell Viability and double cell number after 3 days; close to or better than commercialized control	✓✓✓
Anti-microbial properties	Kirby Bauer Growth Inhibition Assay	No bacterial lawns on inoculated agar gel; close to or better than commercialized control	✓✓✓

****Results subjectively rated from 1-3 checks in comparison to 3M Alginate foam dressing****

5.2 Future work

This project attempted to comprehensively evaluate material properties of arabinoxylan as electrospun fiber and foam platforms and evaluate its potential as a wound dressing material. Although many of the properties in vitro have been successfully characterized, more work needs to be done before it can be evaluated in a clinical setting. The three dimensional porosity of arabinoxylan fibers and foams needs to be accurately measured using mercury porosimetry method. Processing conditions such as AXF gel freezing temperature and lyophilization pressure will need to be explored to examine its effect on its material properties. In vitro, additional cell studies such as looking at macrophage and keratinocyte viability and proliferation should be considered. Extracellular matrix production should also be considered by analyzing fibronectin protein assembly through fluorescent antibody staining.[137] Additionally, a blood clotting test developed by Shih et al. would appropriately assess the clotting properties of AXF scaffolds in a physiologically inclined environment.[138]

Next, in vivo testing models need to be conducted in detail to look at important characteristics such as wound closure rate, neutrophil count, wound histology, extracellular matrix production and mortality rate. Shin-Yeu et al. designed silver loaded chitosan gel dressings for wound healing. This group conducted a study by creating wound incisions on the dorsal region that were infected with *P. aeruginosa* before implanting the dressing on mice for up to 14 days.[139] The wound areas were measured to analyze the degree of closure and its scabs were extracted, homogenized and plated on agar for bacteria quantification at 1, 2, 7 and 14 days. Neutrophils from the mice's blood were counted using a hemostasis analyzer to determine the degree of inflammation after implanting the chitosan scaffold. The wound histology after chitosan implantation was examined using hematoxylin and eosin, Masson Trichrome and Gram-

Twort staining (H&E) to qualitatively assess inflammatory cell infiltration, collagen production and bacteria presence. [139] Histology work from Sun's group also used histology to look at dermal differentiation, epithelial maturation and skin growth after implanting dextran hydrogels on mice burn wound models during a three and five week period.[105] Mortality rate also needs to be considered during these studies. Mouse subjects in Shin-Yeu's work were euthanized when their physical condition deteriorated. Mice that survived after day 7 of the 14 day study were classified as long term survivors while mortality rate was determined by the number of mice that survived after Day 14.[139] Implementing these in vivo studies using arabinoxylan polymers will give a much clearer picture of its clinical effectiveness as a wound dressing material.

In literature, arabinoxylan has been largely unnoticed as potential wound dressing material. However, its material properties, naturally derived origin and abundance are desirable characteristics that warrant further investigation in vivo before clinical trials. This project aimed to highlight the unique properties of arabinoxyan, its fabrication in nanofiber and foam formulations and its application for treatment of acute, moderately exudating wounds. The in vitro data from this project suggests a promising future for these platforms in advancing wound dressing development. So far, there have been no entries of "arabinoxylan" found in any medical device database stored by the United States Food and Drug Administration (FDA), indicating a niche that has yet to be established commercially.[140] This dissertation aimed to contribute and catalyze this area in wound development.

CHAPTER 6: Electrospinning of PEGylated polyamidoamine dendrimer fibers

(Supplemental Dissertation Work)

Donald C. Aduba, Jr. [a], Jefferson W. Overlin[a], Chad D. Frierson[b], Gary L. Bowlin[c], Hu Yang[a,d,*]

[a] *Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284*

[b] *Department of Chemistry, Virginia Commonwealth University, Richmond, VA 23284*

[c] *Department of Biomedical Engineering, The University of Memphis, Memphis, TN 38152*

[d] *Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298*

*Correspondence should be addressed to Hu Yang, Department of Biomedical Engineering, Virginia Commonwealth University, 401 West Main Street, P.O. Box 843067, Richmond, VA 23284, USA. Tel.: 1-804-828-5459; Fax: 1-804-828-4454; E-mail:hyang2@vcu.edu

Accepted into Journal of Materials Science & Engineering: Part C*

Abstract:

Polyamidoamine (PAMAM) dendrimers have emerged as an important class of nanostructured materials and have found a broad range of applications. There is also an ongoing effort to synthesize higher-complexity structures using PAMAM dendrimers as enabling building blocks. Herein, we report for the first time the fabrication of electrospun nanocomposite fibers composed of dendrimer derivatives, namely PEGylated PAMAM dendrimers, blended with a small amount of high-molecular-weight polyethylene oxide (PEO). Morphological features and mechanical properties of the resulting dendrimer fiber mats were assessed.

Keywords: *electrospinning, dendrimer, nanofiber, PEGylation, fast Fourier transform*

6.1. Introduction

Polyamidoamine (PAMAM) dendrimers have emerged as an important class of nanostructured materials and have found a broad range of applications by virtue of their highly branched, nearly perfect monodisperse structures of defined sizes. These distinct nanodomain features include a hydrophobic interior and a relatively hydrophilic surface presenting numerous functional groups [141, 142] The structural versatility of PAMAM dendrimers has led to a vast array of intriguing dendritic architectures as nanocarriers for therapeutic and diagnostic applications.[143, 144] There is also an ongoing effort to synthesize higher-complexity structures using PAMAM dendrimers as enabling building blocks [145-147] Of particular interest is the utility of PAMAM dendrimers in construction of high-dimensional structures for drug delivery and tissue engineering applications.[148] For instance, PAMAM dendrimers are used as a cross-linker or building block to construct cross-linked networks.[149, 150]

Electrospinning has been widely adopted to make fibers with desirable structural features for drug delivery and tissue engineering application.[151-157] A wide range of synthetic and natural linear polymers have been electrospun into fibers with success.[158] Although polymer molecular weight and solution concentration are critical in successful electrospinning, intermolecular chain entanglements within the polymer are very important to stable fiber formation as well. [158] Probably because of the widely recognized steric crowding on the dendrimer periphery precluding chain entanglements, there is scarcity in the literature on the fabrication of dendrimer fibers via electrospinning. Madani et al. reported electrospinning of blends of non-functionalized PAMAM dendrimers and high-molecular-weight polyethylene oxide (PEO), in which PEO, however, accounts for a large proportion (at least 30% by weight) of fiber mass.[159] Our recent work shows that PAMAM dendrimer can be hybridized with linear polymers, e.g., gelatin, and

electrospun into fibers as a secondary component.[60] Alternatively, PAMAM dendrimers can be covalently coupled to fibers in a post-electrospinning step.[160]

In this paper, we report for the first time electrospun nanocomposite fibers composed of dendrimer derivatives, namely PEGylated PAMAM dendrimers, blended with a small amount of high-molecular-weight polyethylene oxide (PEO) (6.25% by weight). The new dendrimer-containing nanocomposite fibers represent a new structure with added complexity of dendrimer and fibrous mat. PEGylation reduces the cytotoxicity of the resulting conjugate of the nanomaterial due to the superb biocompatibility of PEG.[161-163] PEGylated dendrimers may encourage greater retention time in the circulatory system due to the stealth properties of PEG.[164] We envision that new dendrimer-containing fibers will broaden the use of dendrimers in biomedical applications such as drug delivery, and the ease of fabrication via electrospinning will allow this new platform to be readily translatable.

6.2. Experimental section

6.2.1. Synthesis of PEGylated PAMAM dendrimer conjugates

PAMAM dendrimer G3.0 was used as the underlying core for the synthesis because of its combination of possessing low cytotoxicity at high molar concentrations and a relatively large number of surface groups for functionalization. [161-163] Methoxypolyethylene glycol (mPEG, 2000 g/mol) was coupled to PAMAM dendrimer G3.0 at feed molar ratios of 32:1 and 16:1, respectively following the method published by us.[165] These two molar ratios were chosen to ensure the resulting PEGylated dendrimer conjugates with discrete degrees of PEGylation could be achieved. [165] PEGylated G3.0 conjugates were purified using SnakeSkin tubing with 7000 MWCO and freeze dried.

6.2.2. Electrospinning

Electrospinning solutions of mPEG or mPEG-G3.0 with or without high-molecular-weight PEO (M_v = 900,000 g/mol) additive were prepared in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and tested for fiber formation (**Table 6.1**). The electrospinning solution was then drawn up through a blunt-end needle (18G×1½ in) on a 5 ml syringe. The syringe was loaded into a syringe pump, delivering the solution to the needle orifice 30 cm away from the collecting mandrel at a rate of 2 ml/h. The needle and the collection target were connected to a positive electrode (+ 20 kV) and the earth ground of a high voltage power supply (Spellman CZE100R, Spellman High Voltage Electronics Corporation), respectively. Fibers were collected on a rounded, stainless steel mandrel (120 mm length with 6 mm diameter) rotating at 500 rpm.

Table 6.1. Electrospinning conditions tested for fiber formation.

Polymer (A/B)	A (% w/v)	B (% w/v)	Fiber formation
mPEG2000/PEO	20-25	0	No
mPEG2000/PEO	20-25	0.05-0.1	Yes
mPEG-G3.0 (32:1)/PEO	20-40	0	No
mPEG-G3.0 (32:1)/PEO	15	1	Yes
mPEG-G3.0 (16:1)/PEO	15	1	Yes

6.2.3. ^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded on a Varian Mercury 600 MHz spectrometer. Deuterium oxide (D_2O, 99.9%) was used as solvent in ^{1}H NMR measurements. ^{1}H NMR spectroscopy was applied to characterize PEGylated G3.0 conjugates and determine actual degrees of PEGylation.

6.2.4. Scanning electron microscopy (SEM)

Prior to SEM imaging, scaffolds were placed on a 1 cm diameter stub. The stub was placed on a specimen holder and gold sputter coated. SEM images were taken on a JEOL JSM-5610LV scanning electron microscope**.** One hundred randomly chosen fibers in each SEM image were analyzed with UTHSCSA ImageTool™ software for fiber diameter and pore size measurements.

6.2.5. Tensile testing

"Dog-bone" shaped samples (n=8) were obtained using a punch die (ODC Testing & Molds) of the dimensions 19.0, 3.2 and 6.1 mm at its length, narrowest point and widest point, respectively. Mechanical properties of the samples, including peak load, peak stress, modulus, strain at break and energy to break, were tested using the MTS Bionix 200 Mechanical Testing System in conjunction with TestWorks 4.0 software.

6.2.6. Fast fourier transform (FFT)

FFT technique was conducted to analyze the degree of fiber alignment and anisotropy based on the work reported by Ayers and coworkers.[154, 166] This was completed by taking the SEM image of the scaffolds and converting its image information from the time domain to a discrete frequency domain.[154] The output image after FFT is grayscale pixels within a circle that

have varying intensities with respect to its angle about the circle's central point. Image conversion and analysis was done on Image J software.

6.2.7. Statistical analysis

Statistical analysis was completed using unpaired t-test and the Mann-Whitney method for subgroup comparison. A p-value less than 0.05 was considered statistically significant.

6.3. Results and discussion

For the demonstration of proof-of-concept, we chose amine-terminated PAMAM dendrimer G3.0 as the underlying core (**Scheme 6.1**). We coupled mPEG2000 to the dendrimer surface at feed molar ratios of 16:1 and 32:1, respectively, following a procedure previously described.[165] According to ^{1}H NMR spectroscopy characterization (**Figure 6.2**), the degrees of PEGylation of mPEG-G3.0 (16:1) and mPEG-G3.0 (32:1), i.e., percentages of dendrimer PEG surface amines coupled to PEG, were 44% and 92%, respectively. For electrospinning fabrication, coupling mPEG to PAMAM dendrimer G3.0 at 32:1 (i.e. 100% PEGylation) would be more favorable. However, for drug delivery applications, an increased density of mPEG chains can reduce the ability to couple drugs and moieties of interest due to steric hindrance of PEG and reduced surface groups.[165]

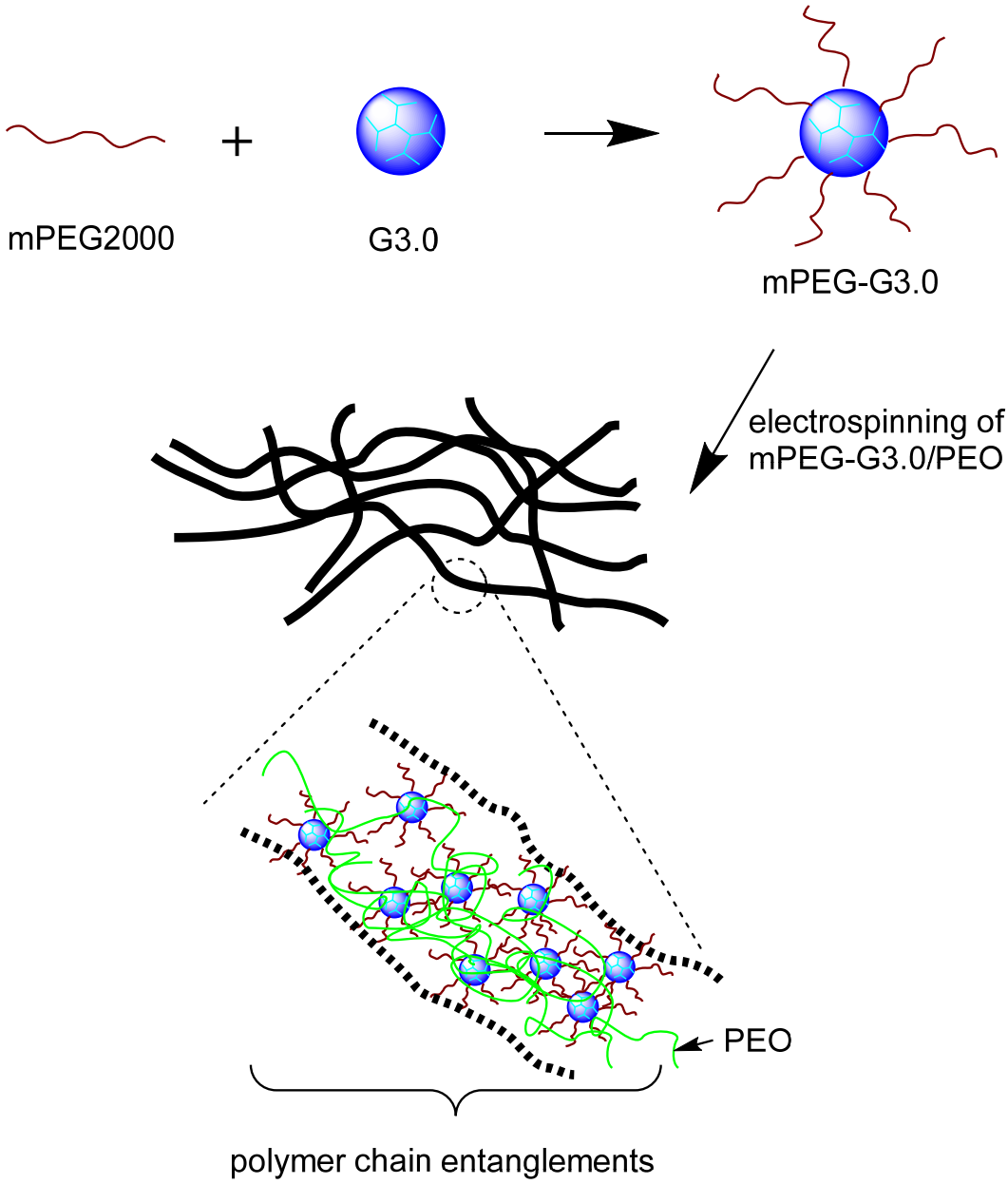


Figure 6.1. Schematic illustration of synthesis and electrospinning of mPEG-G3.0 blended with a small amount of high-molecular-weight PEO 900,000 Da.

