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Electrospun Spiderskin Bandage for Epidermal Protection and Recovery

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ELECTROSPUN SPIDERSKIN BANDAGE FOR EPIDERMAL PROTECTION AND RECOVERY

by

Michael David Paskett

Thesis submitted in partial fulfillment
of the requirements for the degree

of

DEPARTMENTAL HONORS

in

Biological Engineering
in the Department of Biological Engineering

Approved:

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Abstract

Spider silk is one of nature's most promising biomaterials for a variety of applications, however, due to the inability to farm spiders, transgenic hosts are required for large-scale production. With the unique combination of strength, elasticity, and biocompatibility, spider silk has an incredible potential for use in the human body. This study was conducted to merge two major applications of spider silk for the creation of a novel bandaging product. Electrospinning technology was utilized to create a spider silk/polymer bandage matrix to be applied with an aqueous spider silk skin adhesive.

In designing the bandaging matrix, the mechanical properties of the electrospun silk were evaluated against commercially-available products and known values of human skin. The chosen formulation had physical properties more comparable human skin than commercially-available products. The aqueous adhesive was tested in conjunction with the electrospun matrix for its adhesion and found comparable to commercial products. The durability of the bandage was tested via cyclic stresses and found analogous to commercial products. The common antimicrobial chlorhexidine was incorporated into the adhesive and had a release profile lasting about 4 days. With this incorporation into the aqueous adhesive, the adhesive can be reapplied to provide additional antimicrobial protection, a necessity in the healthcare industry. The bandaging showed no signs of inhibiting mammalian cell proliferation under cytotoxicity testing.

The final product, deemed "SpiderSkin," presents a unique bandaging solution capable of providing a healthy environment for the regeneration of epidermal tissue, while protecting the wound from outside infection, and providing mechanical stability similar to that of human skin.

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Project Summary

The purpose of this project was to create a bandaging product by harnessing the mechanical properties of recombinant spider silk proteins (rSSps). Using an electrospinning device, the goal was to create an rSSps/polymer bandage matrix made of nanofibers that can be incorporated with an aqueous rSSps skin adhesive to create an ultra-thin "second skin" to act as a barrier of defense in the event of a skin injury. Furthermore, this bandage may promote healing by the elution of antimicrobials, growth factors, and other healing agents, while taking advantage of the biocompatibility of the rSSps. This product will be mechanically sound under varied mechanical manipulation as well as under different environmental conditions. The resulting electrospun bandage matrix and adhesive will allow for an innovative product to both protect and heal numerous skin injuries and will reduce the need for hospital staff to constantly change dressings (a painful process) since the components of this product will eventually degrade over time or simply peel off. Finally, the design of this product will provide a starting point for numerous future projects including the possibility for a mechanically-stable cell scaffold to regenerate a patient's skin in the event of major injury. The final product was named "SpiderSkin".

Introduction

The human body's first layer of protection against the elements, disease, and regulation of internal processes is the skin. To provide effective protection, the skin must be able to regenerate as quickly as it is damaged. Unfortunately, in cases of extreme injuries, such as burns or large abrasions, or cases of decreased blood flow, as in pressure wounds, natural repair mechanisms prove insufficient. These injuries are both widespread and costly. The American Burn association estimates over 486,000 burn injuries were treated in US burn centers in 2015 ("American Burn Association," n.d.). Approximately 2.5 million people are affected by pressure sores each year of which 159,000 are nursing home residents ("American Burn Association").

In the above circumstances where extensive damage to the skin occurs, an extra layer of defense, effectively a second layer of skin, is needed to maintain the health and wellness of affected individuals. This "second skin" must have similar properties to that of the original: permeability, flexibility, and protection against infection. The product may also serve as a scaffold for re-epithelialization.

The purpose of this study was to develop a second skin capable of providing the environment necessary for natural repair while protecting the skin from further damage. The product was evaluated through testing of mechanical properties and cellular interactions.

Significance and Innovation

Bandages protect wounds and promote healing by creating a moist environment that allows for natural repair without significant disruption. Problems in the wound care industry today are numerous, and there have been many efforts to combat these. The main problems that this study sought to solve are: the occurrence of skin maceration and contact dermatitis in the skin surrounding the wound (made worse by continual dressing changes), the ability to tune a bandage as the wound environment and needs change,

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and combatting the risk of infection in immunocompromised patients. The goal of this project was to create a bandaging process that will decrease the healing time and scarring of wounds like bed sores or burns, without increasing the damage to the skin during dressing changes and wound treatment.

Objectives

The main objective of this project is to create an rSSps electrospun "second skin" with an rSSps-based adhesive that has the ability to match or surpass the properties of skin, while being comparable to the mechanical properties of commercial products. Furthermore, this product will promote healing through the elution of antimicrobials. The following are specific objectives related to the electrospun mat, adhesive, and the mat adhesive complex:

- Use glutaraldehyde as a cross-linking agent to increase the mechanical properties of the adhesive.

- Ensure that the bandage (with and without glutaraldehyde) does not exhibit cytotoxicity.

- Create a mat/adhesive complex with and without cross-linking that stretches without removal in cyclic flexion on pigskin.

- Create a mat/adhesive complex with and without cross-linking that can be manipulated without removal in cyclic compression on pigskin.

- Create an rSSps antimicrobial adhesive using chlorhexidine and exhibit a release of the drug over time.

- Design a mat/adhesive complex that adheres to the skin as well as or better than commercial skin adhesives.

Evaluation Criteria

This project will be evaluated based on the following criteria:

- Test different electrospun mat formulations using different concentrations and combinations of rSSps. Test the workability of these mat combinations by evaluating spinning effectiveness, material availability, and a pressure-sensitive tape test.

- Evaluate rSSps adhesives with and without cross-linking by including these two groups in every mechanical adhesion test as well as an MTS tensile test for each complex.

- Perform SEM imaging to observe the interaction between mat/adhesive to form a complex.

- Perform cytotoxicity testing using both sets of adhesives (with and without glutaraldehyde).

- Complete cyclic MTS fatigue testing with the bandages in flexion under wet, dry, and humid conditions. Use a modified ASTM adhesion scale to evaluate results. Compare to controls Tegaderm and water-proof Band-Aids.

- Complete cyclic MTS testing with the bandage folding in compression under wet, dry, and humid conditions. Use a modified ASTM adhesion scale to evaluate results. Compare to controls Tegaderm and water-proof Band-Aids.

- Compare the mechanical properties of the bandaging product with literature on mechanical properties of human skin.

- Test the bandage's ability to elute chlorhexidine while completely immersed in phosphate-buffered saline solution with and without a cross-linked adhesive. UPLC analysis will be used to quantify the release kinetics.

- Complete MTS T-Peel adhesion testing with the rSSps bandages, Tegaderm, and water-proof Band-Aids.

Background

Injuries to the skin occur by various modes and severities. Two major markets in which bandaging improvements are needed include burns and pressure wounds (bedsores). This study was conducted in order to address specific needs for the treatment of burns, pressure wounds, and other serious health-threatening epithelial injuries.

Target Properties for Epithelial Wound Bandage

Epithelial wounds can be difficult to treat and therefore bandages targeted for these wounds must be capable of meeting several requirements. Among the most important properties to consider are moisture control, oxygen transfer, antimicrobial effect, biocompatibility, and mechanical attributes. The need for moisture control can vary by wound type and severity. The ability of a bandage to absorb secretions of the wound without causing excessive dryness is ideal. Oxygen transfer into the wound is also necessary for proper healing mechanisms to occur, and thus it would be required for the bandage to be semi-permeable. With a semi-permeable bandage, the risk of infection is heightened, and thus bandages must inhibit bacterial infection. The most efficient bandages would also assist in the proliferation of epithelial cells and act as a scaffold to facilitate cell regeneration. The mechanical properties of an ideal bandage would closely mirror that of natural skin.

Pressure Sores and Burns

Pressure sores are one of the fastest growing problems among nursing home residents in the U.S. In a 2004 study it was found that more than 1 in 10 nursing home residents had a pressure ulcer (Lee, Jeong, Kang, Lee, & Park, 2009). This number will continue to increase as the baby-boomer generation reaches retirement age and the nursing home population grows. This will result in a significant financial burden on the health care community and taxpayers. The annual cost for pressure ulcer management was $11 billion in 2006 (Moore & Cowman, 2013). Jointly, the need for effective treatment of pressure sores is going to increase.
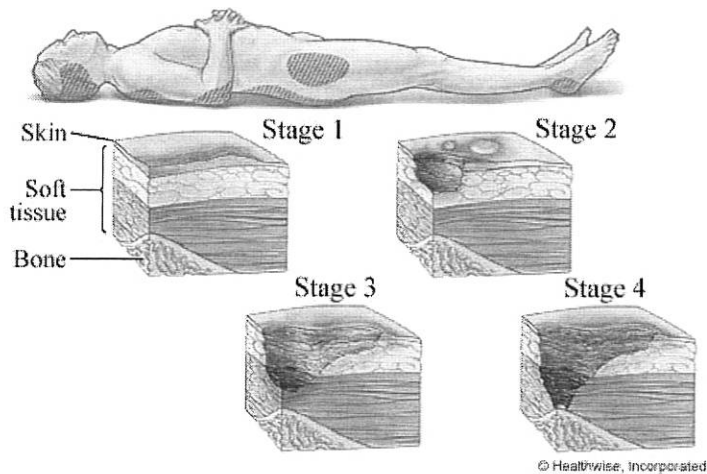
Figure 1. Pressure Wound Stages.

Figure 1 illustrates common areas for pressure wounds to develop. The affected areas are often under constant pressure from bedridden patients. Pressure wounds are most easily managed in Stages 1 and 2 before the wound penetrates to the underlying musculature and bone as in Stages 3 and 4.

Treatment of pressure sores often proves to be very difficult. It is necessary to maintain a healthy moisture balance in the wound. Wounds that release excessive moisture must be capable of draining the excess fluid, and dry wounds must produce a great enough barrier to retain needed moisture (O'Neil, 2004). With bed sores in particular it is important that the bandage is gentle on the surrounding skin to prevent maceration of the skin. As with any wound that breaches the skin, it is important to ensure that the wound does not become infected (Moore & Cowman, 2013). For this reason, antimicrobial bandages are the most advantageous. Finally, the cost of the bandage and how often it needs to be changed are important considerations for the patient and the medical industry.

The American Burn Association has estimated that 486,000 people per year receive treatment for burns at hospitals/burn centers. Costs associated with these burns total approximately $7.5 billion ("American Burn Association," n.d.). Burn treatment is difficult as contact with burn wounds is very painful. Bandaging must be able to cover a large surface area, protect against nosocomial infection, and provide an environment that facilitates cellular proliferation (Rowan et al., 2015). To prevent painful removal of the wound dressing, having a bandage that slowly degrades with time would also be an immense benefit to the patient.