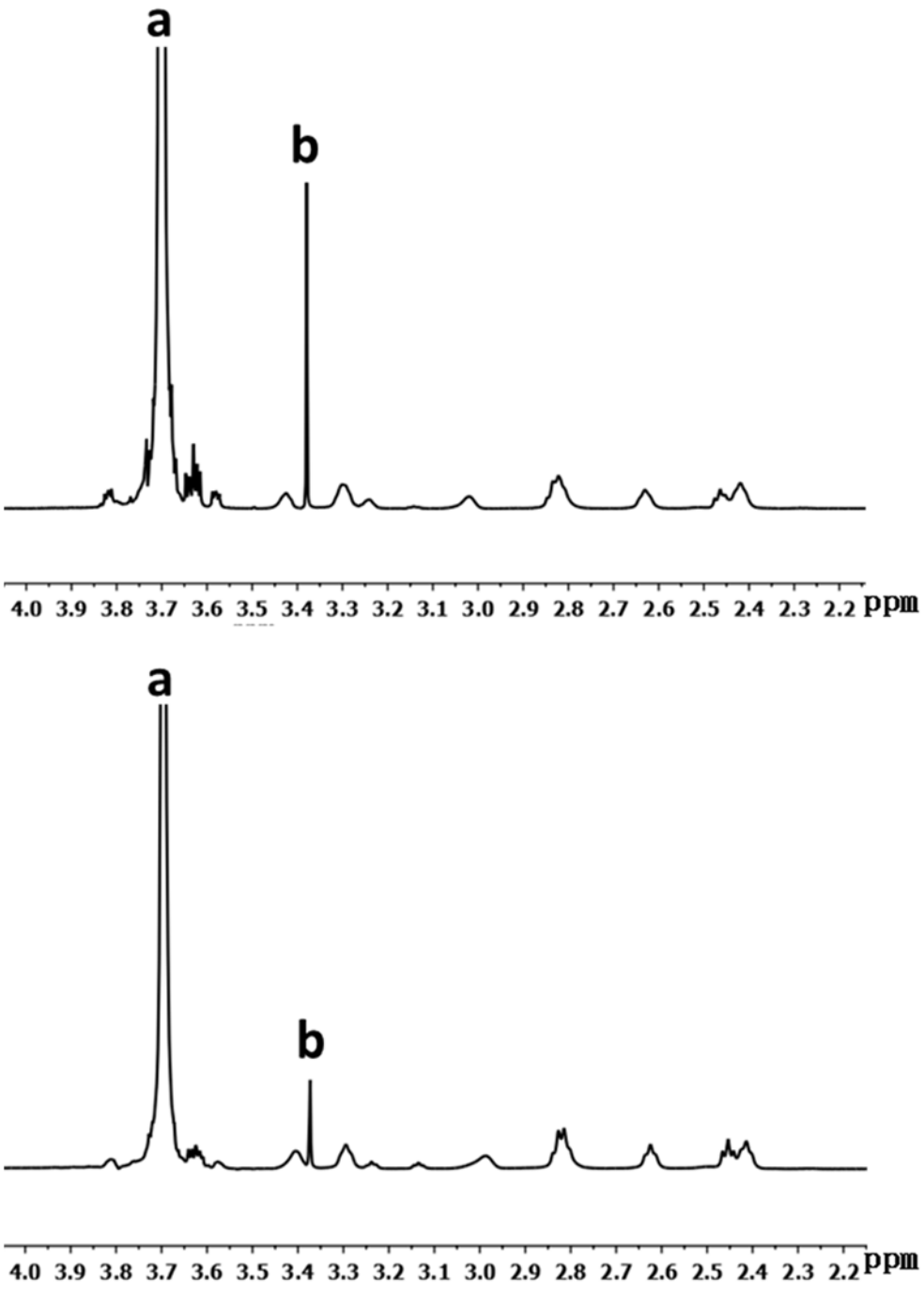


Figure 6.2. ^{1}H NMR spectrum of PEGylated PAMAM dendrimers. (A) mPEG-G3.0 (32:1) and (B) mPEG-G3.0 (16:1). (peak **a**, $-(C\underline{H}_2C\underline{H}_2O)_n-$; peak **b**, $CH_3-CH_2-C\underline{H}_2-O-$; multiple peaks 2.4-3.45 ppm, methylene protons of dendrimer)

It is challenging to make fibers out of pure dendrimers or PEGylated dendrimers because of their highly compact structures, low chain entanglements, and high viscosity. Although it remains controversial as to whether the presence of chain entanglements is essential for fiber formation, it has been shown that a small fraction of PEO in electrospinning solution promotes PEG fiber formation, which was attributed to fluid elasticity increase by PEO other than chain entanglements.[167] Electrospinning solutions of mPEG or mPEG-G3.0 with or without high-molecular-weight PEO additive were tested for electrospinning. After mPEG-G3.0 (16:1 and 32:1) (15% w/v in HFP) was blended with PEO (M_v=900,000 Da) (1% w/v), mPEG-G3.0 was successfully electrospun into fiber mats, presumably as a result of promotion of both chain entanglements and fluid elasticity. Fluid elasticity of mPEG-G3.0 solution was attributed to its ability to adjust to stresses during a longer period of relaxation time. [167] Quantitative analysis of rheological properties of mPEG-G3.0 electrospinning solutions is warranted for electrospinning optimization and will be investigated in future work.

SEM images were used to characterize the electrospun mat's fiber morphology. As shown in **Figure 6.3**, mPEG-G3.0 (32:1) fibers exhibited some beads. The beading formation could be due to applied charges breaking the solution up into droplets, otherwise known as Rayleigh instability. According to the histograms of fiber size and pore size distributions shown in **Figure 6.4**, the average diameters of mPEG-G3.0 (32:1) and mPEG-G3.0 (16:1) fibers were 3.8±2.3 μm and 4.2±2.8 μm, respectively. These relatively large variations in fiber diameter are presumably attributed to high polymer concentrations (10% or higher), which have a tendency to produce a non-normally distributed population of fibers.[168] Average pore sizes of mPEG-G3.0 (32:1) and mPEG-G3.0 (16:1) fiber mats were 209 μm^2 and 135 μm^2, respectively. Typically, electrospun scaffolds exhibit fiber diameters in the micrometer diameter range, but they can

achieve nanometer fiber diameters under proper processing conditions. For this study, these scaffolds were in the micron size range. This is likely due to the high concentration of mPEG-G3.0 (**Table 6.1**) in electrospinning solution. One potential method to create fibers in the nanoscale would be to 1) reduce the mPEG-G3.0 concentration below 10% to create nanofibers as illustrated for PEO in literature and 2) increase the PEO additive concentration from 1% (**Table 6.1**) up to 7% to improve spinnability. [168] The balance of those two parameters could help achieve stable, nano-scaled fibers that can closely mimic the extracellular matrix, encouraging cellular activity for tissue engineering applications. Having a nano-fiber topography can also inspire a well-controlled drug release system for drug delivery applications because of the scaffold's high surface area to volume ratio.[130]

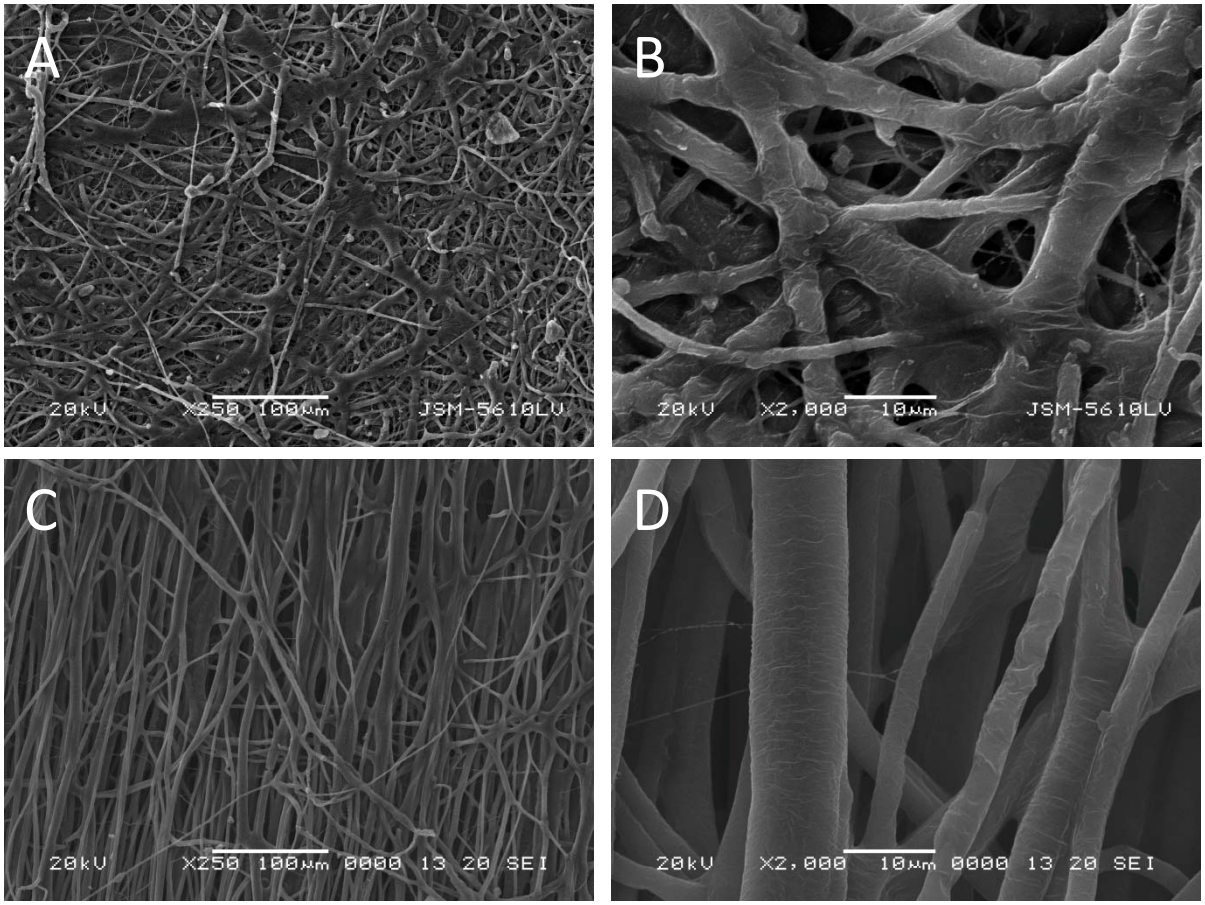
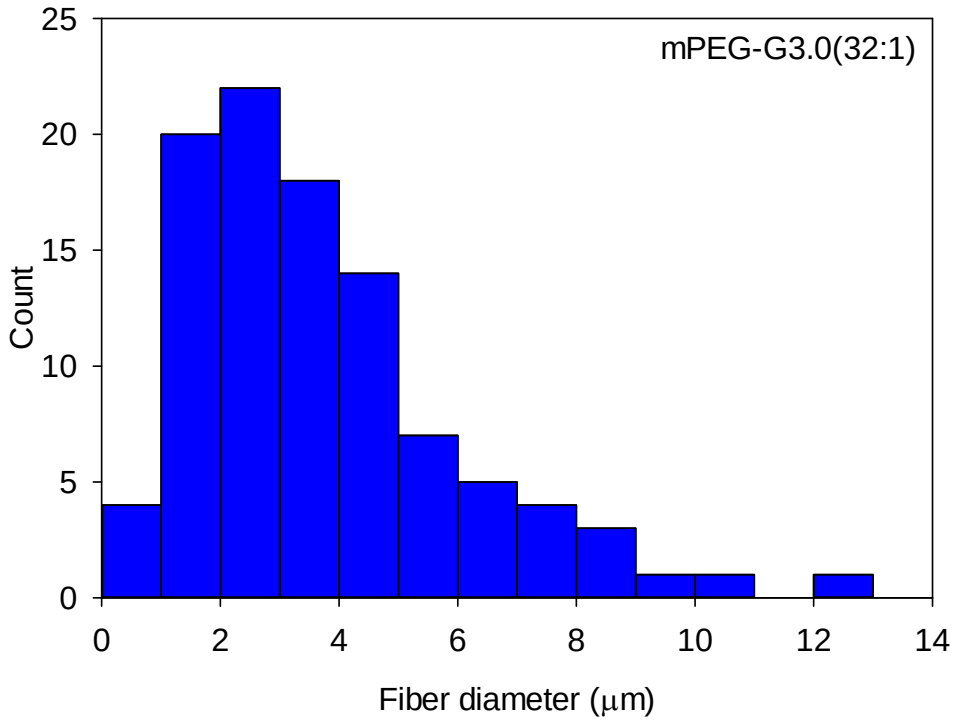
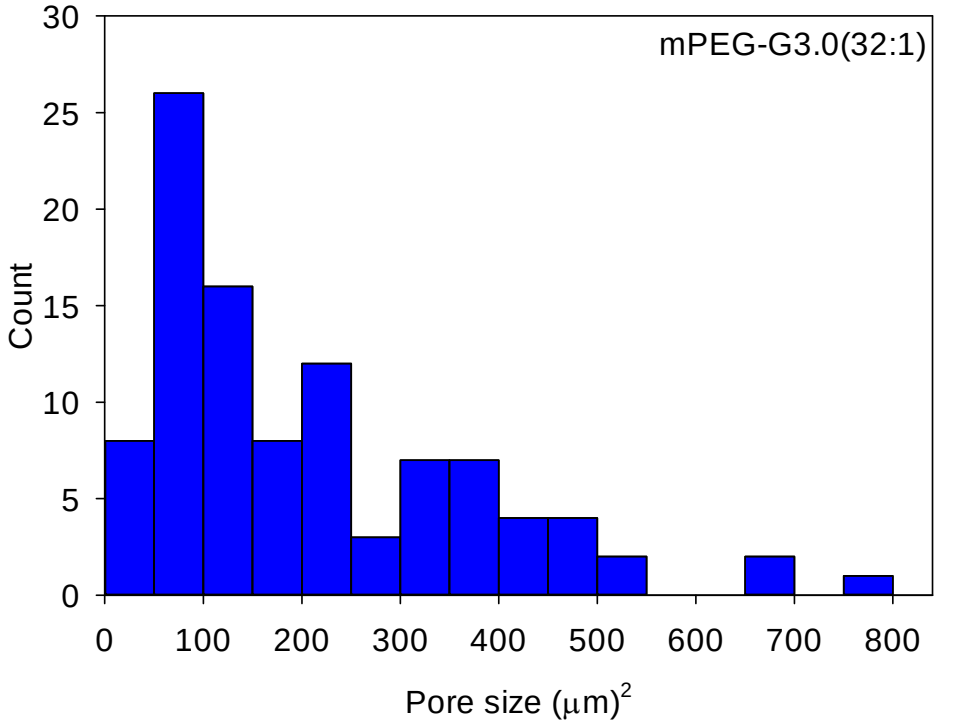


Figure 6.3. SEM images of electrospun fibers on the basis of mPEG-G3.0(32:1)(A, B) and mPEG-G3.0(16:1)(C, D) at different magnifications.


mPEG-G3.0(32:1)
Count
Fiber diameter (μm)
0
5
10
15
20
25
0
2
4
6
8
10
12
14


mPEG-G3.0(32:1)
Count
Pore size (μm)2
0
5
10
15
20
25
30
0
100
200
300
400
500
600
700
800