Electrospun Bandages

Electrospinning is a process by which polar polymeric solutions (called dopes) are used to create nanofiber mats via high voltage application (Figure 2). Electrospinning began in 1934 and allowed the production of nanofibers using a high electoral charge from a syringe needle to a collector plate or drum. The ability to produce mats made up of fibers on the nanoscale offer a number of desirable properties. First is the high surface area of bandages produced by electrospinning. This can be key in encouraging hemostasis which can be vital to critical wounds (Karami, Rezaeian, Zahedi, & Abdollahi, 2013). This surface area to volume ratio also means that the bandages can be extremely absorptive. In the healing process, the body extrudes many liquids and the ability of a bandage to absorb this assists recovery. As

discussed earlier, bandages that allow a wound to breathe are more effective. Electrospun bandages are semi-permeable which means the wound will not dry up, but will also not be vulnerable to further bacterial infection. The fine fiber size of electrospinning also allows electrospun bandages to contour to any type of wound. The better a bandage can conform to a wound the more effective it will be and this could be achieved in a number of ways with electrospun bandages. For example, pre-spun bandages could contour well due to their fine fiber size or bandages could be electrospun directly onto a wound to achieve nearly perfect conformation. An electrospun bandage can use a wide variety of polymers and be loaded with substances such as antimicrobials and growth factors. Electrospun bandages also can easily be made bioactive by incorporating drugs into the fibers. Lastly, the bandages can act as a scaffolding which assists skin and veins in regrowth, leading to less scarring.
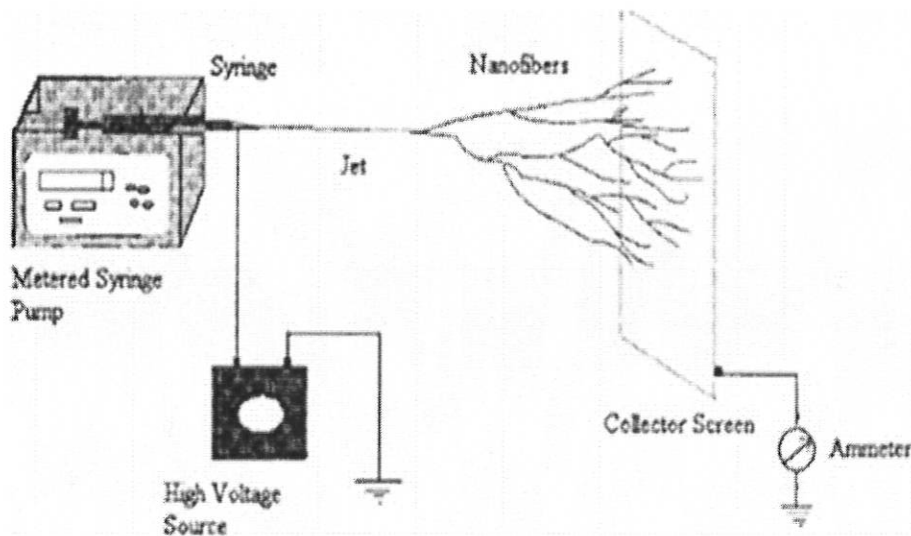
Figure 2. Illustration of electrospinning concepts.

Different polymers (specifically rSSps) will be used to design several applications for wound covering. Spider silk made up of biocompatible proteins known for their elasticity and strength (Lewis, 2006; Panilaitis et al., 2003). By using combinations of polymers, the physical characteristics of the bandage can be modified for specific applications/areas of the body. Dopes can be loaded with compounds before being electrospun. The effectiveness of antimicrobial therapy will be explored when loaded into the rSSps/polymer dopes.

Potential Applications of rSSps Bandage Materials

As a biopolymer, spider silk presents unique characteristics as compared to leading therapies. Spider silk is biocompatible and thus will not interfere with natural healing processes(Lewis, 2006). Antimicrobial compounds and growth factors can be incorporated into the electrospun bandage and/or adhesive to provide protection against infection and induce cellular regrowth. Moisture control can be managed by varying the thickness of the bandage as well as the amount of adhesive applied.

Mechanical properties of rSSps are such that the applied bandage would withstand continued stress without failure. rSSps can be used as both the bandage material as an electrospun mat, and as the adhesive to bind the mat to the wound. Electrospinning produces fibers in the nano-scale, enabling small

molecules, such as oxygen, to pass through with little resistance. Because of the high surface-area-to-volume ratio of nanofibers, bandages functionalized with antimicrobial compounds or growth factors will release quickly and efficiently into the wound (Maleki, Latifi, Amani-Tehran, & Mathur, 2013).

Current Epithelial Wound Treatment

Currently, there are two main classes of bandages. Passive bandages seek to cover a wound to allow the body to heal and recover underneath. They do little to help the body but prevent further infection of injury. A commonly used example is gauze. Passive bandages can inhibit healing because they do not allow air into the wound which can cause the wound to dry up. Bioactive bandages, on the other hand, seek to not only cover a wound but to allow it to breathe. They are more porous and allow water vapor and oxygen to the wound site which can help healing. Biological compounds can also be incorporated to affect the chemical environment of the body and help it to heal. rSSps electrospun bandages would be bioactive bandages which competes with current bandaging options.

Tegaderm is a commonly used product for pressure sores. This bandage boasts a number of advantageous properties. It is clear which enables the user to observe the wound without having to remove the bandage repeatedly. It is water and oxygen permeable allowing the wound to "breathe" naturally. The bandage prevents bacterial contamination into the wound but does not have any properties to treat existing bacterial infection. However, Tegaderm chlorhexidine gluconate (CHG) bandages have been shown to exhibit antibacterial properties.

Literature Review

Electrospun Bandaging for Wound Therapy

Wound dressings have significant potential for improvement as technologies advance in fabrication methods and available materials. Dressings have developed from natural materials designed for bio-inertness and have advanced to bioactive states, improving the healing process beyond the body's natural capabilities. Key players in this progression have been fabrication via electrospinning and the incorporation of anti-microbial and anti-inflammatory agents. The purpose of these newer dressings is to control the biochemical states of the wound.

In 2010 Zahedi et al. published a review on wound dressings with an emphasis on electrospun polymeric bandages (Zahedi, Rezaeian, Ranaei-Siadat, Jafari, & Supaphol, 2010). In this review, a large variety of polymeric compounds, such as alginates, cellulose, chitin, and hyaluronic acid have been extensively studied. A challenge with many of these polymers is their limited solubility (Lee et al., 2009).

From the studies in the above reviews, only a small variety of antimicrobial additives were used, the most common being ionic silver. The review found the release rate of the antimicrobials to be of supreme importance as the large surface area of electrospun fibrous mats causes an initial burst of antimicrobial release followed by a severely lessened steady release rate (Zahedi et al., 2010). In order to combat the initial burst, one group electrospun core-shell nanofibers with the antimicrobial compound inside of the polymer to reduce the initial burst. This method effectively reduced the initial burst release rate (Maleki et al., 2013).

In a novel study published in 2012, Arenbergerova et al. produced a nanofiber textile doped with a tetraphenylporphyrin (TPP) photosensitizer. When this compound is activated by visible light, it releases a reactive oxygen compound that inactivates bacteria at the surface (Arenbergerova, Arenberger, Bednar, Kubat, & Mosinger, 2012).

Several groups began experimenting with alternative biomaterials for preventing infection. In 2012 Karami et al. introduced thymol, an extract from thyme, into poly(e-caprolactone) (PCL), poly(lactic acid) (PLA) electrospun mats. The group found thymol effective against both gram-positive and gram-negative strains of bacteria. Wounds treated with thymol had a closure rate of 92.5% after 14 days (Karami et al., 2013).

In 2015 a novel aspect of bandaging was introduced with the use of zwitterionic nanofibers created to reduce cell adhesion to the dressing. Through their poly (carboxybetaine-co-methyl methacrylate) copolymer, the group demonstrated that blood cells did not attach to the membrane and thus would not cause clotting at the wound site (Unnithan et al., 2016).

Recent studies have had a large focus on anti-microbial additives, as anti-bacterial resistance and nosocomial infection is becoming an ever greater issue in treatment. The other main area of focus is using polymers that will have favorable reactions with living tissue. Arenbergerova presented a novel anti-microbial product; however, a light requirement could be problematic in situations where the patient has limited mobility or the wound is in a hard-to-reach area.

Incorporation of naturally-occurring compounds such as thymol is an area with many possibilities and potential for findings that could vastly improve current technologies in the medical field. Further research should be done using biopolymers in combination with natural anti-microbial compounds for finding effective methods of protecting the wound from infection and inducing healing mechanisms. This study's focus was to understand the interaction between electrospun rSSps mats/adhesives alongside the release kinetics of antimicrobial/growth-inducing compounds.

Spider Silk as a Biomaterial

Recombinant spider silk proteins have captured the attention of researchers for many years now. These highly conserved protein sequences have endured millions of years of evolutionary pressure and, depending on the species, have uses ranging from prey capture to egg sac formation (Lewis, 2006). The most notable and highly sought after silks are the major ampullate silks also known as dragline silk. As nature's strongest biomaterial, dragline silk is tougher than Kevlar and stronger than steel by weight. This remarkable combination of strength and elasticity has ignited numerous research projects to produce large quantities of these proteins in transgenic hosts. The hurdles associated with this are the large size of the native proteins (>250kDa), purification and solvation of these proteins, and expression of these proteins in transgenic hosts without truncation (Xia et al., 2010). Once produced, these naturally insoluble proteins have been solvated using harsh organic solvents. However, using high heat and pressure it was found that rSSps could be solvated in water, opening the door to a wide variety of biomedical applications (Rising, Widhe, Johansson, & Hedhammar, 2011).

Spider silk's impressive mechanical properties have yielded a lot of interest in the production of synthetic spider silk fibers. Beyond fibers alone, it has been found that spider silks can be used to produce a wide

array of materials such as fibrous mats, adhesives, coatings, films, hydrogels, lyogels, and sponges (Jones et al., 2015). The lack of an immune response with respect to spider silk proteins has also led to numerous research projects using silk products as cell scaffolding (Bauer, Wohlrab, & Scheibel, 2013). These scaffolds allow for cells to attach and differentiate on a matrix that can then be transferred to a living system.

Current Bandaging Hurdles and Research Associated with Epidermal Recovery

With new advances in biotechnology and biomaterials, research into wound healing and bandages is rapidly increasing. Normal bandages cannot provide the ideal conditions for wounds to heal properly, and even contribute to conditions in which a wound could be further damaged. Current efforts include probes and sensors to manipulate the conditions of wounds dressed in conventional bandages (Mone, 2015).

One study showed the antimicrobial effects of chitosan acetate bandages on mice with burn wounds. Although the bandages reduced inflammation, they also stuck to the wounds (Burkatovskaya, Castano, Demidova-Rice, Tegos, & Hamblin, 2008). The consequence of a bandage that cannot be easily removed is that the wound could be further damaged and the overall healing process could take longer (Mone, 2015).

Electrospun mats are currently being considered for use in the treatment of large wounds. The wounds can heal relatively quickly if covered in electrospun materials. The porous materials created from electrospinning methods provide excellent barriers between the outside environment and the wounds they are covering. They allow for gases to pass through, such as oxygen, but have small enough pores to prevent bacteria from passing (Wendorff, Agarwal, & Greiner, 2012).

Bandages typically involve some sort of adhesive, and their ability to stick and be removed from the skin can have a big impact on consumer's perception of the products. However, the actual adhesive being used is often overlooked in the design process. The future of smart bandages depends on the combination of bandage and adhesive research ("Pressure-Sensitive Medical Technology").

Medical products, such as adhesives, must go through biocompatibility assessments to be approved for human use. Researchers in India cured polyurethane pressure-sensitive adhesive tape by electron beam and followed ISO guidelines to determine if their method produced a biocompatible product (Singh).

The aim of this project is to create a product prototype which combines innovative materials in bandages and adhesives to create a "second layer of skin" which promotes wound healing, protects against microbial infection, and can be absorbed by the body. The bandaging system will be spider silk based, making it more biocompatible than bandaging systems made from synthetic materials. A nylon-spider silk electrospun mat is to be used in combination with a spider silk adhesive, either of which can be loaded with antimicrobials and various growth factors to further promote healing.

Silk Products used in Epidermal Recovery

The use of silk polymers for wound treatment has existed for thousands of years. However, today, much research is being done to test the efficiency of these polymers as a biocompatible treatment. Some of the advantages of using spider silk are argued to be its ability to be resorbed into the body and to not illicit an immune response from the host. The largest challenge in creating a viable spider silk bandage is the

ability to mass produce the spider silk protein and subsequently, spider silk thread and non-fibrous rSSps products. This review will identify what work has been done and how it can be applied to the use of electrospun spider silk as a viable wound treatment. Articles will be reviewed for their application to this project, supporting evidence, and scope of research.

The toxicity of silk proteins in epidermal applications is important and within the scope of this study to explore. The topical application of a silk protein film has been used to test dermal irritation in rats and hamsters using the Draize test (Padol et al., 2011). This article offers a good animal testing scenario to show that silk protein films are non-toxic when applied to the skin. On the other hand, it has too broad of a scope in that the type of silk protein used is not clearly identified. It would be most valuable if spider silk were the type used. The applications were all on healthy test subjects, not on those with any sort of wounds. It would be valuable to see the dermal response in a compromised area, such as a burn.

Studies have also been done to test the ability of spider silk fibers to serve as a scaffold for skin cell culture. An article by Wendt tested this possible application. This articles strengths came in using spider silk fibers and testing skin culture regrowth, both of which would be valuable to the topic. However, the article uses a very simplistic model with limited replication or statistical analysis. The results show potential for the application but miss the mark of offering definitive evidence (Wendt et al., 2011).

The applications of spider silk as a biomaterial has also been studies. An article by Vepari offers the most extensive research and results. It is beneficial in showing past studies where silk has been used for tissue scaffold applications. However, similar to the Padol article, the scope of this article may be too broad. It uses a variety of silks and a variety of forms of the silk, rather than simply fibers or electrospun mats (Vepari & Kaplan, 2007).

From this review, it can be determined that spider silk offers a novel biomaterial in terms of its strength and biocompatibility. However, there are still many areas which need to be researched. These articles show that spider silk can be used without causing harm to the host and even encourage epidermal cell regrowth. But the research lacks information regarding the use of electrospun spider silk and its potential as a tissue scaffold. Also, no article shows the effects of glutaraldehyde as a crosslinking agent and the potential toxicity associated with that treatment. Both of these would be valuable areas for further study.

Design Process

A. Overview

The design process for this project was orchestrated with mechanical properties, functionalization properties, and biocompatibility in mind. The different tests were designed to evaluate the mechanical properties of the electrospun bandages as well as providing proof of concept for antimicrobial elution. Because glutaraldehyde was used as a cross-linking agent in the formation of some of the electrospun bandages, development of a cytotoxicity test was also necessary to evaluate the product's effectiveness when exposed to living cells.

Figure 3 shows the current concept map of ideas for this project. As the design progress continues, the concept map will adapt to illustrate the decision making and the ideas that were pursued throughout this project.
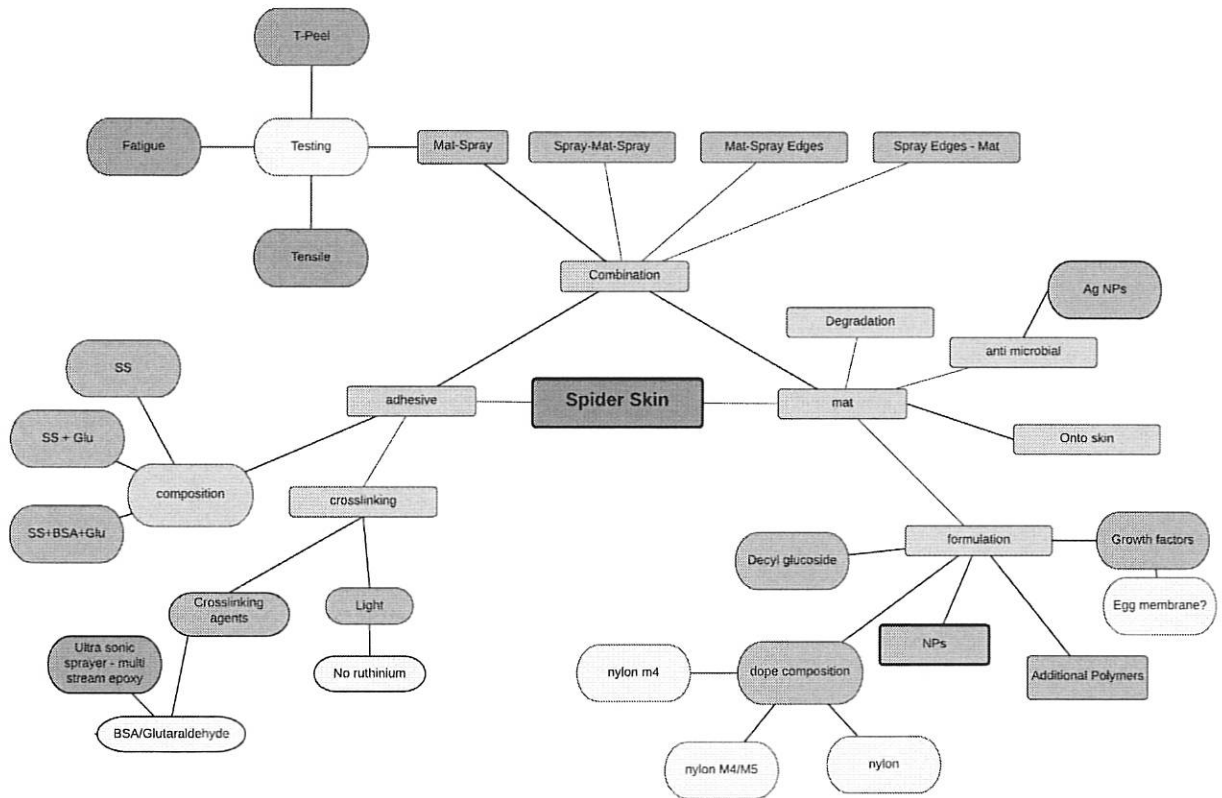
Figure 3. Design process concept map showing the different ideas and methods of testing that were explored in this study.

B. All Materials

- Goat-derived recombinant spider silk proteins (rSSps): major ampullate spidroin 1 (rMaSp1) and major ampullate spidroin 2 (rMaSp2)
- 97% Formic Acid
- Distilled water
- Pigskin
- Electrospinner
- MTS (Mechanical Testing System) Synergie 100
- MTS Cards (8 mm gage distance)
- Optical Microscope

- Digital Camera
- Motic Imaging Software
- Test Works 4 Software
- Sprayer
- Humidifier
- UPLC Chromatographic System
- End-over-end plate mixer
- Centrifugal Dryer
- 8 mL screw cap tubes with a flat base
- Nunclon 24 well plates
- MRC-5 Lung cells
- DMEM + 10%FBS media
- 50% glutaraldehyde in solution
- Trypsin
- ViCell

C. Selection of Mat/rSSps Adhesive Formulation

Rationale
Initial tests were chosen to decide what was the best formulation of electrospun mat and rSSps adhesive to use for the continuation of the project. The following factors were evaluated: ease of spinning, material availability, best adhesive properties when combined with the electrospun mat.

Decisions
This initial thought process led to the decision to test the remaining three mat formulations using the criteria of ease of spinning, material availability, and performance using the on-skin pressure sensitive tape test.
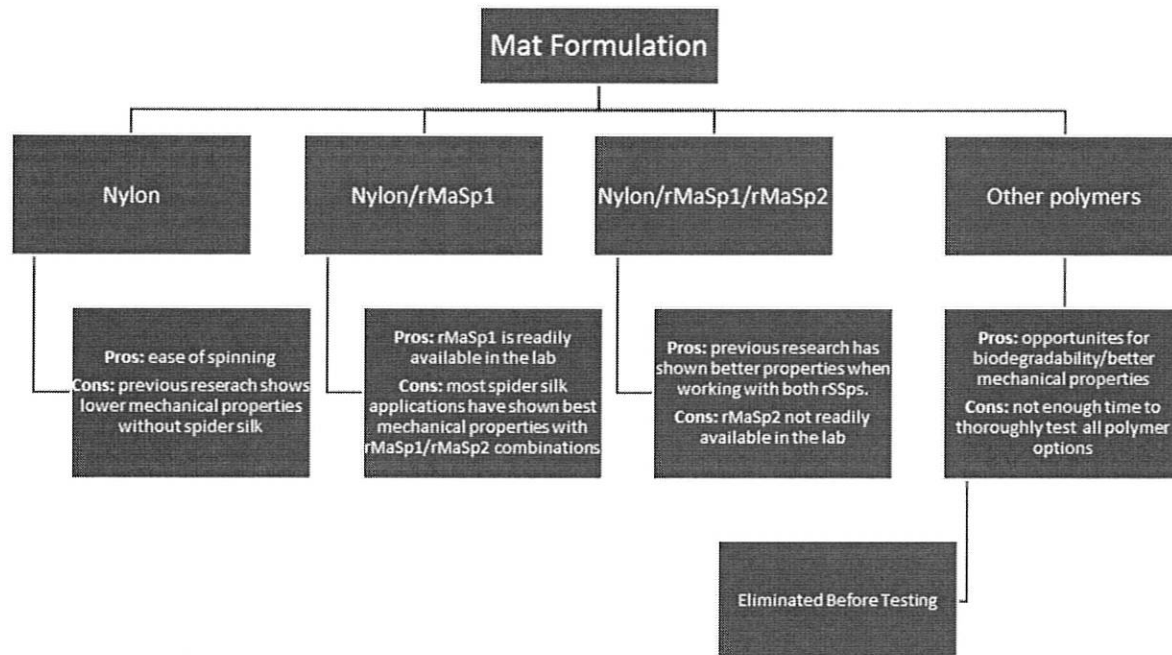
Figure 4. Decision tree for rSSps mat/adhesive formulation

Materials

20% Nylon dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)

10% Nylon + 10% rMaSp1 dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)
- 0.3 g rMaSp1

10% Nylon 10% rSSps 80/20 rMaSp1/rMaSp2 dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)
- 0.24 g rMaSp1 (rSSps protein)
- 0.06 g rMaSp2 (rSSps protein)

12% 50/50 rMaSp1/rMaSp2 rSSps adhesive:

- 3 mL distilled water
- 0.18 g rMaSp1
- 0.18 g rMaSp2

12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde rSSps adhesive:

- 1 mL 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive
- 20 µl 50% glutaraldehyde stock

12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde + 10% Bovine Serum Albumin rSSps adhesive:

- 1 mL 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive
- 20 µl 50% glutaraldehyde stock
- 0.1g 96% bovine serum albumin lyophilized powder

Methods

Mat formulation:

For this test there were 3 different initial mat formulations tested: 20% nylon, 10% nylon 10% rMaSp1 protein, and 10% nylon 10% 80/20 rMaSp1/rMasp2 proteins, all of which were dissolved in formic acid. The method for dope preparation as well as the spinning protocol is explained in detail in Appendix A. Three spins (1 ml dopes for each spin) were completed for each of these formulations and were monitored for ease of spinning. The criteria for this was: the thickness of the mat created, how well the mat could be manipulated by hand without falling apart, and how many drops were created from the dope solution during the spin that altered the effectiveness of the final mat. Availability of materials was also considered during this test.