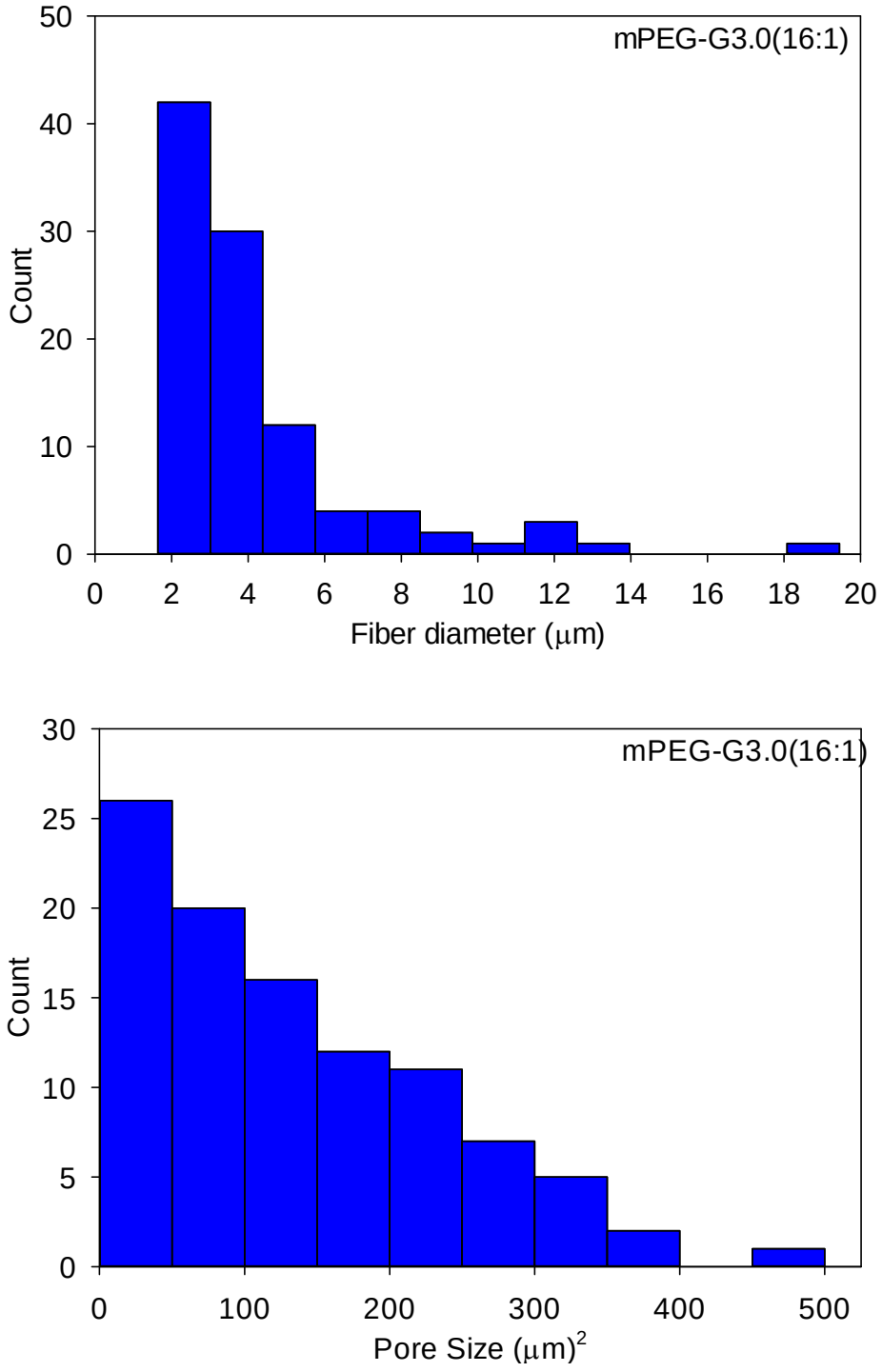


Figure 6.4. Fiber diameter and pore size distributions of mPEG-G3.0 (32:1) and mPEG-G3.0 (16:1) fiber scaffolds. (n=100)

Uniaxial material testing on dendrimer fiber mats was attempted with the MTS Bionix 200 Mechanical Testing System.[60, 124] Stress-strain curves of mPEG-G3.0 (32:1) fiber scaffolds are shown in **Figure 6.5**. Electrospun mPEG-G3.0 (32:1) fibers exhibited poor mechanical properties in terms of peak load (0.19 ± 0.09 N), peak stress (0.11 ± 0.07 MPa), modulus (3.0 ± 1.7 MPa), and energy to break (0.05 ± 0.03 N×mm). However, insufficient data was acquired for mPEG-G3.0 (16:1) fiber mat using the same method because the mat's thickness (0.1 ± 0 mm) was much less than mPEG-G3.0 (32:1) mat's thickness (0.7 ± 0.2 mm). Therefore, it was difficult to preserve mat structure during the sample preparation. As a result, mPEG-G3.0 (16:1) fiber mats were neither thick enough nor reproducible for accurate tensile measurements.

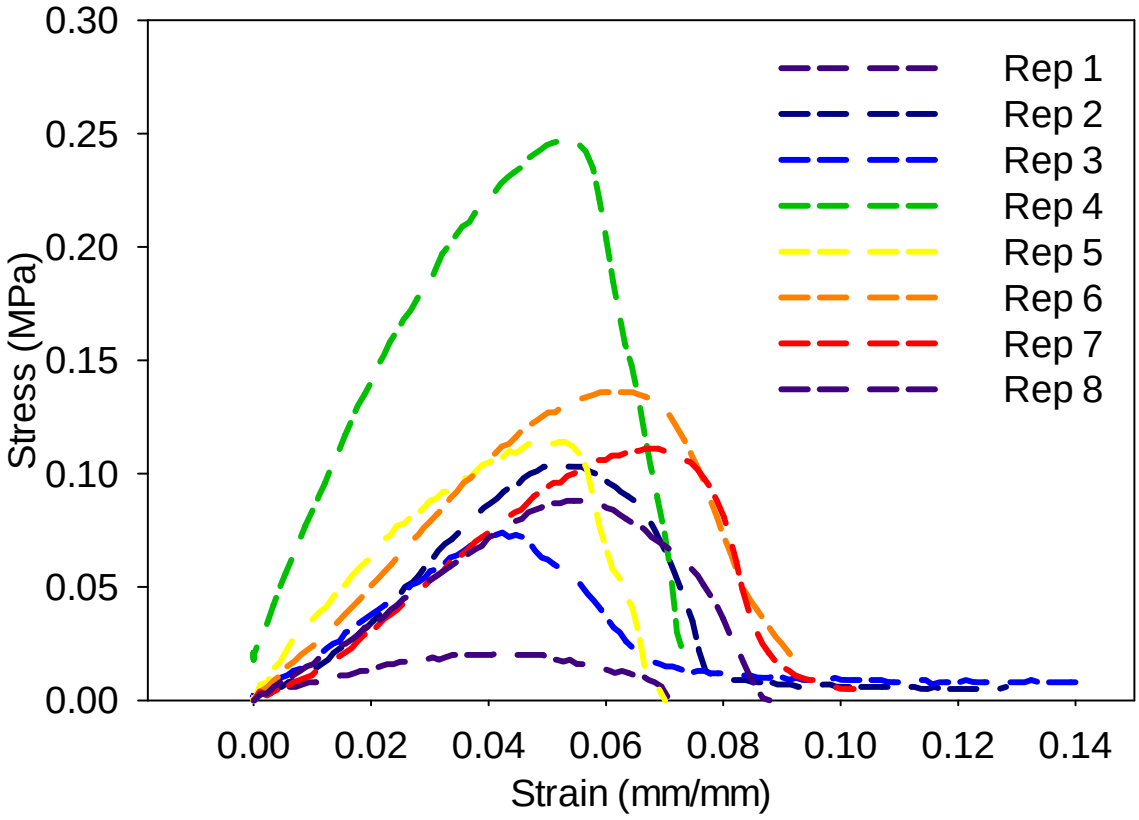


Figure 6.5. Stress-strain curves of mPEG-G3.0 (32:1) fiber scaffolds (n=8).

The fast Fourier transform (FFT) technique was conducted to characterize the degree of fiber alignment and anisotropy following the work by Ayers et al.[166] In particular, this analysis was completed by converting SEM image information from the time domain to a discrete frequency domain. The output image after FFT is grayscale pixels within a circle that has varying intensities with respect to its angle about the circle's central point. [166] Image conversion and analysis was done by using Image J. Distinct peaks in the FFT plots indicates fiber alignment. According to the FFT analysis result (**Figure 6.6**), the peak normalized intensities of mPEG-G3.0 (32:1) and mPEG-G3.0 (16:1) fibers are 0.14 and 0.08, respectively. This result quantitatively confirms that mPEG-G3.0 (16:1) scaffold possesses a higher degree of fiber alignment, which, in turn, leads to a less porous structure as evidenced by smaller pore size. A higher degree of fiber alignment enables an anisotropic scaffold that can better withstand uniform axial loads and provide signaling cues for changes in cell proliferation, migration and phenotype.[154, 169]

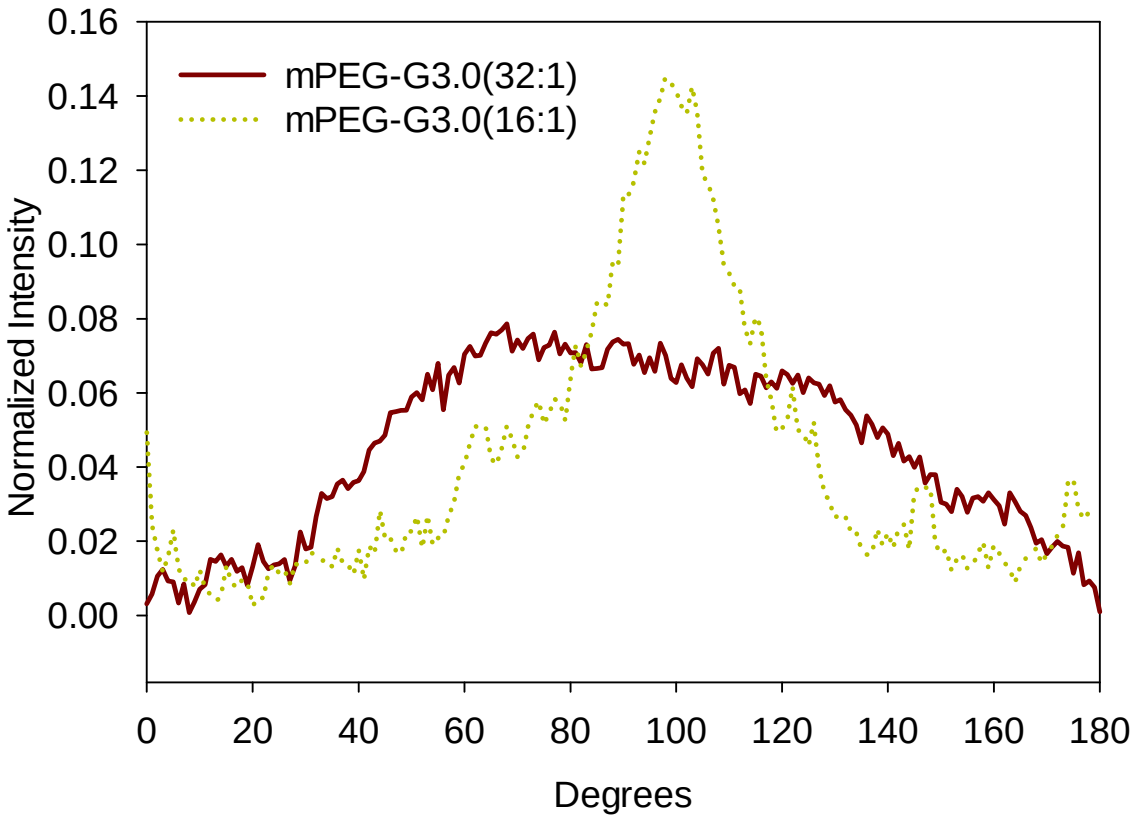


Figure 6.6. Pixel intensity plots with respect to the angle of acquisition for electrospun mPEG-G3.0 fiber scaffold.

Overall, the mPEG-G3.0 fiber scaffolds exhibit poor mechanical properties, which may limit the scaffold's stability to promote cellular activity and controlled release in tissue engineering and drug delivery applications, respectively. To improve the physical properties of dendrimer fiber scaffolds, additional polymers such as *poly(lactic-co-glycolic acid*) (PLGA) can be coupled to PEG to form PLGA-PEG copolymers on the dendrimer surface for drug delivery applications.[170] PLGA has high mechanical strength and elasticity in an early time course. [130] Its material properties such as hydrophilicity and elasticity can be controlled by changing its polymer concentration or ratio of lactic to glycolic acid. However, optimization of these

electrospinning additives is necessary to improve the physical properties while maintaining the original properties of the mPEG-G3.0 conjugates. In addition, dendrimer surface groups may be chemically functionalized to form a cross-linked network following electrospinning to further enhance structural stability and mechanical properties of dendrimer fibrous mats.

By theory, a critical concentration (c*) for chain entanglements in solution should be surpassed for successful fiber formation during electrospinning.[158] This parameter can be theoretically estimated based on **Equation 1**:

$$c^* = 3M/4\pi N_A R_g^3 \quad (1) \text{ ref. [167]}$$

where M is molecular weight, N_A is the Avogadro number, and R_g is the radius of gyration of the polymer and can be estimated using **Equation 2**.

$$R_g = 0.215M^{0.583}(\text{Å}) \quad (2) \text{ ref. [171]}$$

Although PEG chain interpenetration among PEGylated dendritic molecules may help with chain entanglements, PEGylated dendrimers are highly compact. R_g of PAMAM G3.0 fully conjugated with PEG of 5000 Da was reported to be 6.27 nm [172], which was only twice the radius of gyration of linear PEG 5000 Da (3.08 nm according to **Equation 2** [171]). The same is true for PAMAM G3.0 coupled with mPEG2000 due to an even smaller R_g. Not surprisingly, the highest concentration 40% w/v tested for mPEG-G3.0 (32:1) did not generate fibers. Only droplets deposited on the mandrel were observed during the electrospinning process. Therefore, this estimation suggested a slight chance of electrospinning PEGylated dendrimers alone into fibers due to the difficulty of achieving a critical concentration and further substantiated the use of long PEO as a fiber forming additive. Nonetheless, PEO additive contributed to only 6.25% fiber mass, the structure and properties of the resulting fiber mats are predominately influenced by PEGylated PAMAM dendrimers.

In future work, the in vitro cytocompatibility of the scaffolds will be assessed. Anti-microbial tests such as the Kirby-Bauer assay or turbidity measurement will be utilized to validate the sterility of the scaffold before application. The encapsulation and efficacy of relevant drugs, growth factors and anti-microbial agents will be tested to confirm the functions of bioactive molecules within our novel fiber system. Additional polymers such as PLGA may be incorporated to enhance the scaffold's mechanical stability. Lastly, in vivo studies will be planned to examine its pre-clinical potential in physiological conditions.

6.4. Conclusions

In summary, we have successfully fabricated electrospun dendrimer-containing nanocomposite fibers. Morphologically, the mats possessed a uni-modal, non-normal distribution of fibers on the micrometer scale. Fiber alignment is influenced by the degree of PEGylation on the dendrimer surface. The dendrimer fibrous mats show weak mechanical properties that can be improved by adding more stable copolymers such as PLGA without compromising the functionality of dendrimers. In addition, dendrimer surface groups may be chemically functionalized to form a cross-linked network following electrospinning to further enhance structural stability and mechanical properties of dendrimer fibrous mats. Further improvements in the mat's mechanical properties can make it a potential platform for drug delivery and tissue engineering applications.

Acknowledgements

This work was supported, in part, by the National Science Foundation CAREER award (CBET0954957) and National Institutes of Health (R01EY024072). D.A. thanks Southern Regional Education Board (SREB)-State Doctoral Scholars Program.

Literature Cited

1. Carvajal-Millan E, Guilbert S, Doublier J, Micard V. Arabinoxylan/protein gels: Structural, rheological and controlled release properties. Food Hydrocoll 2006;20(1):53-61.

2. Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC, Longaker MT. Human skin wounds: A major and snowballing threat to public health and the economy. Wound Repair and Regeneration 2009;17(6):763-71.

3. Markova A, Mostow EN. US skin disease assessment: Ulcer and wound care. Dermatol Clin 2012;30(1):107-11.

4. Global Health & Aging [Internet]; c2015 [cited 2015 Aug 8]. Available from: https://www.nia.nih.gov/research/publication/global-health-and-aging/humanitys-aging.

5. **Number of Americans with Diabetes Projected to Double or Triple by 2050** [Internet]; c2010 [cited 2015 Aug 4]. Available from: http://www.cdc.gov/media/pressrel/2010/r101022.html.

6. Wysocki A, Mustoe T, Schultz G. Skin, molecular cell biology of. Encyclopedia of Molecular Cell Biology and Molecular Medicine 2006.

7. Odland GF. Structure of the skin. Physiology, Biochemistry, and Molecular Biology of the Skin 1991;1:3-62.

8. Hooper BJ, Goldman MP. Primary dermatologic care. Mosby Inc; 1999. .

9. Jacob SW, Francone CA, Lossow WJ. Structure and function in man. Saunders; 1978. .

10. Haake A, Scott GA, Holbrook KA. Structure and function of the skin: Overview of the epidermis and dermis. The Biology of the Skin 2001;2001:19-45.

11. Griffith LG, Naughton G. Tissue engineering--current challenges and expanding opportunities. Science 2002 Feb 8;295(5557):1009-14.

12. Haake A, Holbrook K. The structure and development of skin. Fitzpatrick's Dermatology in General Medicine 1999;1:70-114.

13. Millington P, Wilkinson R. Skin. biological structure and function. Cambridge University Press, Cambridge 1983.

14. Sams W. Structure and function of the skin. Principles and Practice of Dermatology.New York, NY: Churchill Livingstone 1990.

15. Wysocki AB. A review of the skin and its appendages. Adv Wound Care 1995 Mar-Apr;8(2 Pt 1):53,4, 56-62, 64 passim.

16. Martin P. Wound healing--aiming for perfect skin regeneration. Science 1997;276(5309):75-81.

17. Stryer L. From biochemistry, chapter 2. 1995.

18. Ruszczak Z. Effect of collagen matrices on dermal wound healing. Adv Drug Deliv Rev 2003;55(12):1595-611.

19. Gay S, Miller EJ. Collagen in the physiology and pathology of connective tissue. Fischer; 1978. .

20. Rudowski W. Burn therapy and research. Johns Hopkins University Press; 1976. .

21. Roth RR, James WD. Microbial ecology of the skin. Annual Reviews in Microbiology 1988;42(1):441-64.

22. Spence AP, Mason EB. Human anatomy and physiology. Benjamin-Cummings Publishing Company; 1987. .

23. Weinberg A, Swartz M. General considerations of bacterial diseases. Dermatology in General Medicine, 3rd Ed.McGraw-Hill, New York 1987:2089-100.

24. Hentges DJ. The anaerobic microflora of the human body. Clin Infect Dis 1993 Jun;16 Suppl 4:S175-80.

25. Skin microflora and bacterial infections of the skin. Journal of investigative dermatology symposium proceedingsNature Publishing Group; 2001. .

26. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annual Reviews in Microbiology 1995;49(1):711-45.

27. Headington JT. The dermal dendrocyte. Br J Dermatol 1985;113(s29):15-.

28. Wenger C. Thermoregulation. In: I. Freedberg, editor. Fitzpatrick's dermatology in general medicine. 5th ed. New York: McGraw-Hill; 1999. .

29. Solomons B. Lecture notes on dermatology. 1983.

30. Petreaca M, Martins-Green M. Cell-ECM interactions in repair and regeneration. In: A. Atala, R. Lanza, J. A. Thomson, R. M. Nerem, editors. Foundations of regenerative medicine. Elsevier; 2008. .

31. Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: A review. J Pharm Sci 2008;97(8):2892-923.

32. Shakespeare P. Burn wound healing and skin substitutes. Burns 2001;27(5):517-22.

33. Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: Initiation, maintenance, and regulation. Biochemistry (N Y) 1991;30(43):10363-70.

34. Hübner G, Hu Q, Smola H, Werner S. Strong induction of activin expression after injury suggests an important role of activin in wound repair. Dev Biol 1996;173(2):490-8.

35. Cohen IK, Die-gelmann RF, Lindblad WJ, Hugo NE. Wound healing: Biochemical and clinical aspects. Plastic & Reconstructive Surgery 1992(90):926.

36. Black JM, Hawks JH, Keene AM. Medical-surgical nursing: Clinical management for positive outcomes. WB Saunders Co; 2001. .

37. Xu J, Clark RA. A three-dimensional collagen lattice induces protein kinase C-zeta activity: Role in alpha2 integrin and collagenase mRNA expression. J Cell Biol 1997 Jan 27;136(2):473-83.

38. Salo T, Mäkelä M, Kylmäniemi M. Expression of MMP-2 and MMP-9 (72 kD and 92 kD type IV collagenases) during early human wound healing. Lab Invest 1994;70:176.

39. Broughton G,2nd, Janis JE, Attinger CE. The basic science of wound healing. Plast Reconstr Surg 2006 Jun;117(7 Suppl):12S-34S.

40. Nwomeh BC, Liang H, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. J Surg Res 1999;81(2):189-95.

41. Schultz GS, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, Romanelli M, Stacey MC, Teot L, Vanscheidt W. Wound bed preparation: A systematic approach to wound management. Wound Repair and Regeneration 2003;11(s1):S1-S28.

42. Thomas S. Wound management and dressings. Pharmaceutical Pr; 1990. .

43. Ovington LG. Advances in wound dressings. Clin Dermatol 2007;25(1):33-8.

44. Jones V, Grey JE, Harding KG. Wound dressings. BMJ 2006 Apr 1;332(7544):777-80.

45. Doyle JW, Roth TP, Smith RM, Li Y, Dunn RM. Effect of calcium alginate on cellular wound healing processes modeled in vitro. J Biomed Mater Res 1996;32(4):561-8.

46. Thomas A, Harding K, Moore K. Alginates from wound dressings activate human macrophages to secrete tumour necrosis factor-< i> α. Biomaterials 2000;21(17):1797-802.

47. Thomson T. Foam Composite 2006.

48. Ramos-e-Silva M, Ribeiro de Castro, Maria Cristina. New dressings, including tissue-engineered living skin. Clin Dermatol 2002;20(6):715-23.

49. Ramshaw JA, Werkmeister JA, Glattauer V. Collagen-based biomaterials. Biotechnology and Genetic Engineering Reviews 1996;13(1):335-82.

50. Doillon CJ, Silver FH. Collagen-based wound dressing: Effects of hyaluronic acid and firponectin on wound healing. Biomaterials 1986;7(1):3-8.

51. Ishihara M, Nakanishi K, Ono K, Sato M, Kikuchi M, Saito Y, Yura H, Matsui T, Hattori H, Uenoyama M. Photocrosslinkable chitosan as a dressing for wound occlusion and accelerator in healing process. Biomaterials 2002;23(3):833-40.

52. Sai K P, Babu M. Collagen based dressings—a review. Burns 2000;26(1):54-62.

53. Mian M, Beghe F, Mian E. Collagen as a pharmacological approach in wound healing. Int J Tissue React 1992;14 Suppl:1-9.

54. Schwarzer T. Manufacturing pricinciples of freeze-dried collagen sponges: Characteristics and applications. In: L. Rey, JC May, editors. Lyophilization of pharmaceutical and biological products. New York: Marcel Dekker; 1999. .

55. Voinchet V, Vasseur P, Kern J. Efficacy and safety of hyaluronic acid in the management of acute wounds. American Journal of Clinical Dermatology 2006;7(6):353-7.

56. Ueno H, Mori T, Fujinaga T. Topical formulations and wound healing applications of chitosan. Adv Drug Deliv Rev 2001;52(2):105-15.

57. Alvarez JI, Krishnamurthy J, Teale JM. Doxycycline treatment decreases morbidity and mortality of murine neurocysticercosis: Evidence for reduction of apoptosis and matrix metalloproteinase activity. The American Journal of Pathology 2009;175(2):685-95.

58. Smith Jr GN, Mickler EA, Hasty KA, Brandt KD. Specificity of inhibition of matrix metalloproteinase activity by doxycycline: Relationship to structure of the enzyme. Arthritis & Rheumatism 1999;42(6):1140-6.

59. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH. Antibacterial activity and mechanism of action of the silver ion in staphylococcus aureus and escherichia coli. Appl Environ Microbiol 2008 Apr;74(7):2171-8.

60. Dongargaonkar AA, Bowlin GL, Yang H. Electrospun blends of gelatin and Gelatin–Dendrimer conjugates as a wound-dressing and drug-delivery platform. Biomacromolecules 2013;14(11):4038-45.

61. Anton F. Process and Apparatus for Preparing Artificial Threads 1934.

62. Zahedi P, Rezaeian I, Ranaei-Siadat S, Jafari S, Supaphol P. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. Polym Adv Technol 2010;21(2):77-95.

63. Dabney SE. The use of Electrospinning Technology to Produce Wound Dressings 2002.

64. Schiffman JD, Schauer CL. A review: Electrospinning of biopolymer nanofibers and their applications. Polymer Reviews 2008;48(2):317-52.

65. Chen J, Chang G, Chen J. Electrospun collagen/chitosan nanofibrous membrane as wound dressing. Colloids Surf Physicochem Eng Aspects 2008;313:183-8.

66. Greiner A, Wendorff JH. Electrospinning: A fascinating method for the preparation of ultrathin fibers. Angewandte Chemie International Edition 2007;46(30):5670-703.

67. Noh HK, Lee SW, Kim J, Oh J, Kim K, Chung C, Choi S, Park WH, Min B. Electrospinning of chitin nanofibers: Degradation behavior and cellular response to normal human keratinocytes and fibroblasts. Biomaterials 2006;27(21):3934-44.

68. Cai Z, Mo X, Zhang K, Fan L, Yin A, He C, Wang H. Fabrication of chitosan/silk fibroin composite nanofibers for wound-dressing applications. International Journal of Molecular Sciences 2010;11(9):3529-39.

69. Lee KY, Jeong L, Kang YO, Lee SJ, Park WH. Electrospinning of polysaccharides for regenerative medicine. Adv Drug Deliv Rev 2009;61(12):1020-32.

70. Miraftab M, Qiao Q, Kennedy JF, Anand SC, Groocock MR. Fibres for wound dressings based on mixed carbohydrate polymer fibers. Carb Poly 2003;53:225-231.

71. Shalumon K, Anulekha K, Nair SV, Nair S, Chennazhi K, Jayakumar R. Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. Int J Biol Macromol 2011;49(3):247-54.

72. Lu J, Zhu Y, Guo Z, Hu P, Yu J. Electrospinning of sodium alginate with poly (ethylene oxide). Polymer 2006;47(23):8026-31.

73. Safi S, Morshed M, Hosseini Ravandi S, Ghiaci M. Study of electrospinning of sodium alginate, blended solutions of sodium alginate/poly (vinyl alcohol) and sodium alginate/poly (ethylene oxide). J Appl Polym Sci 2007;104(5):3245-55.

74. Kubota N, Eguchi Y. Facile preparation of water-soluble N-acetylated chitosan and molecular weight dependence of its water-solubility. Polym J 1997;29(2):123-7.

75. Aiba S. Studies on chitosan: 3. evidence for the presence of random and block copolymer structures in partially N-acetylated chitosans. Int J Biol Macromol 1991;13(1):40-4.

76. Rinaudo M. Chitin and chitosan: Properties and applications. Progress in Polymer Science 2006;31(7):603-32.

77. Ohkawa K, Cha D, Kim H, Nishida A, Yamamoto H. Electrospinning of chitosan. Macromolecular Rapid Communications 2004;25(18):1600-5.

78. Spasova M, Manolova N, Paneva D, Rashkov I. Preparation of chitosan-containing nanofibres by electrospinning of chitosan/poly (ethylene oxide) blend solutions. E-Polymers 2004;4(1):624-35.

79. Li J, He A, Han CC, Fang D, Hsiao BS, Chu B. Electrospinning of hyaluronic acid (HA) and HA/gelatin blends. Macromolecular Rapid Communications 2006;27(2):114-20.

80. Ji Y, Ghosh K, Shu XZ, Li B, Sokolov JC, Prestwich GD, Clark RA, Rafailovich MH. Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds. Biomaterials 2006;27(20):3782-92.

81. Bhattarai N, Gunn J, Zhang M. Chitosan-based hydrogels for controlled, localized drug delivery. Adv Drug Deliv Rev 2010;62(1):83-99.

82. Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery. Adv Drug Deliv Rev 2008;60(15):1638-49.

83. Khan F, Tare RS, Oreffo R, Bradley M. Versatile biocompatible polymer hydrogels: Scaffolds for cell growth. Angewandte Chemie International Edition 2009;48(5):978-82.

84. Ladet S, David L, Domard A. Multi-membrane hydrogels. Nature 2008;452(7183):76-9.

85. Denkbas EB, Ottenbrite RM. Perspectives on: Chitosan drug delivery systems based on their geometries. J Bioact Compatible Polym 2006;21(4):351-68.

86. Hoffman AS. Hydrogels for biomedical applications. Adv Drug Deliv Rev 2012;64:18-23.

87. Wichterle O, Lim D. Hydrophilic gels for biological use. 1960.

88. Flory PJ. Principles of polymer chemistry. Cornell University Press; 1953. .

89. Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. Adv Mater 2006;18(11):1345-60.

90. Langer R, Peppas NA. Advances in biomaterials, drug delivery, and bionanotechnology. AIChE J 2003;49(12):2990-3006.

91. Peppas N, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. European Journal of Pharmaceutics and Biopharmaceutics 2000;50(1):27-46.

92. Lou X, Dalton P, Chirila T. Hydrophilic sponges based on 2-hydroxyethyl methacrylate part VII: Modulation of sponge characteristics by changes in reactivity and hydrophilicity of crosslinking agents. J Mater Sci Mater Med 2000;11(5):319-25.

93. Lou X, Chirila TV. Swelling behavior and mechanical properties of chemically cross-linked gelatin gels for biomedical use. J Biomater Appl 1999 Oct;14(2):184-91.

94. Cruise GM, Scharp DS, Hubbell JA. Characterization of permeability and network structure of interfacially photopolymerized poly (ethylene glycol) diacrylate hydrogels. Biomaterials 1998;19(14):1287-94.

95. Lin C, Metters AT. Hydrogels in controlled release formulations: Network design and mathematical modeling. Adv Drug Deliv Rev 2006;58(12):1379-408.

96. Schepetkin IA, Quinn MT. Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. Int Immunopharmacol 2006 3;6(3):317-33.

97. Coviello T, Matricardi P, Marianecci C, Alhaique F. Polysaccharide hydrogels for modified release formulations. J Controlled Release 2007;119(1):5-24.

98. Valenta C, Auner BG. The use of polymers for dermal and transdermal delivery. European Journal of Pharmaceutics and Biopharmaceutics 2004;58(2):279-89.

99. Balakrishnan B, Mohanty M, Umashankar P, Jayakrishnan A. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials 2005;26(32):6335-42.

100. Balakrishnan B, Jayakrishnan A. Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. Biomaterials 2005;26(18):3941-51.

101. Balakrishnan B, Mohanty M, Fernandez AC, Mohanan PV, Jayakrishnan A. Evaluation of the effect of incorporation of dibutyryl cyclic adenosine monophosphate in an in situ-forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials 2006;27(8):1355-61.

102. Shigemasa Y, Minami S. Applications of chitin and chitosan for biomaterials. Biotechnology and Genetic Engineering Reviews 1996;13(1):383-420.

103. Rao SB, Sharma CP. Use of chitosan as a biomaterial: Studies on its safety and hemostatic potential. J Biomed Mater Res 1997;34(1):21-8.

104. Park CJ, Clark SG, Lichtensteiger CA, Jamison RD, Johnson AJW. Accelerated wound closure of pressure ulcers in aged mice by chitosan scaffolds with and without bFGF. Acta Biomaterialia 2009;5(6):1926-36.

105. Sun G, Zhang X, Shen YI, Sebastian R, Dickinson LE, Fox-Talbot K, Reinblatt M, Steenbergen C, Harmon JW, Gerecht S. Dextran hydrogel scaffolds enhance angiogenic responses and promote complete skin regeneration during burn wound healing. Proc Natl Acad Sci U S A 2011 Dec 27;108(52):20976-81.

106. Kubo W, Miyazaki S, Attwood D. Oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations. Int J Pharm 2003;258(1):55-64.

107. Rozier A, Mazuel C, Grove J, Plazonnet B. Functionality testing of gellan gum, a polymeric excipient material for ophthalmic dosage forms. Int J Pharm 1997;153(2):191-8.

108. Jansson B, Hägerström H, Fransén N, Edsman K, Björk E. The influence of gellan gum on the transfer of fluorescein dextran across rat nasal epithelium in vivo. European Journal of Pharmaceutics and Biopharmaceutics 2005;59(3):557-64.

109. Cencetti C, Bellini D, Pavesio A, Senigaglia D, Passariello C, Virga A, Matricardi P. Preparation and characterization of antimicrobial wound dressings based on silver, gellan, PVA and borax. Carbohydr Polym 2012;90(3):1362-70.

110. Prehm P. Hyaluronan. In: S. De Baets, E. J. Vandamme, A. Steinbüchel, editors. Biopolymers. Weinheim: Wiley-VCH; 2002. .

111. Tomihata K, Ikada Y. Crosslinking of hyaluronic acid with water-soluble carbodiimide. J Biomed Mater Res 1997;37(2):243-51.

112. Luo Y, Kirker KR, Prestwich GD. Cross-linked hyaluronic acid hydrogel films: New biomaterials for drug delivery. J Controlled Release 2000;69(1):169-84.

113. Zhang F, He C, Cao L, Feng W, Wang H, Mo X, Wang J. Fabrication of gelatin–hyaluronic acid hybrid scaffolds with tunable porous structures for soft tissue engineering. Int J Biol Macromol 2011;48(3):474-81.

114. Seidlits SK, Drinnan CT, Petersen RR, Shear JB, Suggs LJ, Schmidt CE. Fibronectin–hyaluronic acid composite hydrogels for three-dimensional endothelial cell culture. Acta Biomaterialia 2011;7(6):2401-9.