Adhesive formulation:

There were 3 different initial adhesive formulations tested: 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive, 12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde adhesive, 12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde + 10% bovine serum albumin adhesive. The method for the adhesive preparation is further explained in Appendix A. The ease of production and application of each adhesive formulation, in combination with each mat formulation was the initial criteria in deciding which adhesive to use.

Pressure-sensitive tape test:

This test was conducted to narrow the choices for the mat and rSSps adhesive. The methods for this test were derived from ASTM D3359-09. Fresh pigskin was cleaned and shaved as detailed in Appendix C and electrospun mats were prepared according to the protocols in Appendix A. The rSSps adhesives were prepared in three different formulations: 50/50 rMaSp1:rMaSp2, 50/50 rMaSp1:rMaSp2 with 1% glutaraldehyde, and 50/50 rMaSp1:rMaSp2 with 10% bovine serum albumin (BSA). Different ratios of

rMaSp1 and rMaSp2 were not explored because previous research has shown these goat-derived rSSps adhesives constitute the best mechanical properties (Jones et al., 2015). The different electrospun mats were cut into 5 cm x 2.5 cm rectangular samples. Five samples of each of the three mats were applied and adhered to the fresh pigskin with each of the three rSSps adhesives and left to dry for approximately 35 minutes. Once the mats were dry, 1 cm X-shaped incisions were made to the mats and covered in small pieces of painting tape. The tape was peeled off from the mat and results were based on a 0-5 scale as follows:

Table 1. Pressure-Sensitive Tape Test Grading Scale

5A	No peeling or removal
4A	Trace peeling or removal along incisions or at their intersection
3A	Jagged removal along incisions up to 1.6 mm on either side
2A	Jagged removal along most of incisions up to 3.2 mm on either side
1A	Removal from most of the area of the X under the tape
0A	Removal beyond the area of the X

Scanning Electron Microscopy:
SEM imaging was used to evaluate the surface conditions of the electrospun mat/rSSps adhesive complex. The qualitative analysis of the bandage material was to see the adhesive/mat matrix and how the adhesive permeated the fibers. This was one of the first steps in the initial characterization of the bandaging product.

Results

The SEM images indicated that there was an infiltration of the rSSps adhesive into the fibrous pores of the electrospun mat, causing it to change from a white material with no transparency to a material with complete transparency. This imaging was the first step in the characterization of the bandaging product. No major difference was seen in the surface characteristics of the different materials, however it became clear that the adhesive saturated the surface to the point that the individual fibers were no longer visible (Figure 5).

Table 1 illustrates how the different mat/rSSps adhesive combinations performed for the three criteria: ease of electrospinning, material/protein availability, and the pressure-sensitive tape test performance. All were analyzed on a 0-5 scale. The pressure-sensitive tape test scale is shown above and the numbers reported below are an average score with n=5. From these results, it was decided to move forward with the rest of the study using a 10% Nylon: 10% rMaSp1 mat due to the fact that it was relatively easy to spin and interacted better than the other two mats with the rSSps adhesives. As for the rSSps adhesives, the rest of the study moved forward using two adhesives: the 50/50 rMaSp1:rMaSp2 adhesive and this same adhesive with 1% glutaraldehyde added as a cross-linking agent. It was noted during these experiments that the 1% glutaraldehyde adhesive was easier to work with than the other adhesives and it was given a good score on the pressure-sensitive tape tests. It was decided that this cross-linking agent was of interest in this study, therefore all future study includes an analysis of both these adhesives.

Table 2. Criteria Comparisons for Mat/rSSps Adhesive

	Criteria Weight [a]	20% Nylon Mat	10% Nylon/10% rMaSp1	10% Nylon/ 10% 50/50 rMaSp1:rMaSp2
50/50 rMaSp1:rMaSp2 Adhesive	Spinning: 0.3	Spinning: 5 Availability: 4 Tape Test: 3 TOTAL: 3.9	Spinning: 4 Availability: 4 Pressure-sensitive Tape Test: 4.5 TOTAL: 4.2	Spinning: 3 Availability: 2 Pressure-sensitive Tape Test: 4 TOTAL: 3.1
50/50 rMaSp1:rMaSp2 Adhesive w/ 1% glutaraldehyde	Availability: 0.3	Spinning: 5 Availability: 4 Tape Test: 3 TOTAL: 3.9	Spinning: 4 Availability: 4 Pressure-sensitive Tape Test: 5 TOTAL: 4.4	Spinning: 3 Availability: 2 Pressure-sensitive Tape Test: 4 TOTAL: 3.1
50/50 rMaSp1:rMaSp2 Adhesive w/ 10% BSA	Tape Test: 0.4	Spinning: 5 Availability: 4 Tape Test: 1 TOTAL: 3.1	Spinning: 4 Availability: 4 Tape Test: 1 TOTAL: 2.8	Spinning: 3 Availability: 2 Tape Test: 1 TOTAL: 1.9

a. Ease of spinning , availability of material, and the pressure-sensitive tape test were given weights of importance of 30%, 30%, and 40% respectively.
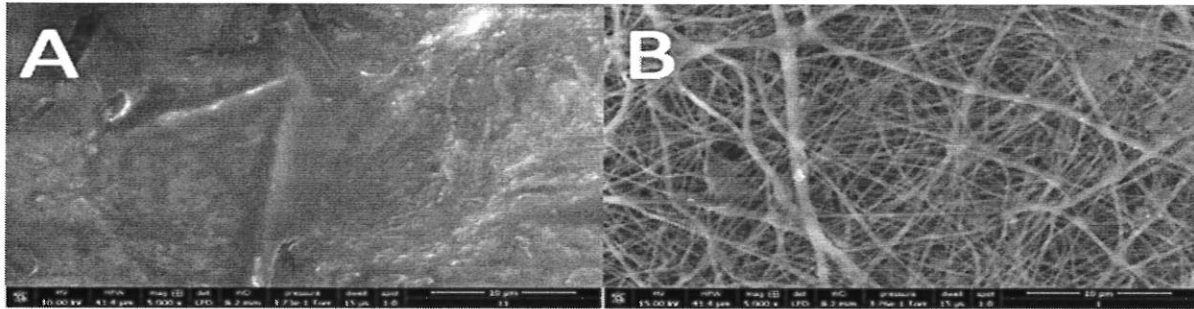
Figure 5. SEM images at 5000x magnification. (A) Nylon-rMaSp1 electrospun mat with 50/50 rMaSp1:rMaSp2 adhesive, (B) Nylon-rMaSp2 mat control with no adhesive

Conclusion

By doing a criteria comparison test using the criteria: ease of spinning, material availability, and the pressure sensitive tape test results it was possible to eliminate the mat formulations of 20% nylon, and 10% 50/50 rMaSp1/rMaSp2 with 10% nylon. Using a combination of all of these criteria, the project was able to move forward with a mat formulation that performed well on skin, was easy to electrospin, and all materials for this formulation were readily available. Of course, much more research could be done to optimize this mat formulation, perhaps creating a mat that is made of only rSSps or including polymers that have different levels of biodegradability and testing how these polymers interact with the rSSps.

The SEM imaging allowed for the qualitative analysis of the bandaging product and allowed for a visual representation of the interaction between the nanofibers of the electrospun mat and the rSSps adhesive. The adhesive covers and encompasses these nanofibers as was hypothesized.

D. T-Peel Testing

Rationale

T-peel testing was conducted to test the strength of the adhesives compared to already existing medical adhesives. This test was derived from ASTM F2256. Water-proof Band-Aids and Tegaderm tape were used as controls for comparison. Only the clear adhesive portions of these control bandages were used so that a direct adhesive comparison was possible.

Decisions

Due to the fact that this was the first on-skin quantitative test the decisions made during this testing period were carried through for the remainder of the design process. Therefore, the same controls and the same environmental testing conditions were carried out in all mechanical testing thereafter.

Figure 6. Decision Tree for T-Peel Testing.

Materials

- MTS (Mechanical Testing System) Synergie 100
- Humidifier
- Distilled water
- Water-proof Band-Aids
- Tegagerm dressing
- Pigskin
- Sprayer

10% Nylon + 10% rMaSp1 dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)
- 0.3 g rMaSp1

12% 50/50 rMaSp1/rMaSp2 rSSps adhesive:

- 3 mL distilled water

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- 0.18 g rMaSp1
- 0.18 g rMaSp2

12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde rSSps adhesive:

- 1 mL 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive
- 20 μl 50% glutaraldehyde stock

Methods

Strips of pigskin were prepared according to Appendix C so that one 0.5 cm x 2.5 cm electrospun mat or control strip fit on each strip of skin. Of the 60 mat strips glued onto the strips of pigskin, 30 were glued with the 50/50 rMaSp1:rMasp2 adhesive and 30 were glued with the rMaSp1:rMasp2 + 1% glutaraldehyde adhesive. The properties of the adhesives were subjected to three different conditions: dry, humid, and wet. These conditions are provided in more detail in Appendix D.

As seen in Figure 7 an edge of each mat or test control was pulled up from the skin to fit into the top MTS grips, while the skin was secured to the bottom grip. As the top grip moved up the bandage system was peeled off of the skin. The samples were pulled off at a rate of 2.5 mm/min, and the average load to peel the sample off of the skin was measured. This test was derived from ASTM F2256.
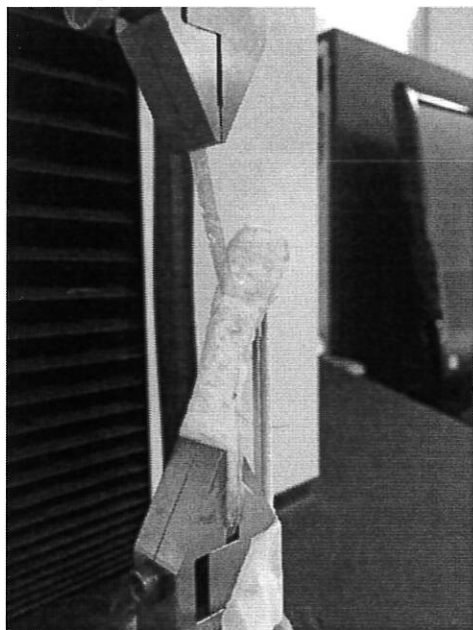
Figure 7. Illustration of T-Peel Adhesive Testing.