115. Iravani S, Fitchett CS, Georget DMR. Physical characterization of arabinoxylan powder and its hydrogel containing a methyl xanthine. Carbohydr Polym 2011 4/22;85(1):201-7.

116. Bhatia M, Ahuja M. Psyllium arabinoxylan: Carboxymethylation, characterization and evaluation for nanoparticulate drug delivery. Int J Biol Macromol 2015 1;72(0):495-501.

117. Weightman RM, Fitchett CS, Greenshields R, inventors. Improvements relating to bran gels. Cabridgeshire Cambridge Biopolymers Limited, editor. United States patent 6482430 2002 Nov 19.

118. Neyrinck AM, Possemiers S, Druart C, Van de Wiele T, De Backer F, Cani PD, Larondelle Y, Delzenne NM. Prebiotic effects of wheat arabinoxylan related to the increase of bifidobacteria, roseburia and bacteroides/prevotella in diet-induced obese mice. Plos One 2011;6(6):1-12.

119. Neyrinck AM, Van Hee VF, Piront N, De Backer F, Toussaint O, Cani PD, Delzenne NM. Wheat-derived arabinoxylan oligosaccharides with prebiotic effect increase satietogenic gut peptides and reduce metabolic endotoxemia in diet-induced obese mice. Clin Nutr and Diab 2012;2:1-9.

120. Jayakumar R, Prabaharan M, Nair S, Tamura H. Novel chitin and chitosan nanofibers in biomedical applications. Biotechnol Adv 2010;28(1):142-50.

121. Höije A, Sternemalm E, Heikkinen S, Tenkanen M, Gatenholm P. Material properties of films from enzymatically tailored arabinoxylans. Biomacromolecules 2008;9(7):2042-7.

122. 3M™ tegaderm™ alginate ag silver dressing. St. Paul, MN: 3M Healthcare; 2009.

123. 3M^{TM} Tegaderm^{TM} alginate document Product Information. Jan 2011]. .

124. Aduba Jr DC, Hammer JA, Yuan Q, Andrew Yeudall W, Bowlin GL, Yang H. Semi-interpenetrating network (sIPN) gelatin nanofiber scaffolds for oral mucosal drug delivery. Acta Biomaterialia 2013;9(5):6576-84.

125. Yoganarasimha S, Trahan WR, Best AM, Bowlin GL, Kitten TO, Moon PC, Madurantakam PA. Peracetic acid: A practical agent for sterilizing heat-labile polymeric tissue-engineering scaffolds. Tissue Engineering Part C: Methods 2014;20(9):714-23.

126. Hudzicki J. Kirby-bauer disk diffusion susceptibility test protocol. Am Soc Microbiol 2009.

127. Giacometti A, Cirioni O, Schimizzi A, Del Prete M, Barchiesi F, D'errico M, Petrelli E, Scalise G. Epidemiology and microbiology of surgical wound infections. J Clin Microbiol 2000;38(2):918-22.

128. Glasser JS, Guymon CH, Mende K, Wolf SE, Hospenthal DR, Murray CK. Activity of topical antimicrobial agents against multidrug-resistant bacteria recovered from burn patients. Burns 2010;36(8):1172-84.

129. Feng Q, Wu J, Chen G, Cui F, Kim T, Kim J. A mechanistic study of the antibacterial effect of silver ions on escherichia coli and staphylococcus aureus. J Biomed Mater Res 2000;52(4):662-8.

130. Barnes CP, Sell SA, Boland ED, Simpson DG, Bowlin GL. Nanofiber technology: Designing the next generation of tissue engineering scaffolds. Adv Drug Deliv Rev 2007 12/10;59(14):1413-33.

131. Mogoşanu GD, Grumezescu AM. Natural and synthetic polymers for wounds and burns dressing. Int J Pharm 2014;463(2):127-36.

132. Das N, Bera T, Mukherjee A. Biomaterial hydrogels for different biomedical applications. Int J Pharm Bio Sci 2012;3(3):586-97.

133. Gelling protocol for arabinoxylan ferulate - AXF September 2008]. .

134. Zuidema JM, Rivet CJ, Gilbert RJ, Morrison FA. A protocol for rheological characterization of hydrogels for tissue engineering strategies. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2013.

135. Fu Y, Kao WJ. Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. Expert Opinion on Drug Delivery 2010;7(4):429-44.

136. Fournier D, De Geest BG, Du Prez FP. On-demand click functionalizaiton of polyurethane films and foams . Polymer 2009;50(23):5362-5367.

137. Sevilla CA, Dalecki D, Hocking DC. Regional fibronectin and collagen fibril co-assembly directs cell proliferation and microtissue morphology. PloS One 2013;8(10).

138. Shih M, Shau M, Chang M, Chiou S, Chang J, Cherng J. Platelet adsorption and hemolytic properties of liquid crystal/composite polymers. Int J Pharm 2006;327(1):117-25.

139. Ong S, Wu J, Moochhala SM, Tan M, Lu J. Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials 2008 11;29(32):4323-32.

140. Medical Device Databases [Internet]: US Food and Drug Administration; c2015 [cited 2015 July 7]. Available from: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Databases/default.htm.

141. Tomalia D.A., Baker H., Dewald J., Hall M., Kallos G., Martin S., Roeck J., Ryder J., and Smith P. . Polymer 1985;17(1):117-132.

142. Tomalia DA, Baker H, Hall M, Kallos G, Martin S, Roeck J, Ryder J, Smith P. **Dendritic macromolecules: Synthesis of starburst dendrimers**. Macromolecules 1986;19(9):2466-2468.

143. Yang H, Kao WJ. Dendrimers for pharmaceutical and biomedical applications. Journal of Biomaterials Science, Polymer Edition 2006;17(1-2):3-19.

144. Yang H. Nanoparticle-mediated brain-specific drug delivery, imaging, and diagnosis. Pharm Res 2010;27(9):1759-71.

145. Tomalia DA, Uppuluri S, Swanson DR, Li J. Dendrimers as reactive modules for the synthesis of new structure-controlled, higher-complexity megamers. Pure and Applied Chemistry 2000;72(12):2343-58.

146. Uppuluri S, Swanson D, Piehler L, Li J, Hagnauer G, Tomalia D. Core–shell tecto (dendrimers): I. synthesis and characterization of saturated shell models. Adv Mater 2000;12(11):796-800.

147. Tomalia DA. Birth of a new macromolecular architecture: Dendrimers as quantized building blocks for nanoscale synthetic polymer chemistry. Progress in Polymer Science 2005;30(3):294-324.

148. Kailasan A, Yuan Q, Yang H. Synthesis and characterization of thermoresponsive polyamidoamine–polyethylene glycol–poly (d, l-lactide) core–shell nanoparticles. Acta Biomaterialia 2010;6(3):1131-9.

149. Zhong S, Yung LYL. Enhanced biological stability of collagen with incorporation of PAMAM dendrimer. Journal of Biomedical Materials Research Part A 2009;91(1):114-22.

150. Desai PN, Yuan Q, Yang H. Synthesis and characterization of photocurable polyamidoamine dendrimer hydrogels as a versatile platform for tissue engineering and drug delivery. Biomacromolecules 2010;11(3):666-73.

151. Pham QP, Sharma U, Mikos AG. Electrospun poly (ε-caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: Characterization of scaffolds and measurement of cellular infiltration. Biomacromolecules 2006;7(10):2796-805.

152. Wnek GE, Carr ME, Simpson DG, Bowlin GL. Electrospinning of nanofiber fibrinogen structures. Nano Letters 2003;3(2):213-6.

153. Liang D, Hsiao BS, Chu B. Functional electrospun nanofibrous scaffolds for biomedical applications. Adv Drug Deliv Rev 2007 12/10;59(14):1392-412.

154. Ayres CE, Jha BS, Meredith H, Bowman JR, Bowlin GL, Henderson SC, Simpson DG. Measuring fiber alignment in electrospun scaffolds: A user's guide to the 2D fast fourier transform approach. Journal of Biomaterials Science, Polymer Edition 2008;19(5):603-21.

155. Pakravan M, Heuzey M, Ajji A. A fundamental study of chitosan/PEO electrospinning. Polymer 2011;52(21):4813-24.

156. Kriegel C, Kit K, McClements DJ, Weiss J. Electrospinning of chitosan–poly (ethylene oxide) blend nanofibers in the presence of micellar surfactant solutions. Polymer 2009;50(1):189-200.

157. Agarwal S, Wendorff JH, Greiner A. Use of electrospinning technique for biomedical applications. Polymer 2008;49(26):5603-21.

158. Shenoy SL, Bates WD, Frisch HL, Wnek GE. Role of chain entanglements on fiber formation during electrospinning of polymer solutions: Good solvent, non-specific polymer–polymer interaction limit. Polymer 2005 4/25;46(10):3372-84.

159. Madani M, Sharifi-Sanjani N, Iraji-Rad R. Aureole nanofibers by electrospinning of PAMAM-PEO solution. J Appl Polym Sci 2009;113(5):3005-11.

160. Zhao Y, Zhu X, Liu H, Luo Y, Wang S, Shen M, Zhu M, Shi X. Dendrimer-functionalized electrospun cellulose acetate nanofibers for targeted cancer cell capture applications. Journal of Materials Chemistry B 2014;2(42):7384-93.

161. Malik N, Wiwattanapatapee R, Klopsch R, Lorenz K, Frey H, Weener J, Meijer E, Paulus W, Duncan R. Dendrimers:: Relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. J Controlled Release 2000;65(1):133-48.

162. Jevprasesphant R, Penny J, Jalal R, Attwood D, McKeown N, D'emanuele A. The influence of surface modification on the cytotoxicity of PAMAM dendrimers. Int J Pharm 2003;252(1):263-6.

163. Yang H, Lopina ST, DiPersio LP, Schmidt SP. Stealth dendrimers for drug delivery: Correlation between PEGylation, cytocompatibility, and drug payload. J Mater Sci Mater Med 2008;19(5):1991-7.

164. Ryan SM, Mantovani G, Wang X, Haddleton DM, Brayden DJ. Advances in PEGylation of important biotech molecules: Delivery aspects. 2008.

165. Yang H, Morris JJ, Lopina ST. Polyethylene glycol–polyamidoamine dendritic micelle as solubility enhancer and the effect of the length of polyethylene glycol arms on the solubility of pyrene in water. J Colloid Interface Sci 2004 5/1;273(1):148-54.

166. Ayres C, Bowlin GL, Henderson SC, Taylor L, Shultz J, Alexander J, Telemeco TA, Simpson DG. Modulation of anisotropy in electrospun tissue-engineering scaffolds: Analysis of fiber alignment by the fast fourier transform. Biomaterials 2006;27(32):5524-34.

167. Yu JH, Fridrikh SV, Rutledge GC. The role of elasticity in the formation of electrospun fibers. Polymer 2006 6/14;47(13):4789-97.

168. Deitzel J, Kleinmeyer J, Harris D, Tan NB. The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer 2001;42(1):261-72.

169. Yang F, Murugan R, Wang S, Ramakrishna S. Electrospinning of nano/micro scale poly (L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials 2005;26(15):2603-10.

170. Cheng J, Teply BA, Sherifi I, Sung J, Luther G, Gu FX, Levy-Nissenbaum E, Radovic-Moreno AF, Langer R, Farokhzad OC. Formulation of functionalized PLGA–PEG nanoparticles for in vivo targeted drug delivery. Biomaterials 2007;28(5):869-76.

171. Devanand K, Selser J. Asymptotic behavior and long-range interactions in aqueous solutions of poly (ethylene oxide). Macromolecules 1991;24(22):5943-7.

172. Hedden RC, Bauer BJ. Structure and dimensions of PAMAM/PEG dendrimer-star polymers. Macromolecules 2003;36(6):1829-35.

Appendix A – Statistical analysis: Electrospinning of arabinoxylan as a novel fiber scaffold:

Morphology: Fiber Diameter & Pore Size

One Way Analysis of Variance Monday, October 14, 2013, 10:03:33 AM

Data source: Data 1 in Fiber diameter Gel_AXF

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Monday, October 14, 2013, 10:03:33 AM

Data source: Data 1 in Fiber diameter Gel_AXF

Group	N	Missing	Median	25%	75%
Gel-AXF 1:1	100	0	0.410	0.350	0.490
Gel-AXF 2:1	100	0	0.560	0.430	0.670
Gel-AXF 4:1	100	0	1.015	0.610	1.350

H = 103.971 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
Gel-AXF 4:1 vs Gel-AXF 1:1	12488.000	14.396	Yes
Gel-AXF 4:1 vs Gel-AXF 2:1	6767.500	7.801	Yes
Gel-AXF 2:1 vs Gel-AXF 1:1	5720.500	6.594	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Monday, October 14, 2013, 10:15:48 AM

Data source: Data 1 in Pore Size Gel_AXF

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Monday, October 14, 2013, 10:15:48 AM

Data source: Data 1 in Pore Size Gel_AXF

Group	N	Missing	Median	25%	75%
Gel-AXF 1:1	100	0	0.505	0.333	0.808
Gel-AXF 2:1	100	0	1.195	0.725	1.795
Gel-AXF 4:1	100	0	2.620	1.215	4.845

H = 117.749 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
Gel-AXF 4:1 vs Gel-AXF 1:1	13280.000	15.309	Yes
Gel-AXF 4:1 vs Gel-AXF 2:1	5843.500	6.736	Yes
Gel-AXF 2:1 vs Gel-AXF 1:1	7436.500	8.573	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Tensile Properties

One Way Analysis of Variance Monday, October 14, 2013, 10:45:06 AM

Data source: Thickness in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.238)

Equal Variance Test: Passed (P = 0.258)

Group Name	N	Missing	Mean	Std Dev	SEM
1:1 Gel-AXF	12	0	0.00842	0.00133	0.000384
2:1 Gel-AXF	12	0	0.0113	0.00152	0.000437
4:1 Gel-AXF	12	0	0.0169	0.000772	0.000223

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.000445	0.000222	143.277	<0.001
Residual	33	0.0000512	0.00000155		
Total	35	0.000496			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
4:1 Gel-AXF vs. 1:1 Gel-AXF	0.00846	16.629	<0.001	Yes
4:1 Gel-AXF vs. 2:1 Gel-AXF	0.00562	11.059	<0.001	Yes
2:1 Gel-AXF vs. 1:1 Gel-AXF	0.00283	5.570	<0.001	Yes

One Way Analysis of Variance Monday, October 14, 2013, 10:43:04 AM

Data source: Peak Load in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.905)

Equal Variance Test: Passed (P = 0.161)

Group Name	N	Missing	Mean	Std Dev	SEM
1:1 Gel-AXF	12	0	0.288	0.0932	0.0269
2:1 Gel-AXF	12	0	0.588	0.162	0.0468
4:1 Gel-AXF	12	0	1.380	0.182	0.0524

Source of Variation	DF	SS	MS	F	P
Between Groups	2	7.643	3.822	168.687	<0.001
Residual	33	0.748	0.0227		
Total	35	8.391			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
4:1 Gel-AXF vs. 1:1 Gel-AXF	1.092	17.778	<0.001	Yes
4:1 Gel-AXF vs. 2:1 Gel-AXF	0.792	12.888	<0.001	Yes
2:1 Gel-AXF vs. 1:1 Gel-AXF	0.300	4.890	<0.001	Yes

One Way Analysis of Variance Monday, October 14, 2013, 10:44:07 AM

Data source: Peak Stress in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.848)

Equal Variance Test: Passed (P = 0.813)

Group Name	N	Missing	Mean	Std Dev	SEM
1:1 Gel-AXF	12	0	0.503	0.156	0.0452
2:1 Gel-AXF	12	0	0.774	0.186	0.0536

4:1 Gel-AXF	12	0	1.207	0.149	0.0429

Source of Variation	DF	SS	MS	F	P
Between Groups	2	3.027	1.513	56.009	<0.001
Residual	33	0.892	0.0270		
Total	35	3.918			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

One Way Analysis of Variance Monday, October 14, 2013, 10:46:54 AM

Data source: Modulus in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.987)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Monday, October 14, 2013, 10:46:54 AM

Data source: Modulus in Notebook1

Group	N	Missing	Median	25%	75%
1:1 Gel-AXF	12	0	5.731	4.395	7.851
2:1 Gel-AXF	12	0	22.148	16.307	32.483
4:1 Gel-AXF	12	0	46.463	40.584	51.656

H = 30.709 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
4:1 Gel-AXF vs 1:1 Gel-AXF	286.000	7.836	Yes
4:1 Gel-AXF vs 2:1 Gel-AXF	146.000	4.000	Yes
2:1 Gel-AXF vs 1:1 Gel-AXF	140.000	3.836	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Monday, October 14, 2013, 10:47:22 AM