Results

The maximum load recorded was compared between the groups in dry, humid, and wet conditions (Figure 8). Statistical analysis was performed via JMP® software. The visual outputs of JMP® are explained in Appendix F.
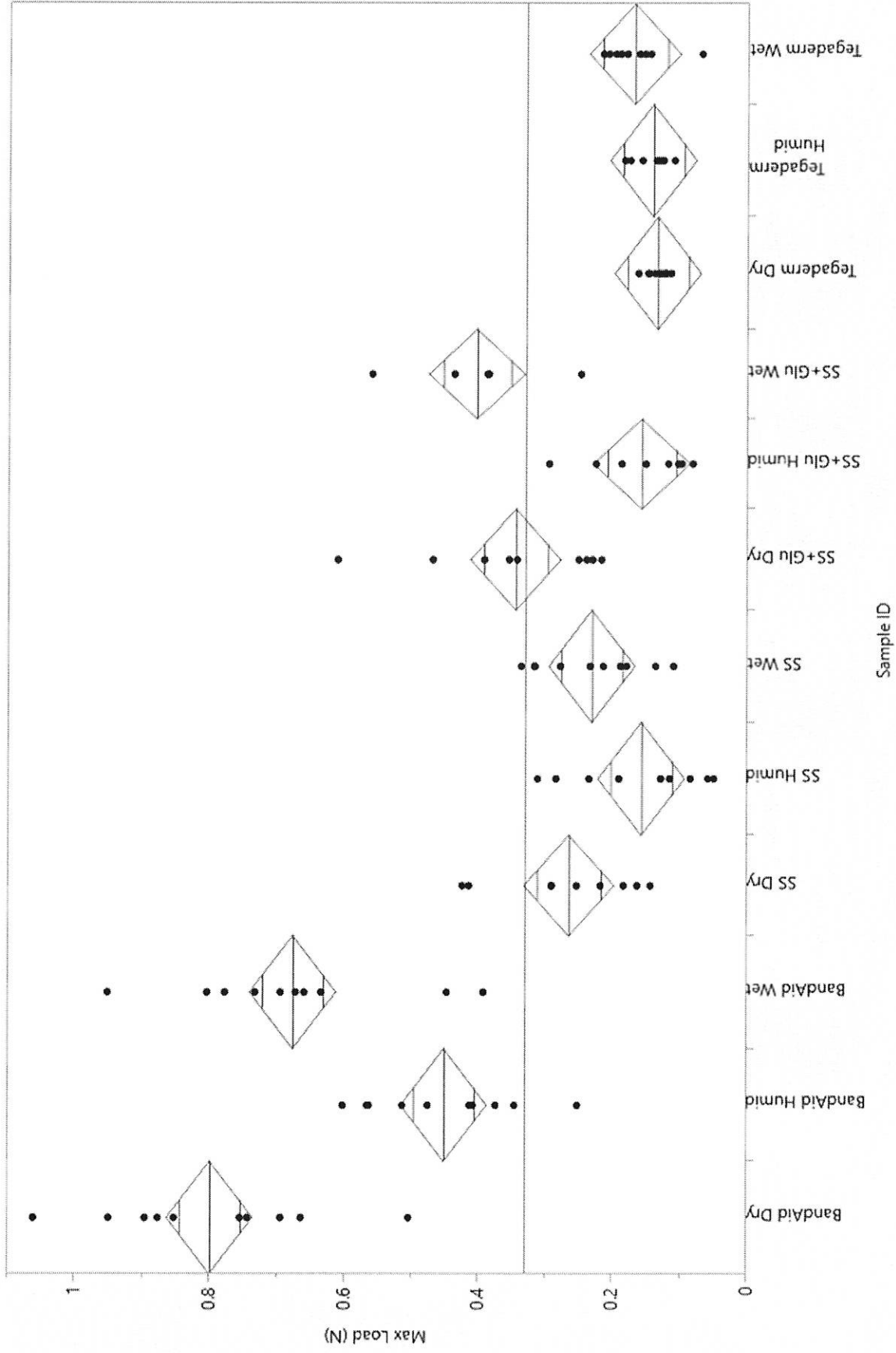
Figure 8. Comparison of Max Load in T-Peel testing

The rSSps adhesives with and without glutaraldehyde were shown to have an adhesive strength between that of Tegaderm and Band-Aids. Band-Aids proved to have the greatest adhesion to the skin, while Tegaderm was consistently one of the lowest. The rSSps samples showed no significant difference in adhesive strength between the wet, dry, and humid conditions tested. However, the rSSps and glutaraldehyde adhesive samples tested under dry and wet conditions exhibited higher adhesive strength than the rest of the samples, except for the Band-Aid samples tested under the same conditions. The samples are arranged into statistically significant groups in Figure 9.

◢ Connecting Letters Report

Level					Mean
BandAid Dry	A				0.79910950
BandAid Wet	A				0.67611520
BandAid Humid		B			0.45042420
SS+Glu Wet		B	C		0.40291775
SS+Glu Dry		B	C	D	0.34410333
SS Dry			C	D	E	0.26340756
SS Wet				D	E	0.22964380
Tegaderm Wet					E	0.16771344
SS+Glu Humid					E	0.15617375
SS Humid					E	0.15597560
Tegaderm Humid					E	0.13997490
Tegaderm Dry					E	0.13303520

Figure 9. Statistical Groupings of T-Peel tests

Conclusion

The rSSps and rSSps with glutaraldehyde adhesive samples matched or exceeded the adhesive strength of commercially-available Tegaderm, but did not adequately compare to the Band-Aid samples for this specific test. The results indicate that the rSSps adhesives are capable of competing with commercial products in the adhesive realm.

E. Cyclic Fatigue Testing with Bandages in Compression

Rationale

Cyclic fatigue testing in compression was conducted in order to evaluate the efficacy of the bandage under cyclic compression. This scenario would be seen in real-life application when the bandage is applied near or on joints. The ability of the bandaging material to stay intact and adhered onto the skin when compression lines are formed was considered vital for the stability of the product.

Decisions

Figure 10. Decision tree for cyclic fatigue testing with bandages in compression.
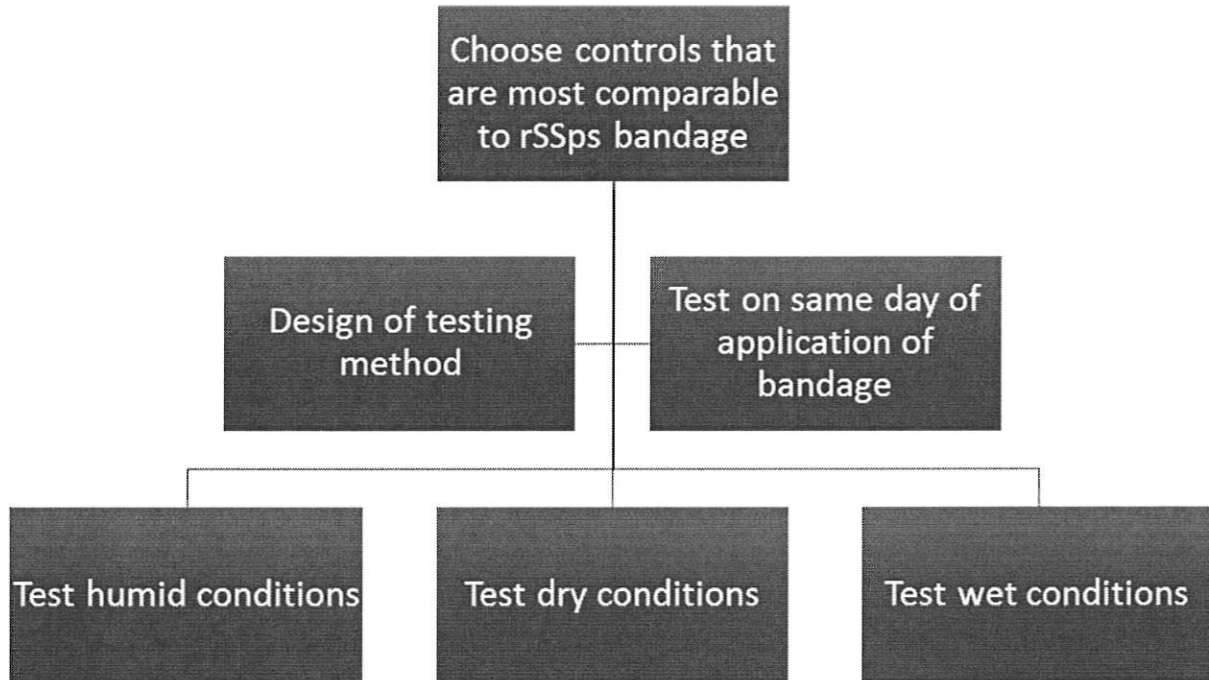
Materials

- MTS (Mechanical Testing System) Synergie 100
- Humidifier
- Distilled water
- Water-proof Band-Aids
- Tegagerm dressing
- Pigskin
- MTS Cards (8 mm gage distance)
- Sprayer

10% Nylon + 10% rMaSp1 dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)
- 0.3 g rMaSp1

12% 50/50 rMaSp1/rMaSp2 rSSps adhesive:

- 3 mL distilled water

- 0.18 g rMaSp1

- 0.18 g rMaSp2

- 0.03 μl Glutaraldehyde (50% stock solution)

- 0.1 g Bovine Serum Albumin (BSA)

12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde rSSps adhesive:

- 1 mL 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive

- 20 μl 50% glutaraldehyde stock

Methods

Nylon/rMaSp1 mats were cut into 0.5 cm x 2.5 cm strips and glued in groups of 3-4 unto prepared pieces of skin with the rSSps adhesives (with and without glutaraldehyde). Once dry, the pieces of skin were secured on the MTS by grips on the top and bottom sides, with the mats oriented upright (Figure 11). The MTS was programmed to move the top grip at a rate of 5 mm/sec up and down 100 times bending the skin to about 90° to create cyclic stress, compressing the bandage. The testing was conducted under three different conditions (wet, dry, humid) according to Appendix D. After the testing was completed the adhesion of the bandage material (either control bandange or electrospun mat formulation) were evaluated according to the scale detailed in Table 3. The ratings were done by the same individual to avoid differences in rating style and personal preference. Statistical analysis was performed via JMP® software. The visual outputs of JMP® are explained in Appendix F.

Table 3. Cyclic fatigue in compression grading scale

5A	No peeling or removal
4A	Trace peeling or separation from the substrate
3A	Half of the bandage is separated
2A	More than half of the bandage is separated
1A	Most of the bandage is separated
0A	Full separation
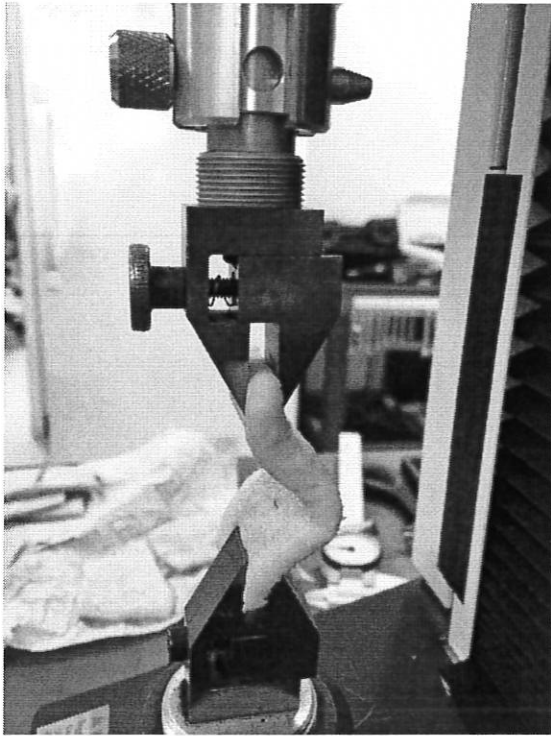
Figure 11. Illustration of compression cyclic testing using the MTS.

Results

Following statistical analysis, all variations of SpiderSkin (with and without glutaraldehyde) were in the same statistical grouping as the commercial bandage material Tegaderm. Furthermore, under this compressive testing, water-proof Band-Aids were significantly worse in wet conditions than all other groups due to the separation of the the bandage material as the compression lines were manipulated and moisture got underneath the bandage.
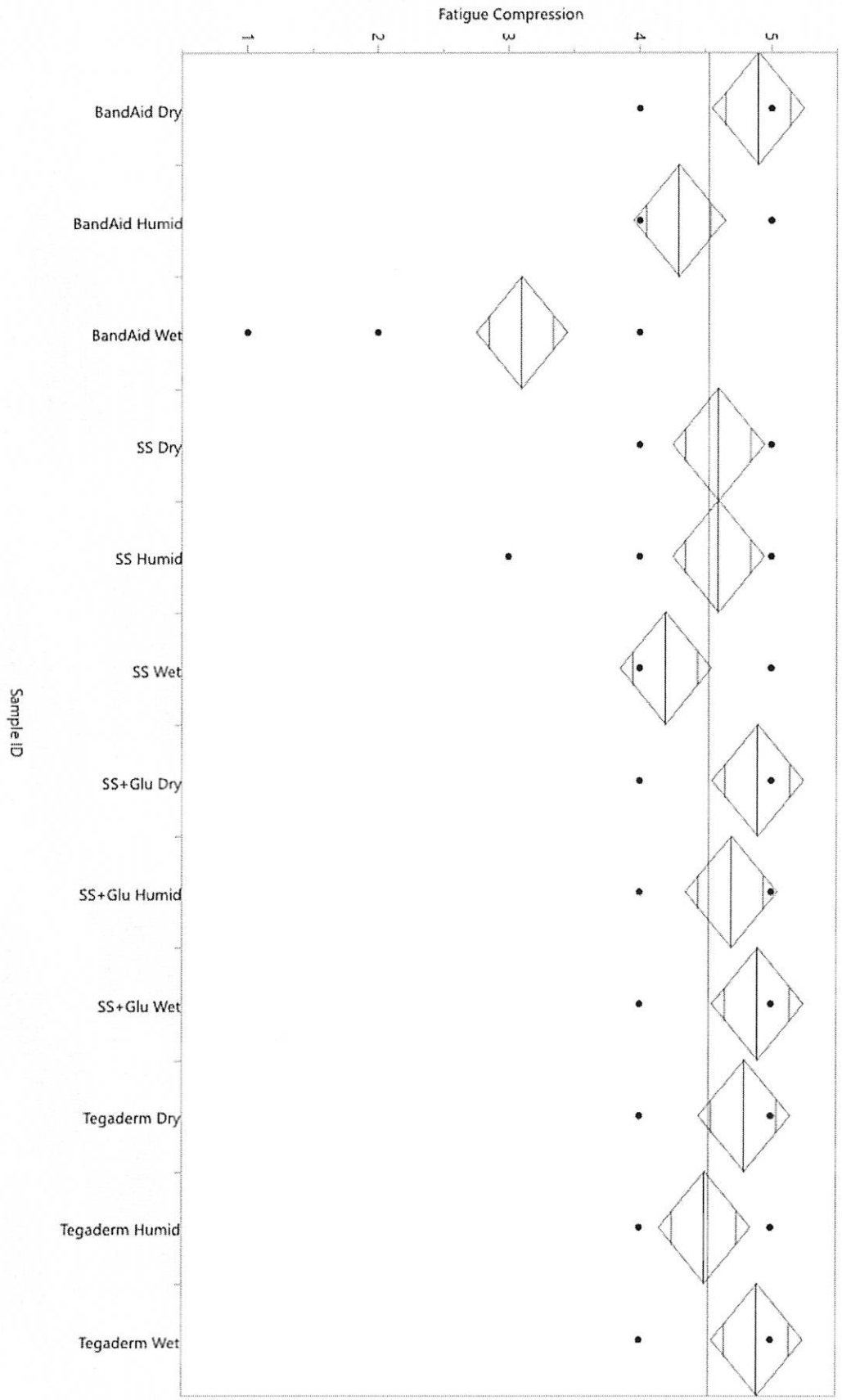
Figure 12. Comparison of Grades for Compressive Fatigue Testing

Connecting Letters Report

Level		Mean
BandAid Dry	A	4.9000000
SS+Glu Dry	A	4.9000000
SS+Glu Wet	A	4.9000000
Tegaderm Wet	A	4.9000000
Tegaderm Dry	A	4.8000000
SS+Glu Humid	A	4.7000000
SS Dry	A	4.6000000
SS Humid	A	4.6000000
Tegaderm Humid	A	4.5000000
BandAid Humid	A	4.3000000
SS Wet	A	4.2000000
BandAid Wet	B	3.1000000

Levels not connected by same letter are significantly different.

Figure 13. Connecting Letters Report for Compressive Fatigue Testing

Conclusion

All groups performed similarly, with the exception of Band-Aid which had significantly reduced adhesion under cyclic compression in wet conditions. Both glutaraldehyde and non-glutaraldehyde bandages were comparable to commercially available products. The rSSps adhesives with and without glutaraldehyde exhibited an ability to maintain adhesion of the nylon/rSSps mat under compression even in wet and humid conditions.

F. Cyclic Fatigue Testing with Bandages in Flexion

Rationale

This test, similar to the cyclic fatigue testing in compression, will be used in evaluating the efficacy of the bandage under repeated flexion. This will occur most intensely when the bandage is applied to a joint.
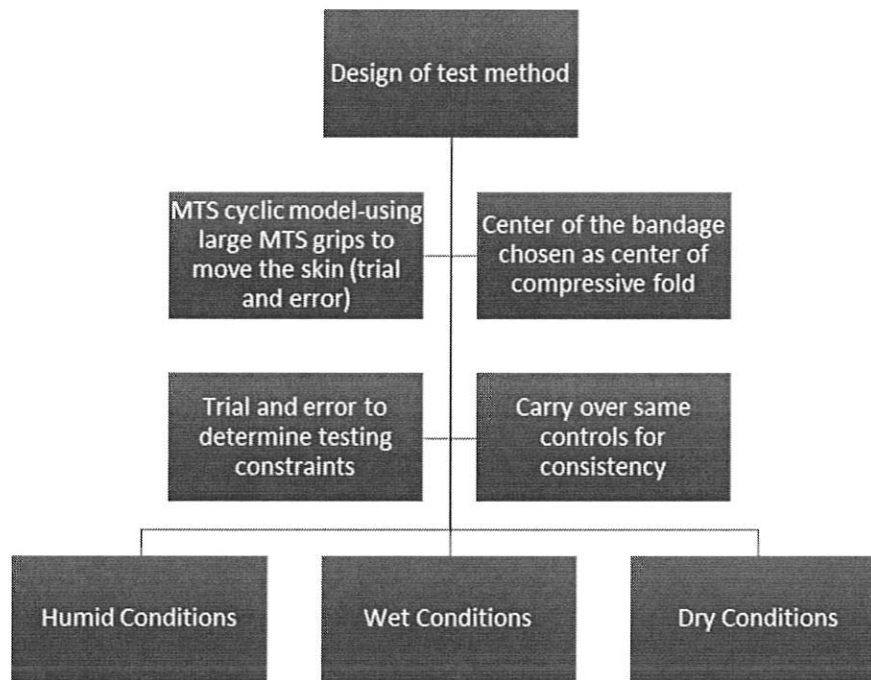
Decisions
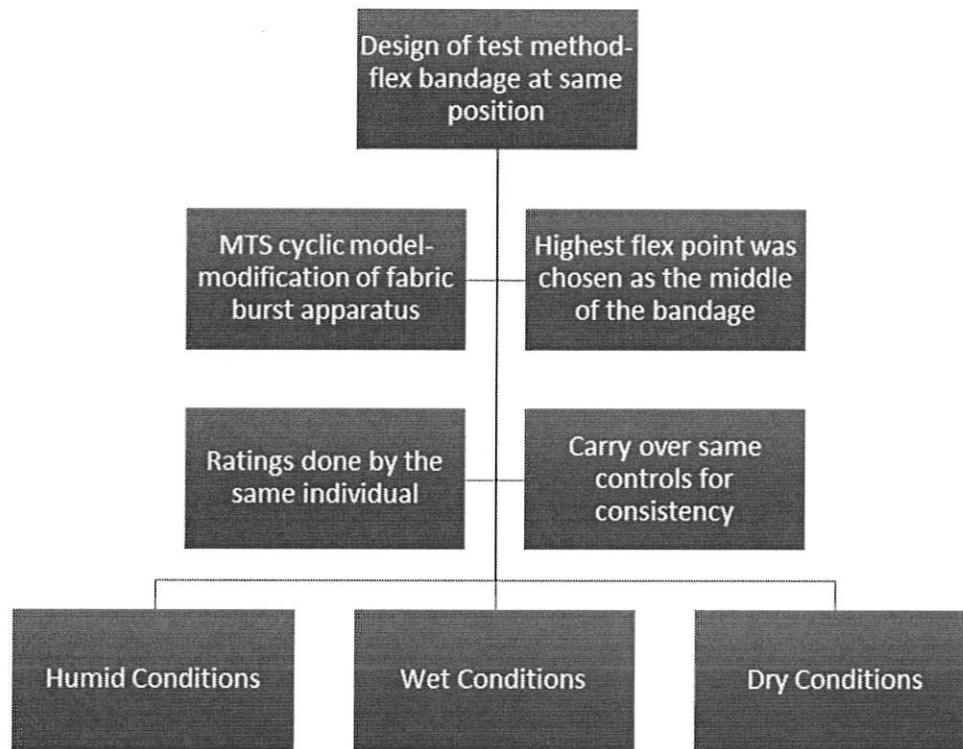
Figure 14. Decision tree for cyclic fatigue testing with bandages in flexion

Materials

- MTS (Mechanical Testing System) Synergie 100
- Humidifier
- Distilled water
- Water-proof Band-Aids
- Tegagerm dressing
- Pigskin
- MTS Cards (8 mm gage distance)
- Sprayer

10% Nylon + 10% rMaSp1 dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)
- 0.3 g rMaSp1

12% 50/50 rMaSp1/rMaSp2 rSSps adhesive:

- 3 mL distilled water
- 0.18 g rMaSp1
- 0.18 g rMaSp2
- 0.03 µl Glutaraldehyde (50% stock solution)
- 0.1 g Bovine Serum Albumin (BSA)

12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde rSSps adhesive:

- 1 mL 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive
- 20 µl 50% glutaraldehyde stock