Data source: Strain at Break in Notebook1

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Monday, October 14, 2013, 10:47:22 AM

Data source: Strain at Break in Notebook1

Group	N	Missing	Median	25%	75%
1:1 Gel-AXF	12	0	0.124	0.107	0.154
2:1 Gel-AXF	12	0	0.0645	0.0432	0.0952
4:1 Gel-AXF	12	0	0.0330	0.0273	0.0365

H = 25.607 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
1:1 Gel-AXF vs 4:1 Gel-AXF	260.500	7.138	Yes
1:1 Gel-AXF vs 2:1 Gel-AXF	114.500	3.137	No
2:1 Gel-AXF vs 4:1 Gel-AXF	146.000	4.000	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Monday, October 14, 2013, 10:41:57 AM

Data source: Energy to Break in Notebook1

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Monday, October 14, 2013, 10:41:57 AM

Data source: Energy to Break in Notebook1

Group	N	Missing	Median	25%	75%
1:1 Gel-AXF	12	0	0.145	0.0895	0.186
2:1 Gel-AXF	12	0	0.128	0.0847	0.136
4:1 Gel-AXF	12	0	0.183	0.134	0.191

H = 7.576 with 2 degrees of freedom. (P = 0.023)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.023)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
4:1 Gel-AXF vs 2:1 Gel-AXF	142.000	3.891	Yes
4:1 Gel-AXF vs 1:1 Gel-AXF	74.000	2.028	No
1:1 Gel-AXF vs 2:1 Gel-AXF	68.000	1.863	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Carbohydrate Assay

One Way Analysis of Variance Saturday, March 28, 2015, 9:43:12 PM

Data source: Data 1 in Carbohydrate Assay compiled

Normality Test (Shapiro-Wilk) Passed (P = 0.457)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Saturday, March 28, 2015, 9:43:12 PM

Data source: Data 1 in Carbohydrate Assay compiled

Group	N	Missing	Median	25%	75%
Gel-AXF 1:1	10	0	9.695	7.833	15.637
Gel-AXF 2:1	10	0	3.895	2.163	5.235
Gel-AXF 4:1	10	0	1.615	0.578	2.740

H = 21.399 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
Gel-AXF 1:1 vs Gel-AXF 4:1	180.000	6.466	Yes
Gel-AXF 1:1 vs Gel-AXF 2:1	114.000	4.095	Yes

Gel-AXF 2:1 vs Gel-AXF 4:1	66.000	2.371	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Saturday, March 28, 2015, 9:44:04 PM

Data source: Data 1 in Carbohydrate Assay compiled

Normality Test (Shapiro-Wilk) Passed (P = 0.458)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Saturday, March 28, 2015, 9:44:04 PM

Data source: Data 1 in Carbohydrate Assay compiled

Group	N	Missing	Median	25%	75%
AXF Pct Loss Gel-AXF 1:1	10	0	40.305	34.363	42.167
AXF Pct Loss Gel-AXF 2:1	10	0	29.405	28.065	31.137
AXF Pct Loss Gel-AXF 4:1	10	0	18.385	17.260	19.422

H = 25.806 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
GEL-AXF 1:1 Pct Loss vs GEL-AXF 4:1 Pct Loss	200.000	7.184	Yes
GEL-AXF 1:1 Pct Loss vs GEL-AXF 2:1 Pct Loss	100.000	3.592	Yes
GEL-AXF 2:1 Pct Loss vs GEL-AXF 4:1 Pct Loss	100.000	3.592	Yes

Drug Release Kinetics

One Way Analysis of Variance Wednesday, March 25, 2015, 3:24:16 PM

Data source: Data 1 in Drug Release Study March

Normality Test (Shapiro-Wilk) Passed (P = 0.475)

Equal Variance Test: Passed (P = 0.159)

Group Name	N	Missing	Mean	Std Dev	SEM
1:1 Gel-AXF 1 h	6	0	5.045	1.727	0.705

2:1 Gel-AXF 1 h	6	0	4.103	0.807	0.329
4:1 Gel-AXF 1 h	6	0	2.193	0.450	0.184

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	25.340	12.670	9.914	0.002
Residual	15	19.170	1.278		
Total	17	44.510			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.002).

Power of performed test with alpha = 0.050: 0.944

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
1:1 Gel-AXF vs. 4:1 Gel-AXF	2.852	4.370	0.002	Yes
2:1 Gel-AXF vs. 4:1 Gel-AXF	1.910	2.926	0.021	Yes
1:1 Gel-AXF vs. 2:1 Gel-AXF	0.942	1.443	0.170	No

One Way Analysis of Variance Wednesday, March 25, 2015, 3:34:35 PM

Data source: Data 1 in Drug Release Study March

Normality Test (Shapiro-Wilk) Passed (P = 0.889)

Equal Variance Test: Passed (P = 0.126)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
1:1 Gel-AXF 2 h	6	0	8.495	2.259	0.922
2:1 Gel-AXF 2 h	6	0	7.291	1.120	0.457
4:1 Gel-AXF 2 h	6	0	4.186	0.773	0.316

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	2	59.316	29.658	12.792	<0.001
Residual	15	34.778	2.319		
Total	17	94.094			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 0.986

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	**Diff of Means**	**t**	**P**	**P<0.050**
1:1 Gel-AXF vs. 4:1 Gel-AXF	4.309	4.901	<0.001	Yes
2:1 Gel-AXF vs. 4:1 Gel-AXF	3.105	3.532	0.006	Yes
1:1 Gel-AXF vs. 2:1 Gel-AXF	1.204	1.369	0.191	No

One Way Analysis of Variance Wednesday, March 25, 2015, 3:35:09 PM

Data source: Data 1 in Drug Release Study March

Normality Test (Shapiro-Wilk) Passed (P = 0.889)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, March 25, 2015, 3:35:09 PM

Data source: Data 1 in Drug Release Study March

Group	N	Missing	Median	25%	75%
1:1 Gel-AXF 6 h	6	0	12.564	8.273	15.215
2:1 Gel-AXF 6 h	6	0	8.837	8.045	10.733
4:1 Gel-AXF 6 h	6	0	6.338	4.648	7.351

H = 10.889 with 2 degrees of freedom. (P = 0.004)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.004)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
1:1 Gel-AXF 6 vs 4:1 Gel-AXF 6	59.000	4.512	Yes
1:1 Gel-AXF 6 vs 2:1 Gel-AXF 6	16.000	1.224	No
2:1 Gel-AXF 6 vs 4:1 Gel-AXF 6	43.000	3.288	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Wednesday, March 25, 2015, 3:35:41 PM

Data source: Data 1 in Drug Release Study March

Normality Test (Shapiro-Wilk) Passed (P = 0.671)

Equal Variance Test: Passed (P = 0.332)

Group Name	N	Missing	Mean	Std Dev	SEM
1:1 Gel-AXF 12 h	6	0	14.541	4.084	1.667
2:1 Gel-AXF 12 h	6	0	10.978	2.320	0.947

4:1 Gel-AXF 12 h	6	0	7.832	1.600	0.653

Source of Variation	DF	SS	MS	F	P
Between Groups	2	135.204	67.602	8.236	0.004
Residual	15	123.123	8.208		
Total	17	258.326			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.004).

Power of performed test with alpha = 0.050: 0.885

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
1:1 Gel-AXF vs. 4:1 Gel-AXF	6.709	4.056	0.003	Yes
1:1 Gel-AXF vs. 2:1 Gel-AXF	3.563	2.154	0.093	No
2:1 Gel-AXF vs. 4:1 Gel-AXF	3.146	1.902	0.077	No

One Way Analysis of Variance Wednesday, March 25, 2015, 3:36:14 PM

Data source: Data 1 in Drug Release Study March

Normality Test (Shapiro-Wilk) Passed (P = 0.935)

Equal Variance Test: Passed (P = 0.338)

Group Name	N	Missing	Mean	Std Dev	SEM
1:1 Gel-AXF 24 h	6	0	17.283	5.332	2.177
2:1 Gel-AXF 24 h	6	0	13.488	4.211	1.719
4:1 Gel-AXF 24 h	6	0	9.487	1.847	0.754

Source of Variation	DF	SS	MS	F	P
Between Groups	2	182.413	91.207	5.520	0.016
Residual	15	247.849	16.523		
Total	17	430.262			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.016).

Power of performed test with alpha = 0.050: 0.680

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
1:1 Gel-AXF vs. 4:1 Gel-AXF	7.797	3.322	0.014	Yes
2:1 Gel-AXF vs. 4:1 Gel-AXF	4.001	1.705	0.206	No
1:1 Gel-AXF vs. 2:1 Gel-AXF	3.796	1.617	0.127	No

One Way Analysis of Variance Wednesday, March 25, 2015, 3:37:00 PM

Data source: Data 1 in Drug Release Study March

Normality Test (Shapiro-Wilk) Passed (P = 0.912)

Equal Variance Test: Passed (P = 0.057)

Group Name	N	Missing	Mean	Std Dev	SEM
1:1 Gel-AXF 48 h	6	0	20.894	7.882	3.218
2:1 Gel-AXF 48 h	6	0	16.965	6.977	2.848
4:1 Gel-AXF 48 h	6	0	10.596	1.957	0.799

Source of Variation	DF	SS	MS	F	P
Between Groups	2	324.125	162.062	4.241	0.035
Residual	15	573.210	38.214		
Total	17	897.335			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.035).

Power of performed test with alpha = 0.050: 0.523

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
1:1 Gel-AXF vs. 4:1 Gel-AXF	10.298	2.885	0.034	Yes
2:1 Gel-AXF vs. 4:1 Gel-AXF	6.370	1.785	0.180	No
1:1 Gel-AXF vs. 2:1 Gel-AXF	3.929	1.101	0.288	No

WST-1 Assay

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Thursday, June 18, 2015, 10:02:55 AM

Data source: Data 1 in wst compiled run

Normality Test (Shapiro-Wilk) Passed (P = 0.121)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Thursday, June 18, 2015, 10:02:55 AM

Data source: Data 1 in wst compiled run

Group	N	Missing	Median	25%	75%
1-1 0.05 mg/ml	8	0	1.035	1.023	1.142

1-1 0.5 mg/ml	8	0	1.430	1.188	1.533
1-1 5 mg/ml	8	0	1.615	1.363	1.730
1-1 25 mg/ml	8	0	2.325	1.635	2.598

H = 20.590 with 3 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	**Diff of Ranks**	**q**	**P<0.05**
1-1 25 mg/ml vs 1-1 0.05 mg/ml	162.000	6.106	Yes
1-1 25 mg/ml vs 1-1 0.5 mg/ml	103.000	3.882	Yes
1-1 25 mg/ml vs 1-1 5 mg/ml	51.000	1.922	No
1-1 5 mg/ml vs 1-1 0.05 mg/ml	111.000	4.183	Yes
1-1 5 mg/ml vs 1-1 0.5 mg/ml	52.000	1.960	No
1-1 0.5 mg/ml vs 1-1 0.05 mg/m	59.000	2.224	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Thursday, June 18, 2015, 10:04:05 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Thursday, June 18, 2015, 10:04:05 AM

Data source: Data 2 in wst compiled run

Group	**N**	**Missing**	**Median**	**25%**	**75%**
2-1 0.05 mg/ml	8	0	1.075	0.972	1.360
2-1 0.5 mg/ml	8	0	1.625	1.355	1.775
2-1 5 mg/ml	8	0	1.760	1.672	1.838
2-1 25 mg/ml	8	0	1.735	1.445	1.992

H = 13.778 with 3 degrees of freedom. (P = 0.003)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.003)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	**Diff of Ranks**	**q**	**P<0.05**
2-1 5 mg/ml vs 2-1 0.05 mg/ml	129.500	4.881	Yes

2-1 5 mg/ml vs 2-1 0.5 mg/ml	43.500	1.639	No
2-1 5 mg/ml vs 2-1 25 mg/ml	21.000	0.791	Do Not Test
2-1 25 mg/ml vs 2-1 0.05 mg/ml	108.500	4.089	Yes
2-1 25 mg/ml vs 2-1 0.5 mg/ml	22.500	0.848	Do Not Test
2-1 0.5 mg/ml vs 2-1 0.05 mg/m	86.000	3.241	No

Note: The multiple comparisons on ranks do not include an adjustment for ties.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison. For example, if you had four rank sums sorted in order, and found no significant difference between rank sums 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed rank sums is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the rank sums, even though one may appear to exist.

One Way Analysis of Variance Thursday, June 18, 2015, 10:04:52 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Passed (P = 0.985)

Equal Variance Test: Passed (P = 0.156)

Group Name	N	Missing	Mean	Std Dev	SEM
4-1 0.05 mg/ml	8	0	1.050	0.246	0.0869
4-1 0.5 mg/ml	8	0	1.365	0.270	0.0955
4-1 5 mg/ml	8	0	1.643	0.473	0.167
4-1 25 mg/ml	8	0	1.375	0.437	0.154

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1.409	0.470	3.430	0.030
Residual	28	3.834	0.137		
Total	31	5.243			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.030).

Power of performed test with alpha = 0.050: 0.538

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
4-1 5 mg/ml vs. 4-1 0.05 mg/ml	0.593	3.202	0.020	Yes
4-1 25 mg/ml vs. 4-1 0.05 mg/	0.325	1.757	0.376	No
4-1 0.5 mg/m vs. 4-1 0.05 mg/	0.315	1.702	0.343	No
4-1 5 mg/ml vs. 4-1 0.5 mg/ml	0.278	1.500	0.375	No
4-1 5 mg/ml vs. 4-1 25 mg/ml	0.268	1.446	0.293	No
4-1 25 mg/ml vs. 4-1 0.5 mg/ml	0.01000	0.0540	0.957	No

One Way Analysis of Variance Thursday, June 18, 2015, 10:05:58 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Passed (P = 0.066)

Equal Variance Test: Passed (P = 0.420)

Group Name	N	Missing	Mean	Std Dev	SEM
1-1 0.05 mg/ml	8	0	1.084	0.149	0.0528
2-1 0.05 mg/ml	8	0	1.130	0.216	0.0763
4-1 0.05 mg/ml	8	0	1.050	0.246	0.0869

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.0258	0.0129	0.299	0.745
Residual	21	0.906	0.0431		
Total	23	0.932			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.745).

Power of performed test with alpha = 0.050: 0.049

The power of the performed test (0.049) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Thursday, June 18, 2015, 10:06:40 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Passed (P = 0.588)

Equal Variance Test: Passed (P = 0.850)

Group Name	N	Missing	Mean	Std Dev	SEM
1-1 0.5 mg/ml	8	0	1.371	0.220	0.0779
2-1 0.5 mg/ml	8	0	1.603	0.229	0.0809
4-1 0.5 mg/ml	8	0	1.365	0.270	0.0955

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.293	0.147	2.527	0.104
Residual	21	1.218	0.0580		
Total	23	1.511			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.104).

Power of performed test with alpha = 0.050: 0.278

The power of the performed test (0.278) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Thursday, June 18, 2015, 10:07:12 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Passed (P = 0.573)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Thursday, June 18, 2015, 10:07:12 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
1-1 5 mg/ml	8	0	1.615	1.363	1.730
2-1 5 mg/ml	8	0	1.760	1.672	1.838
4-1 5 mg/ml	8	0	1.695	1.265	2.070

H = 2.117 with 2 degrees of freedom. (P = 0.347)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.347)

One Way Analysis of Variance Thursday, June 18, 2015, 10:07:54 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Passed (P = 0.637)

Equal Variance Test: Passed (P = 0.515)

Group Name	N	Missing	Mean	Std Dev	SEM
1-1 25 mg/ml	8	0	2.250	0.613	0.217
2-1 25 mg/ml	8	0	1.668	0.427	0.151
4-1 25 mg/ml	8	0	1.375	0.437	0.154

Source of Variation	DF	SS	MS	F	P
Between Groups	2	3.175	1.587	6.355	0.007
Residual	21	5.246	0.250		
Total	23	8.420			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.007).

Power of performed test with alpha = 0.050: 0.789

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
1-1 25 mg/ml vs. 4-1 25 mg/ml	0.875	3.501	0.006	Yes
1-1 25 mg/ml vs. 2-1 25 mg/ml	0.583	2.331	0.059	No
2-1 25 mg/ml vs. 4-1 25 mg/ml	0.292	1.170	0.255	No

t-test Thursday, June 18, 2015, 10:11:12 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:11:12 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
1-1 0.05 mg/ml	8	0	1.035	1.023	1.142
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 8.000

T = 92.000 n(small)= 8 n(big)= 8 P(est.)= 0.008 P(exact)= 0.010

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.010)

t-test Thursday, June 18, 2015, 10:11:34 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:11:34 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
1-1 0.5 mg/ml	8	0	1.430	1.188	1.533
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 8.000

T = 92.000 n(small)= 8 n(big)= 8 P(est.)= 0.008 P(exact)= 0.010

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.010)

t-test Thursday, June 18, 2015, 10:12:00 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:12:00 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
1-1 5 mg/ml	8	0	1.615	1.363	1.730
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 0.000

T = 100.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

t-test Thursday, June 18, 2015, 10:12:20 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:12:20 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
1-1 25 mg/ml	8	0	2.325	1.635	2.598
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 0.000

T = 100.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

t-test Thursday, June 18, 2015, 10:12:36 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:12:36 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
2-1 0.05 mg/ml	8	0	1.075	0.972	1.360
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 16.000

T = 84.000 n(small)= 8 n(big)= 8 P(est.)= 0.082 P(exact)= 0.105

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.105)

t-test Thursday, June 18, 2015, 10:13:24 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:13:24 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
2-1 0.5 mg/ml	8	0	1.625	1.355	1.775
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 0.000

T = 100.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

t-test Thursday, June 18, 2015, 10:13:52 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:13:52 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
2-1 5 mg/ml	8	0	1.760	1.672	1.838
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 0.000

T = 100.000 n(small)= 8 n(big)= 8 P(est.)= <0.001 P(exact)= <0.001

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

t-test Thursday, June 18, 2015, 10:14:41 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:14:41 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
2-1 25 mg/ml	8	0	1.735	1.445	1.992
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 8.000

T = 92.000 n(small)= 8 n(big)= 8 P(est.)= 0.008 P(exact)= 0.010

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.010)

t-test Thursday, June 18, 2015, 10:15:01 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:15:01 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
4-1 0.05 mg/ml	8	0	1.035	0.920	1.252
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 32.000

T = 68.000 n(small)= 8 n(big)= 8 P(est.)= 1.000 P(exact)= 1.000

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 1.000)

t-test Thursday, June 18, 2015, 10:15:20 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:15:20 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
4-1 0.5 mg/ml	8	0	1.355	1.100	1.580
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 8.000

T = 92.000 n(small)= 8 n(big)= 8 P(est.)= 0.008 P(exact)= 0.010

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.010)

t-test Thursday, June 18, 2015, 10:15:43 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Passed (P = 0.062)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:15:43 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
4-1 5 mg/ml	8	0	1.695	1.265	2.070
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 8.000

T = 92.000 n(small)= 8 n(big)= 8 P(est.)= 0.008 P(exact)= 0.010

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = 0.010)

t-test Thursday, June 18, 2015, 10:16:13 AM

Data source: Data 2 in wst compiled run

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Thursday, June 18, 2015, 10:16:13 AM

Data source: Data 2 in wst compiled run

Group	N	Missing	Median	25%	75%
4-1 25 mg/ml	8	0	1.250	1.018	1.667
Normalized Control	8	0	1.000	1.000	1.000

Mann-Whitney U Statistic= 16.000

T = 84.000 n(small)= 8 n(big)= 8 P(est.)= 0.082 P(exact)= 0.105

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.105)

Trypan Blue Assay: Cell Viability

One Way Analysis of Variance Wednesday, June 10, 2015, 12:39:14 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.862)

Equal Variance Test: Passed (P = 0.608)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 0 mg/ml	3	0	97.800	0.557	0.321
Gel-AXF 1:1 blend 0.05 mg/ml	3	0	95.133	2.937	1.695

Gel-AXF 1:1 blend 0.5 mg/ml	3	0	98.600	1.389	0.802
Gel-AXF 1:1 blend 5 mg/ml	3	0	97.767	2.223	1.284
Gel-AXF 1:1 blend 25 mg/ml	3	0	94.467	2.173	1.255

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	4	40.157	10.039	2.445	0.115
Residual	10	41.060	4.106		
Total	14	81.217			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.115).

Power of performed test with alpha = 0.050: 0.296

The power of the performed test (0.296) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:39:50 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.742)

Equal Variance Test: Passed (P = 0.396)

Group Name	**N**	**Missing**	**Mean**	**Std Dev**	**SEM**
Gel-AXF 2:1 blend 0 mg/ml	3	0	97.800	0.557	0.321
Gel-AXF 2:1 blend 0.05 mg/ml	3	0	98.367	0.950	0.549
Gel-AXF 2:1 blend 0.5 mg/ml	3	0	97.500	0.721	0.416
Gel-AXF 2:1 blend 5 mg/ml	3	0	97.100	1.873	1.082
Gel-AXF 2:1 blend 25 mg/ml	3	0	97.200	0.854	0.493

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Between Groups	4	3.143	0.786	0.658	0.635
Residual	10	11.947	1.195		
Total	14	15.089			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.635).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:40:27 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.618)

Equal Variance Test: Passed (P = 0.312)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 4:1 blend 0 mg/ml	3	0	97.800	0.557	0.321
Gel-AXF 4:1 blend 0.05 mg/ml	3	0	94.633	3.800	2.194
Gel-AXF 4:1 blend 0.5 mg/ml	3	0	96.200	2.553	1.474
Gel-AXF 4:1 blend 5 mg/ml	3	0	97.733	1.266	0.731
Gel-AXF 4:1 blend 25 mg/ml	3	0	97.100	3.143	1.815

Source of Variation	DF	SS	MS	F	P
Between Groups	4	20.876	5.219	0.797	0.554
Residual	10	65.513	6.551		
Total	14	86.389			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.554).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:42:20 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.811)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 12:42:20 PM

Data source: Raw Data in 6_9_15 compiled data

Group	N	Missing	Median	25%	75%
Gel-AXF 1:1 blend 0.05 mg/ml	3	0	94.600	92.500	98.300
Gel-AXF 2:1 blend 0.05 mg/ml	3	0	98.400	97.400	99.300
Gel-AXF 4:1 blend 0.05 mg/ml	3	0	94.700	90.800	98.400

H = 2.891 with 2 degrees of freedom. P(est.)= 0.236 P(exact)= 0.254

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.254)

One Way Analysis of Variance Wednesday, June 10, 2015, 12:43:12 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.502)

Equal Variance Test: Passed (P = 0.397)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 0.5 mg/ml	3	0	98.600	1.389	0.802
Gel-AXF 2:1 blend 0.5 mg/ml	3	0	97.500	0.721	0.416
Gel-AXF 4:1 blend 0.5 mg/ml	3	0	96.200	2.553	1.474

Source of Variation	DF	SS	MS	F	P
Between Groups	2	8.660	4.330	1.448	0.307
Residual	6	17.940	2.990		
Total	8	26.600			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.307).

Power of performed test with alpha = 0.050: 0.094

The power of the performed test (0.094) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:43:54 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.084)

Equal Variance Test: Passed (P = 0.913)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 5 mg/ml	3	0	97.767	2.223	1.284
Gel-AXF 2:1 blend 5 mg/ml	3	0	97.100	1.873	1.082
Gel-AXF 4:1 blend 5 mg/ml	3	0	97.733	1.266	0.731

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.847	0.423	0.126	0.884
Residual	6	20.113	3.352		
Total	8	20.960			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.884).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:44:41 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.701)

Equal Variance Test: Passed (P = 0.570)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 25 mg/ml	3	0	94.467	2.173	1.255
Gel-AXF 2:1 blend 25 mg/ml	3	0	97.200	0.854	0.493
Gel-AXF 4:1 blend 25 mg/ml	3	0	97.100	3.143	1.815

Source of Variation	DF	SS	MS	F	P
Between Groups	2	14.416	7.208	1.410	0.315
Residual	6	30.667	5.111		
Total	8	45.082			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.315).

Power of performed test with alpha = 0.050: 0.090

The power of the performed test (0.090) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Trypan Blue Assay: Cell Proliferation

One Way Analysis of Variance Wednesday, June 10, 2015, 12:45:25 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.900)

Equal Variance Test: Passed (P = 0.370)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 0 mg/ml	3	0	101266.667	25562.733	14758.651
Gel-AXF 1:1 blend 0.05 mg/ml	3	0	116133.333	32751.997	18909.375
Gel-AXF 1:1 blend 0.5 mg/ml	3	0	131933.333	21822.313	12599.118
Gel-AXF 1:1 blend 5 mg/ml	3	0	141933.333	71978.423	41556.762
Gel-AXF 1:1 blend 25 mg/ml	3	0	125400.000	53556.325	30920.759

Source of Variation	DF	SS	MS	F	P
Between Groups	4	2888906666.667	722226666.667	0.352	0.837
Residual	10	20503066666.667	2050306666.667		
Total	14	23391973333.333			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.837).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:46:07 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.631)

Equal Variance Test: Passed (P = 0.487)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 2:1 blend 0 mg/ml	3	0	101266.667	25562.733	14758.651
Gel-AXF 2:1 blend 0.05 mg/ml	3	0	126133.333	53787.111	31054.003
Gel-AXF 2:1 blend 0.5 mg/ml	3	0	134333.333	35947.369	20754.223
Gel-AXF 2:1 blend 5 mg/ml	3	0	154933.333	15738.276	9086.498
Gel-AXF 2:1 blend 25 mg/ml	3	0	73533.333	27943.753	16133.333

Source of Variation	DF	SS	MS	F	P
Between Groups	4	11862842666.667	2965710666.667	2.527	0.107
Residual	10	11734533333.333	1173453333.333		
Total	14	23597376000.000			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.107).

Power of performed test with alpha = 0.050: 0.312

The power of the performed test (0.312) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:46:52 PM

Data source: Raw Data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.062)

Equal Variance Test: Passed (P = 0.986)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 4:1 blend 0 mg/ml	3	0	101266.667	25562.733	14758.651
Gel-AXF 4:1 blend 0.05 mg/ml	3	0	178066.667	34368.784	19842.827
Gel-AXF 4:1 blend 0.5 mg/ml	3	0	116066.667	30675.289	17710.386
Gel-AXF 4:1 blend 5 mg/ml	3	0	151266.667	25738.169	14859.939
Gel-AXF 4:1 blend 25 mg/ml	3	0	110266.667	29342.347	16940.812

Source of Variation	DF	SS	MS	F	P
Between Groups	4	12486624000.000	3121656000.000	3.631	0.045
Residual	10	8598133333.333	859813333.333		
Total	14	21084757333.333			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.045).

Power of performed test with alpha = 0.050: 0.518

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
Gel-AXF 4:1 vs. Gel-AXF 4:1	76800.000	3.208	0.090	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	67800.000	2.832	0.149	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	62000.000	2.590	0.196	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	50000.000	2.088	0.367	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	41000.000	1.712	0.528	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	35200.000	1.470	0.611	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	26800.000	1.119	0.745	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	14800.000	0.618	0.909	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	9000.000	0.376	0.919	No
Gel-AXF 4:1 vs. Gel-AXF 4:1	5800.000	0.242	0.813	No

One Way Analysis of Variance Wednesday, June 10, 2015, 12:49:48 PM

Data source: raw data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.506)

Equal Variance Test: Passed (P = 0.804)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 0.05 mg/ml	3	0	116133.333	32751.997	18909.375
Gel-AXF 2:1 blend 0.05 mg/ml	3	0	126133.333	53787.111	31054.003
Gel-AXF 4:1 blend 0.05 mg/ml	3	0	178066.667	34368.784	19842.827

Source of Variation	DF	SS	MS	F	P
Between Groups	2	6632808888.889	3316404444.444	1.933	0.225
Residual	6	10293920000.000	1715653333.333		
Total	8	16926728888.889			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.225).

Power of performed test with alpha = 0.050: 0.144

The power of the performed test (0.144) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:50:29 PM

Data source: raw data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.627)

Equal Variance Test: Passed (P = 0.825)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 0.5 mg/ml	3	0	131933.333	21822.313	12599.118
Gel-AXF 2:1 blend 0.5 mg/ml	3	0	134333.333	35947.369	20754.223
Gel-AXF 4:1 blend 0.5 mg/ml	3	0	116066.667	30675.289	17710.386

Source of Variation	DF	SS	MS	F	P
Between Groups	2	591182222.222	295591111.111	0.327	0.733
Residual	6	5418800000.000	903133333.333		
Total	8	6009982222.222			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.733).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:51:01 PM

Data source: raw data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.654)

Equal Variance Test: Passed (P = 0.124)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 5 mg/ml	3	0	141933.333	71978.423	41556.762
Gel-AXF 2:1 blend 5 mg/ml	3	0	154933.333	15738.276	9086.498
Gel-AXF 4:1 blend 5 mg/ml	3	0	151266.667	25738.169	14859.939

Source of Variation	DF	SS	MS	F	P
Between Groups	2	269555555.556	134777777.778	0.0664	0.936
Residual	6	12182080000.000	2030346666.667		
Total	8	12451635555.556			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.936).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 12:51:53 PM

Data source: raw data in 6_9_15 compiled data

Normality Test (Shapiro-Wilk) Passed (P = 0.607)

Equal Variance Test: Passed (P = 0.405)

Group Name	N	Missing	Mean	Std Dev	SEM
Gel-AXF 1:1 blend 25 mg/ml	3	0	125400.000	53556.325	30920.759
Gel-AXF 2:1 blend 25 mg/ml	3	0	73533.333	27943.753	16133.333
Gel-AXF 4:1 blend 25 mg/ml	3	0	110266.667	29342.347	16940.812

Source of Variation	DF	SS	MS	F	P
Between Groups	2	4268506666.667	2134253333.333	1.420	0.313
Residual	6	9020213333.333	1503368888.889		
Total	8	13288720000.000			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.313).

Power of performed test with alpha = 0.050: 0.091

The power of the performed test (0.091) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Kirby-Bauer Growth Inhibition Assay

One Way Analysis of Variance Friday, July 17, 2015, 2:28:18 PM

Data source: P. aeruginosa in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.958)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, July 17, 2015, 2:28:18 PM

Data source: P. aeruginosa in Notebook1

Group	N	Missing	Median	25%	75%
Positive Control	2	0	15.375	15.000	15.750
4:1 GEL-AXF	2	0	16.240	15.230	17.250
Negative Control	2	0	0.000	0.000	0.000

H = 3.824 with 2 degrees of freedom. P(est.)= 0.148 P(exact)= 0.200

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.200)

One Way Analysis of Variance Friday, July 17, 2015, 2:29:03 PM

Data source: S. aureus in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.773)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, July 17, 2015, 2:29:03 PM

Data source: S. aureus in Notebook1

Group	N	Missing	Median	25%	75%
Positive Control	2	0	23.830	23.440	24.220
4:1 GEL-AXF	2	0	9.510	9.250	9.770
Negative Control	2	0	0.000	0.000	0.000

H = 4.706 with 2 degrees of freedom. P(est.)= 0.095 P(exact)= 0.067

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.067)

One Way Analysis of Variance Friday, July 17, 2015, 2:29:38 PM

Data source: E. faecalis in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.954)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, July 17, 2015, 2:29:38 PM

Data source: E. faecalis in Notebook1

Group	N	Missing	Median	25%	75%
Positive Control	2	0	28.250	27.500	29.000
4:1 GEL-AXF	2	0	9.385	9.000	9.770
Negative Control	2	0	0.000	0.000	0.000

H = 4.706 with 2 degrees of freedom. P(est.)= 0.095 P(exact)= 0.067

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.067)

Appendix B – Statistical analysis: Arabinoxylan foams for wound healing applications

Rheometry

One Way Analysis of Variance Friday, December 05, 2014, 12:40:39 PM

Data source: Data 1 in Rheometry

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, December 05, 2014, 12:40:39 PM

Data source: Data 1 in Rheometry

Group	N	Missing	Median	25%	75%
3M Tegaderm Alginate at 25 deg	16	0	3030305.500	2809754.750	3276940.250
AXF foam at 25 degrees Cels	16	0	41721206.500	36002237.500	47338803.500

H = 23.273 with 1 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
AXF foam vs 3M Tegaderm	256.000	6.822	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Friday, December 05, 2014, 12:56:54 PM

Data source: Data 1 in Rheometry

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, December 05, 2014, 12:56:54 PM

Data source: Data 1 in Rheometry

Group	N	Missing	Median	25%	75%
3M Tegaderm Alginate at 37 deg	16	0	2933280.500	2704148.750	3154513.000
AXF foam at 37 degrees Cels	16	0	36400194.000	30817059.500	41458269.250

H = 23.273 with 1 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
AXF foam vs 3M Tegaderm	256.000	6.822	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Friday, December 05, 2014, 12:59:51 PM

Data source: Data 2 in Rheometry

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, December 05, 2014, 12:59:51 PM

Data source: Data 2 in Rheometry

Group	N	Missing	Median	25%	75%
3M Tegaderm Alginate at 25 deg	16	0	526542.000	368287.275	751232.925
AXF foam at 25 degrees Cels	16	0	7478.929	6015.412	48108.995

H = 18.138 with 1 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
3M Tegaderm vs AXF foam	226.000	6.023	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Friday, December 05, 2014, 1:00:24 PM

Data source: Data 2 in Rheometry

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, December 05, 2014, 1:00:24 PM

Data source: Data 2 in Rheometry

Group	N	Missing	Median	25%	75%
3M Tegaderm Alginate at 37 deg	16	0	486225.800	341389.825	691757.200
AXF foam at 37 degrees Cels	16	0	7075.829	5940.793	48064.872

H = 17.501 with 1 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
3M Tegaderm A vs AXF foam	222.000	5.916	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Porosity

One Way Analysis of Variance Friday, December 05, 2014, 1:25:17 PM

Data source: Data 1 in Porosity

Normality Test (Shapiro-Wilk) Passed (P = 0.195)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, December 05, 2014, 1:25:17 PM

Data source: Data 1 in Porosity

Group	N	Missing	Median	25%	75%
Tegaderm 3M Alginate	8	0	1.000	1.000	1.000
AXF foam	8	0	1.000	1.000	1.000

H = 3.579 with 1 degrees of freedom. P(est.)= 0.059 P(exact)= 0.065

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.065)

Swelling

One Way Analysis of Variance Friday, December 05, 2014, 1:04:39 PM

Data source: Copy of Data 1 in Swelling_revised

Normality Test (Shapiro-Wilk) Passed (P = 0.407)

Equal Variance Test: Passed (P = 0.877)

Group Name	N	Missing	Mean	Std Dev	SEM
3M Tegaderm at 25 degrees Cels	8	0	1316.851	303.442	107.283
AXF foam at 25 degrees Cels	8	0	1848.899	257.950	91.199

Source of Variation	DF	SS	MS	F	P
Between Groups	1	1132300.537	1132300.537	14.277	0.002
Residual	14	1110306.534	79307.610		
Total	15	2242607.070			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.002).