Methods

The nylon/rSSps electrospun mats were cut into 0.5 cm x 1.5 cm strips and two mats were applied to the center of square shaped pieces of pigskin with the rSSps, or rSSps + glutaraldehyde adhesives and left to dry. To set up the flexion test, each piece of skin was secured between two frames, screwed into place to prevent movement of the skin, and a rod with a rounded top was used to push the skin 10 mm upward. Figure 15 shows this setup. The thickness of these skin pieces averaged 13 mm.
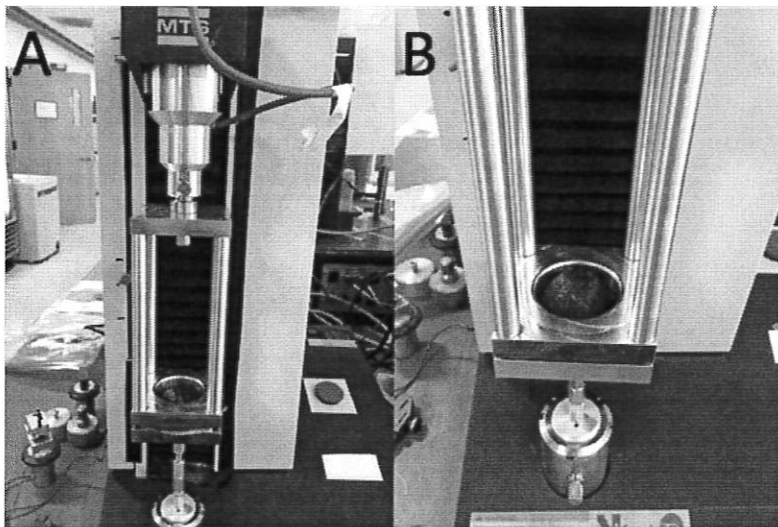
Figure 15. Flexion Testing (A) illustrates the flexion apparatus. (B) Illustrates the testing with the pigskin loaded in the apparatus.

Results

Grades were given to the bandages in dry, humid, and wet conditions (Figure 16). Statistical analysis was performed via JMP® software. The visual outputs of JMP® are explained in Appendix F.

Figure 16. Comparison of Grades for Flexion Fatigue Testing.
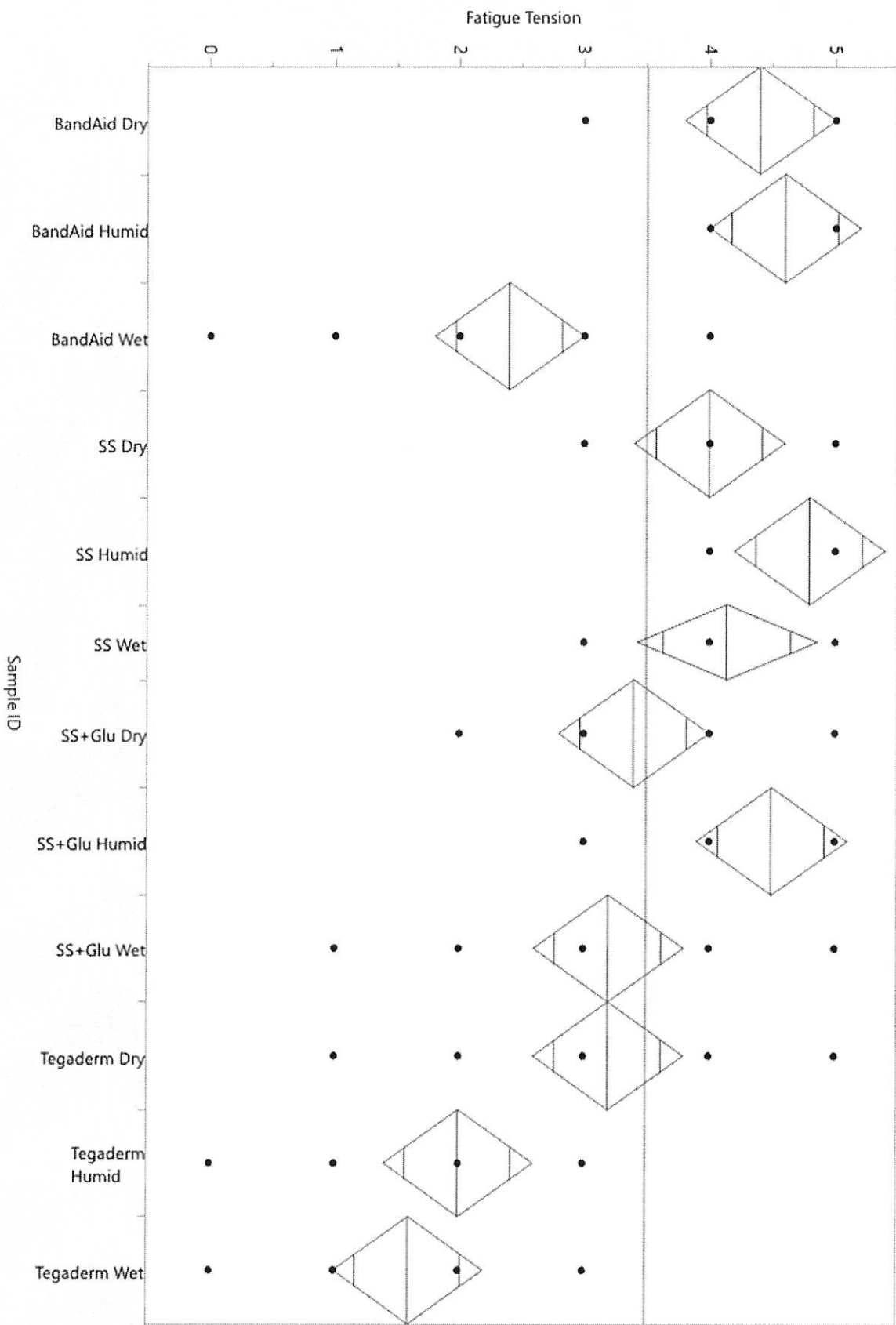
In contrast to the compressive fatigue test detailed above, there was much more variation between groups in flexion fatigue testing. The rSSps adhesive withstood fatigue testing similar to Band-Aid and exceeded the results of Tegaderm. The groups are further detailed in Figure 17.

◿ **Connecting Letters Report**

Level				Mean
SS Humid	A			4.8000000
BandAid Humid	A	B		4.6000000
SS+Glu Humid	A	B		4.5000000
BandAid Dry	A	B		4.4000000
SS Wet	A	B		4.1428571
SS Dry	A	B		4.0000000
SS+Glu Dry	A	B	C	3.4000000
SS+Glu Wet		B	C	3.2000000
Tegaderm Dry		B	C	3.2000000
BandAid Wet			C D	2.4000000
Tegaderm Humid			C D	2.0000000
Tegaderm Wet			D	1.6000000

Levels not connected by same letter are significantly different.

Figure 17. Connecting letters report for cyclic flexion testing.

With the exception of the rSSps adhesive with glutaraldehyde as a cross-linker, the rSSps adhesive alone was not statistically differentiable from water-proof Band-Aid. The rSSps adhesive with glutaraldehyde in the wet condition was significantly different from Band-Aid in wet conditions, as well as matches or exceeds Tegaderm and Band-Aid in every condition tested, respectively.

Conclusion

This test showed that the adhesive properties of both the rSSps and the rSSps + glutaraldehyde bandages under dry, wet, and humid conditions were not affected to the same degree as the Band-Aid and Tegaderm. Although adhesive strength not increased with the addition of glutaraldehyde, the performance of the rSSps adhesive under wet and humid conditions suggests it would be a good alternative for dressing wound with very high moisture levels.

G. Tensile Testing of Materials

Rationale

Tensile testing was conducted to compare four basic mechanical properties: toughness, elastic modulus, strain at failure, and ultimate tensile strength. The properties of the electrospun mat with applied adhesives were compared to Tegaderm and water-proof Band-Aid, as those products contain similar mechanical properties to those desired of the final product.

Decisions
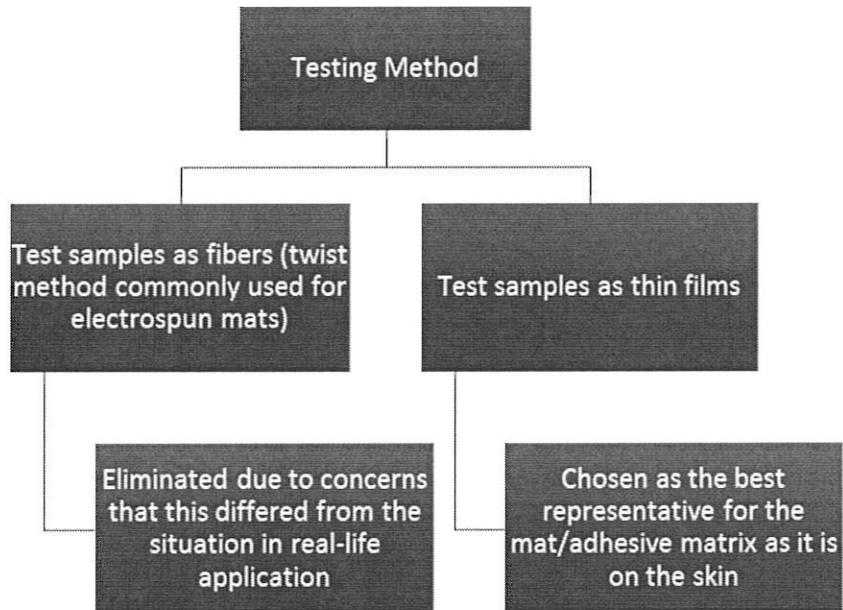
Figure 18. Decision tree for tensile testing.

Materials

- MTS (Mechanical Testing System) Synergie 100
- Humidifier
- Distilled water
- Water-proof Band-Aids
- Tegagerm dressing
- Pigskin
- MTS Cards (8 mm gage distance)
- Optical Microscope
- Digital Camera
- Motic Imaging Software
- Test Works 4 Software
- Sprayer

10% Nylon + 10% rMaSp1 dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)
- 0.3 g rMaSp1

12% 50/50 rMaSp1/rMaSp2 rSSps adhesive:

- 3 mL distilled water
- 0.18 g rMaSp1
- 0.18 g rMaSp2
- 0.03 µl Glutaraldehyde (50% stock solution)
- 0.1 g Bovine Serum Albumin (BSA)

12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde rSSps adhesive:

- 1 mL 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive
- 20 µl 50% glutaraldehyde stock

Methods

Ten samples were collected per nylon/rSSps mat and five per control (Tegaderm and Band-Aid). Thin films were cut and applied to an 8 mm gage before being loaded in tensile. Testing was conducted in dry, humid (approximately 100%), and wet conditions, to mimic possible states of wounds in patient therapy. The testing was continued until failure of the sample, with samples being pulled at 5 mm/min.

Results

Data were converted into stress/strain curves, and various mechanical properties were calculated in their individual spheres. These data were passed on for mean comparison via Analysis of Variance (ANOVA), and individual t-Tests. Statistical analysis was performed via JMP® software. The visual outputs of JMP® are explained in Appendix F.

Toughness:

Toughness is an important mechanical property of the bandaging complex, as the bandage must be resistant to failure upon excessive stresses. Tougher skin is more durable, and therefore able to withstand continuous motion and manipulation by the patient. Human skin was found to have an average toughness of 4.9 ± 1.5 MJ/m^3 (Gallagher et al.).