Power of performed test with alpha = 0.050: 0.936

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

Comparison	Diff of Means	t	P	P<0.050
AXF foam vs. 3M Tegaderm	532.048	3.779	0.002	Yes

One Way Analysis of Variance Friday, December 05, 2014, 1:06:02 PM

Data source: Data 1 in Swelling_revised

Normality Test (Shapiro-Wilk) Passed (P = 0.440)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, December 05, 2014, 1:06:02 PM

Data source: Data 1 in Swelling_revised

Group	N	Missing	Median	25%	75%
3M Tegaderm at 37 degrees Cels	8	0	1017.442	960.002	1036.382
AXF foam at 37 degrees Cels	8	0	1672.819	1533.197	1879.197

H = 11.294 with 1 degrees of freedom. P(est.)= <0.001 P(exact)= <0.001

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
AXF foam vs 3M Tegaderm	64.000	4.753	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

LAL Endotoxin Assay

t-test Wednesday, July 01, 2015, 1:37:57 PM

Data source: Data 1 in Notebook1

Normality Test (Shapiro-Wilk) Passed (P = 0.655)

Equal Variance Test: Passed (P = 0.573)

Group Name	N	Missing	Mean	Std Dev	SEM
Unsterilized AXF	8	0	5.301	1.099	0.388
Sterilized AXF	8	0	3.416	0.871	0.308

Difference 1.885

t = 3.803 with 14 degrees of freedom. (P = 0.002)

95 percent confidence interval for difference of means: 0.822 to 2.948

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.002).

Power of performed test with alpha = 0.050: 0.940

Trypan Blue: Fibroblast Cell Viability

One Way Analysis of Variance Wednesday, June 10, 2015, 1:06:55 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:06:55 PM

Data source: Data 2 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
24 hr AXF	8	0	97.750	94.950	98.850
24 hr 3M Teg	8	0	97.350	94.650	98.175
24 hr None	8	0	96.300	94.450	97.550

H = 2.008 with 2 degrees of freedom. (P = 0.366)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.366)

One Way Analysis of Variance Wednesday, June 10, 2015, 1:07:17 PM

Data source: Data 1 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:07:17 PM

Data source: Data 1 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
72 hr AXF	8	0	97.100	96.025	97.575
72 hr 3M Teg	8	0	97.500	97.050	97.750
72 hr None	8	0	97.300	96.550	97.675

H = 0.925 with 2 degrees of freedom. (P = 0.630)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.630)

One Way Analysis of Variance Wednesday, June 10, 2015, 1:07:51 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:07:51 PM

Data source: Data 2 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
24 hr AXF	8	0	97.750	94.950	98.850
72 hr AXF	8	0	97.100	96.025	97.575

H = 0.799 with 1 degrees of freedom. P(est.)= 0.371 P(exact)= 0.382

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.382)

One Way Analysis of Variance Wednesday, June 10, 2015, 1:08:17 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:08:17 PM

Data source: Data 2 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
24 hr 3M Teg	8	0	97.350	94.650	98.175
72 hr 3M Teg	8	0	97.500	97.050	97.750

H = 0.0249 with 1 degrees of freedom. P(est.)= 0.875 P(exact)= 0.878

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.878)

One Way Analysis of Variance Wednesday, June 10, 2015, 1:08:49 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Passed (P = 0.057)

Equal Variance Test: Passed (P = 0.343)

Group Name	N	Missing	Mean	Std Dev	SEM

24 hr None	8	0	95.787	1.919	0.678
72 hr None	8	0	96.925	1.335	0.472

Source of Variation	DF	SS	MS	F	P
Between Groups	1	5.176	5.176	1.895	0.190
Residual	14	38.244	2.732		
Total	15	43.419			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.190).

Power of performed test with alpha = 0.050: 0.135

The power of the performed test (0.135) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

Trypan Blue: Fibroblast Cell Proliferation

One Way Analysis of Variance Wednesday, June 10, 2015, 1:11:41 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:11:41 PM

Data source: Data 2 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
0 hr AXF	8	0	50000.000	50000.000	50000.000
0 hr 3M Teg	8	0	50000.000	50000.000	50000.000
0 hr None	8	0	50000.000	50000.000	50000.000

H = 0.000 with 2 degrees of freedom. (P = 1.000)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 1.000)

One Way Analysis of Variance Wednesday, June 10, 2015, 1:12:08 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Passed (P = 0.192)

Equal Variance Test: Passed (P = 0.687)

Group Name	N	Missing	Mean	Std Dev	SEM
24 hr AXF	8	0	166350.000	56132.369	19845.789

24 hr 3M Teg	8	0	128825.000	79448.056	28089.130
24 hr None	8	0	205350.000	75518.380	26699.779

Source of Variation	DF	SS	MS	F	P
Between Groups	2	23427203333.333	11713601666.667	2.317	0.123
Residual	21	106161035000.000	5055287380.952		
Total	23	129588238333.333			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.123).

Power of performed test with alpha = 0.050: 0.244

The power of the performed test (0.244) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 1:12:34 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Passed (P = 0.293)

Equal Variance Test: Passed (P = 0.250)

Group Name	N	Missing	Mean	Std Dev	SEM
72 hr AXF	8	0	212025.000	47514.141	16798.786
72 hr 3M Teg	8	0	302000.000	76911.823	27192.436
72 hr None	8	0	288718.750	108982.272	38531.052

Source of Variation	DF	SS	MS	F	P
Between Groups	2	37743529375.000	18871764687.500	2.824	0.082
Residual	21	140351104687.500	6683385937.500		
Total	23	178094634062.500			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.082).

Power of performed test with alpha = 0.050: 0.327

The power of the performed test (0.327) is below the desired power of 0.800.
Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously.

One Way Analysis of Variance Wednesday, June 10, 2015, 1:13:03 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Passed (P = 0.257)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:13:03 PM

Data source: Data 2 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
0 hr AXF	8	0	50000.000	50000.000	50000.000
24 hr AXF	8	0	161900.000	111000.000	205500.000
72 hr AXF	8	0	220000.000	163700.000	241000.000

H = 17.041 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
72 hr AXF vs 0 hr AXF	110.500	5.525	Yes
72 hr AXF vs 24 hr AXF	29.000	1.450	No
24 hr AXF vs 0 hr AXF	81.500	4.075	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Wednesday, June 10, 2015, 1:14:25 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:14:25 PM

Data source: Data 2 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
0 hr 3M Teg	8	0	50000.000	50000.000	50000.000
24 hr 3M Teg	8	0	94500.000	65250.000	193300.000
72 hr 3M Teg	8	0	287000.000	254000.000	357000.000

H = 19.725 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
72 hr 3M Teg vs 0 hr 3M Teg	123.000	6.150	Yes
72 hr 3M Teg vs 24 hr 3M Teg	54.000	2.700	No
24 hr 3M Teg vs 0 hr 3M Teg	69.000	3.450	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

One Way Analysis of Variance Wednesday, June 10, 2015, 1:15:27 PM

Data source: Data 2 in Compiled Data 6_8

Normality Test (Shapiro-Wilk) Passed (P = 0.110)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Wednesday, June 10, 2015, 1:15:27 PM

Data source: Data 2 in Compiled Data 6_8

Group	N	Missing	Median	25%	75%
0 hr None	8	0	50000.000	50000.000	50000.000
24 hr None	8	0	201700.000	140150.000	265500.000
72 hr None	8	0	279575.000	186000.000	394500.000

H = 17.197 with 2 degrees of freedom. (P = <0.001)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
72 hr None vs 0 hr None	111.500	5.575	Yes
72 hr None vs 24 hr None	31.000	1.550	No
24 hr None vs 0 hr None	80.500	4.025	Yes

Note: The multiple comparisons on ranks do not include an adjustment for ties.

Kirby-Bauer Growth Inhibition Assay

One Way Analysis of Variance Friday, July 17, 2015, 2:39:33 PM

Data source: P. aeruginosa in Growth Inhibition data.JNB

Normality Test (Shapiro-Wilk) Passed (P = 0.897)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, July 17, 2015, 2:39:33 PM

Data source: P. aeruginosa in Growth Inhibition data.JNB

Group	N	Missing	Median	25%	75%
Positive Control	2	0	14.375	10.500	18.250
AXF Foam	2	0	22.170	21.090	23.250
3M Alginate	2	0	10.040	8.750	11.330
Negative Control	2	0	0.000	0.000	0.000

H = 6.241 with 3 degrees of freedom. P(est.)= 0.100 P(exact)= 0.038

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.038)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
AXF Foam vs Negative Control	12.000	3.464	No
AXF Foam vs 3M Alginate	7.000	2.021	Do Not Test
AXF Foam vs Positive Control	5.000	1.443	Do Not Test
Positive Cont vs Negative Cont	7.000	2.021	Do Not Test
Positive Cont vs 3M Alginate	2.000	0.577	Do Not Test
3M Alginate vs Negative Cont	5.000	1.443	Do Not Test

Note: The multiple comparisons on ranks do not include an adjustment for ties.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison. For example, if you had four rank sums sorted in order, and found no significant difference between rank sums 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed rank sums is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the rank sums, even though one may appear to exist.

One Way Analysis of Variance Friday, July 17, 2015, 2:40:49 PM

Data source: S. aureus in Growth Inhibition data.JNB

Normality Test (Shapiro-Wilk) Passed (P = 0.523)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, July 17, 2015, 2:40:49 PM

Data source: S. aureus in Growth Inhibition data.JNB

Group	N	Missing	Median	25%	75%
Positive Control	2	0	23.020	22.600	23.440
AXF Foam	2	0	12.390	11.500	13.280
3M Alginate	2	0	0.000	0.000	0.000
Negative Control	2	0	5.750	5.750	5.750

H = 6.829 with 3 degrees of freedom. P(est.)= 0.078 P(exact)= 0.010

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.010)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Ranks	q	P<0.05
Positive Cont vs 3M Alginate	12.000	3.464	No
Positive Cont vs Negative Cont	8.000	2.309	Do Not Test
Positive Control vs AXF Foam	4.000	1.155	Do Not Test
AXF Foam vs 3M Alginate	8.000	2.309	Do Not Test
AXF Foam vs Negative Control	4.000	1.155	Do Not Test
Negative Cont vs 3M Alginate	4.000	1.155	Do Not Test

Note: The multiple comparisons on ranks do not include an adjustment for ties.

A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison. For example, if you had four rank sums sorted in order, and found no significant difference between rank sums 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1 (4 vs. 3 and 3 vs. 2 are enclosed by 4 vs. 2: 4 3 2 1). Note that not testing the enclosed rank sums is a procedural rule, and a result of Do Not Test should be treated as if there is no significant difference between the rank sums, even though one may appear to exist.

One Way Analysis of Variance Friday, July 17, 2015, 2:41:10 PM

Data source: E. faecalis in Growth Inhibition data.JNB

Normality Test (Shapiro-Wilk) Passed (P = 0.120)

Equal Variance Test: Failed (P < 0.050)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, July 17, 2015, 2:41:10 PM

Data source: E. faecalis in Growth Inhibition data.JNB

Group	N	Missing	Median	25%	75%
Positive Control	2	0	27.615	27.500	27.730
AXF Foam	2	0	12.320	11.750	12.890
3M Alginate	2	0	0.000	0.000	0.000
Negative Control	2	0	0.000	0.000	0.000

H = 6.811 with 3 degrees of freedom. P(est.)= 0.078 P(exact)= 0.067

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.067)

Appendix C – Statistical analysis: Electrospinning of PEGylated polyamidoamine dendrimer fibers

Morphology: Fiber Diameter

t-test Friday, December 20, 2013, 10:05:56 AM

Data source: Data 1 in fiber diameter_mPEG_G3.0

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, December 20, 2013, 10:05:56 AM

Data source: Data 1 in fiber diameter_mPEG_G3.0

Group	N	Missing	Median	25%	75%
Col 1	101	1	3.210	2.060	4.803
Col 2	101	1	3.235	2.518	4.500

Mann-Whitney U Statistic= 4552.500

T = 9602.500 n(small)= 100 n(big)= 100 (P = 0.275)

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.275)

Morphology: Pore Size

t-test Friday, December 20, 2013, 9:54:39 AM

Data source: Data 1 in Pore Size

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Friday, December 20, 2013, 9:54:39 AM

Data source: Data 1 in Pore Size

Group	N	Missing	Median	25%	75%
32:1 PEGylation	100	0	153.360	87.120	311.440
16:1 PEGylation	100	0	112.140	49.560	208.050

Mann-Whitney U Statistic= 3582.000

T = 11468.000 n(small)= 100 n(big)= 100 (P = <0.001)

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

VITA

Donald Chukwuemeka Aduba, Jr. was born in Maryville, MO on January 28, 1986 to Nigerian born immigrants. Don attended St. Francis Xavier grade school and Rockhurst High school in Kansas City, MO before moving to Virginia to attend college in 2004. In the fall 2004 semester, Don matriculated at the University of Virginia in Charlottesville, VA. He joined student organizations: the National Society of Black Engineers, PRIDE magazine & UVA Intramural-Recreational Sports as a participant and official. He also participated in undergraduate research in Dr. Cato Laurencin's orthopedics biomaterials laboratory in his third and fourth years. In Spring 2008, Don joined the Zeta Eta chapter Phi Beta Sigma Fraternity and served as Executive Vice President in the 2008-09 year after graduating in Summer 2008. Don received his Bachelor of Science in Sports Medicine with a minor in Biomedical Engineering. He took a year off to stay in Charlottesville to gain research experience under Dr. Roy Ogle before matriculating at Virginia Commonwealth University in Fall 2009 as a Master's student. Don started a research assistant under Dr. Hu Yang in January 2010 and received his Master of Science in Biomedical Engineering in Spring 2012. He continued under Dr. Yang's guidance as a doctoral student and as a selected Southern Regional Education Board scholar in Fall 2012, working on multiple projects while mentoring undergraduate students. In Summer 2014, Don was elected as President of the Engineering Graduate Student Association and helped organize events for graduate students in the 2014-15 academic year. Don will receive his doctorate in Biomedical Engineering in Summer 2015. His ultimate goals are to work in a national laboratory and teach as an adjunct faculty member.