The toughness of the variations of SpiderSkin as compared to commercial products is shown in Figure 19.
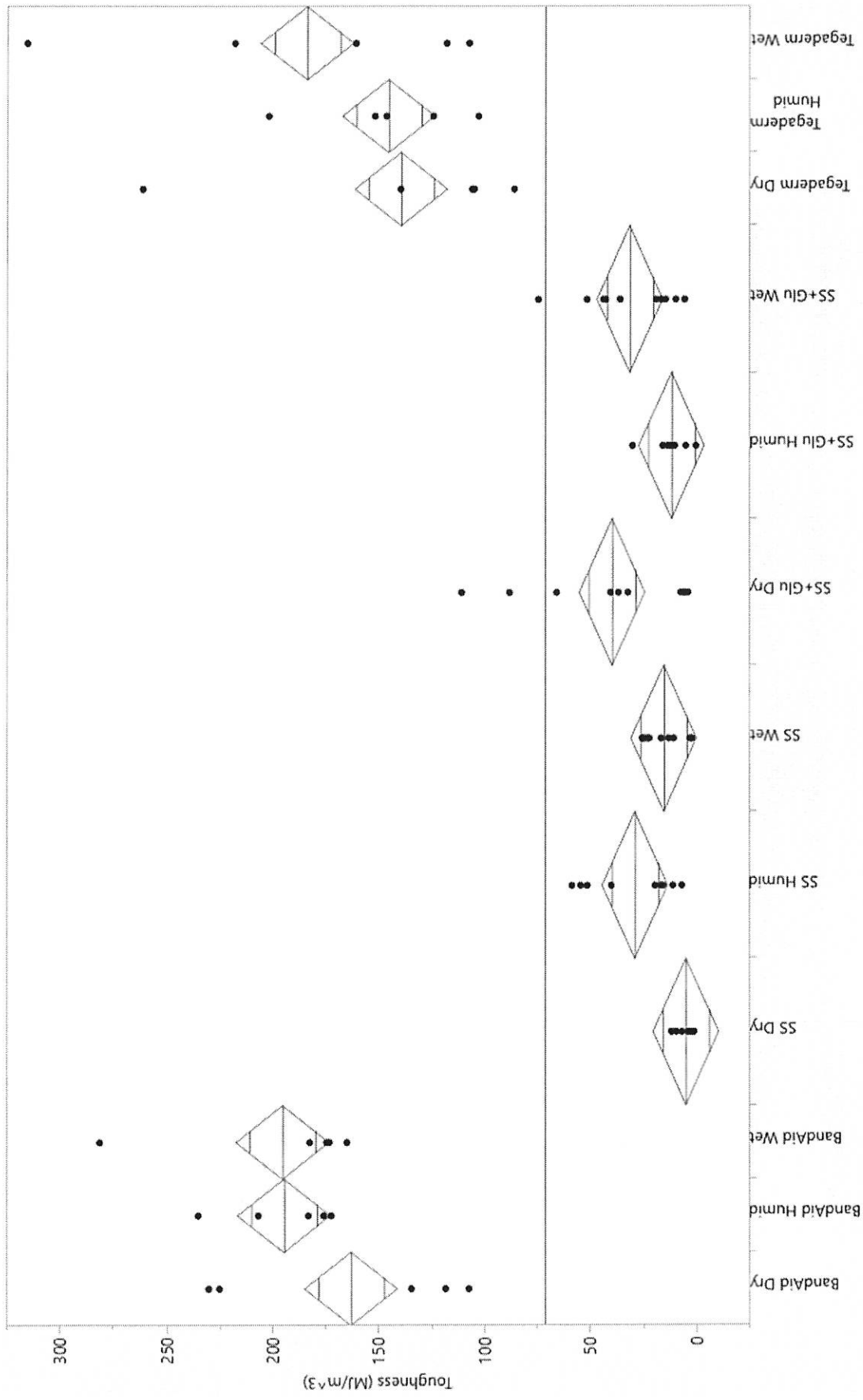
Figure 19. Comparison of Toughness.

It was noted that the rSSps mat and adhesive complexes did not demonstrate toughness similar to that of currently available products, but exhibited toughness more comparable to human skin. This result is mainly due to the relative rigidity of the electrospun mat. The Tegaderm and Band-Aid controls were capable of absorbing a considerably higher amount of energy before failure, and thus are more likely to withstand the patient's motion. In the following Connecting Letters Report, the samples are grouped by their statistical significance (Figure 20).

⊿ Connecting Letters Report

Level			Mean
BandAid Wet	A		195.32290
BandAid Humid	A		194.60469
Tegaderm Wet	A	B	183.96677
BandAid Dry	A	B	163.04293
Tegaderm Humid	A	B	145.47042
Tegaderm Dry		B	139.53501
SS+Glu Dry		C	39.61657
SS+Glu Wet		C	31.30083
SS Humid		C	28.88110
SS Wet		C	15.27153
SS+Glu Humid		C	11.59025
SS Dry		C	4.82244

Figure 20. Toughness grouped by statistical significance.

The mean toughness for the rSSps bandaging complex experimental groups were considerably less than that of Band-Aid or Tegaderm, however they have similar toughness to that of human skin in all cases. Similar toughness to skin could potentially provide better mechanical support to a larger wound.

Elastic Modulus:

The modulus of elasticity measures the material's resistance to elastic deformation. A high elastic modulus denotes a material that is inelastic, usually in the GPa range. For a bandaging application, a very inelastic material would bring discomfort as the bandage would not change with the patient. A very low modulus of elasticity would also be undesirable, as the bandage would not provide necessary support/protection to the wound. Human skin was found to have an average elastic modulus of 98.97 ± 97 MPa, a highly variable value due to the anisotropic nature of human skin as well as the differences of skin properties in different areas of the body (Gallagher et al., n.d.). The elastic moduli of the rSSPs bandaging system and commercially-available products is shown in Figure 21.
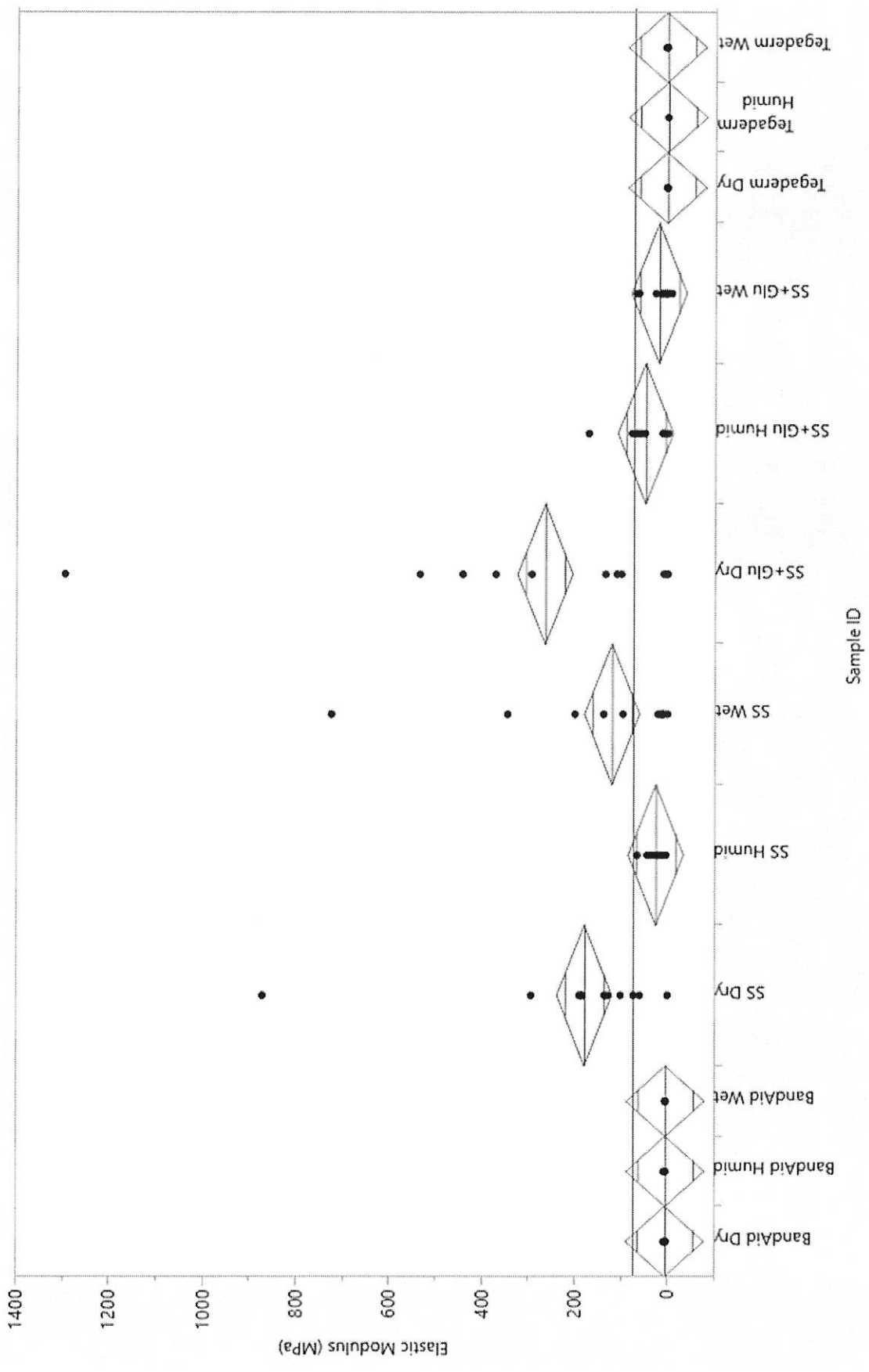
Figure 21. Comparison of Elastic Modulus.

The elastic moduli for the rSSps bandages were much greater than Tegaderm/Band-Aid. In dry conditions, this difference was accentuated. Commercial products, likely due to their water-proof nature, did not show much variance in wet, dry, or humid conditions. As the nylon/rSSps mat is semi-permeable, the water could be absorbed by the bandage and result in a lower modulus of elasticity more similar to the elastic modulus observed in human skin. Significantly different samples are labeled in Figure 22.

◢ Connecting Letters Report

Level				Mean
SS+Glu Dry	A			264.13537
SS Dry	A	B		178.35313
SS Wet		B	C	119.51718
SS+Glu Humid		B	C	48.30444
SS Humid			C	25.02537
SS+Glu Wet			C	20.16460
BandAid Dry			C	4.98768
BandAid Humid			C	3.83313
BandAid Wet			C	3.63242
Tegaderm Wet			C	3.36926
Tegaderm Dry			C	2.89122
Tegaderm Humid			C	1.64317

Figure 22. Modulus of elasticity grouped by statistical significance.

Strain at failure:

Strain at failure denotes the extent to which a material can be deformed without failure. In a bandaging application, the bandage should be able to withstand strain at failure equal to or greater than what the natural skin can withstand. In human skin, average strain at failure values were found to be $25.45 \pm 5.07\%$ (Gallagher et al.). The strain at failure of the rSSps bandages and commercially-available products is shown in Figure 23.
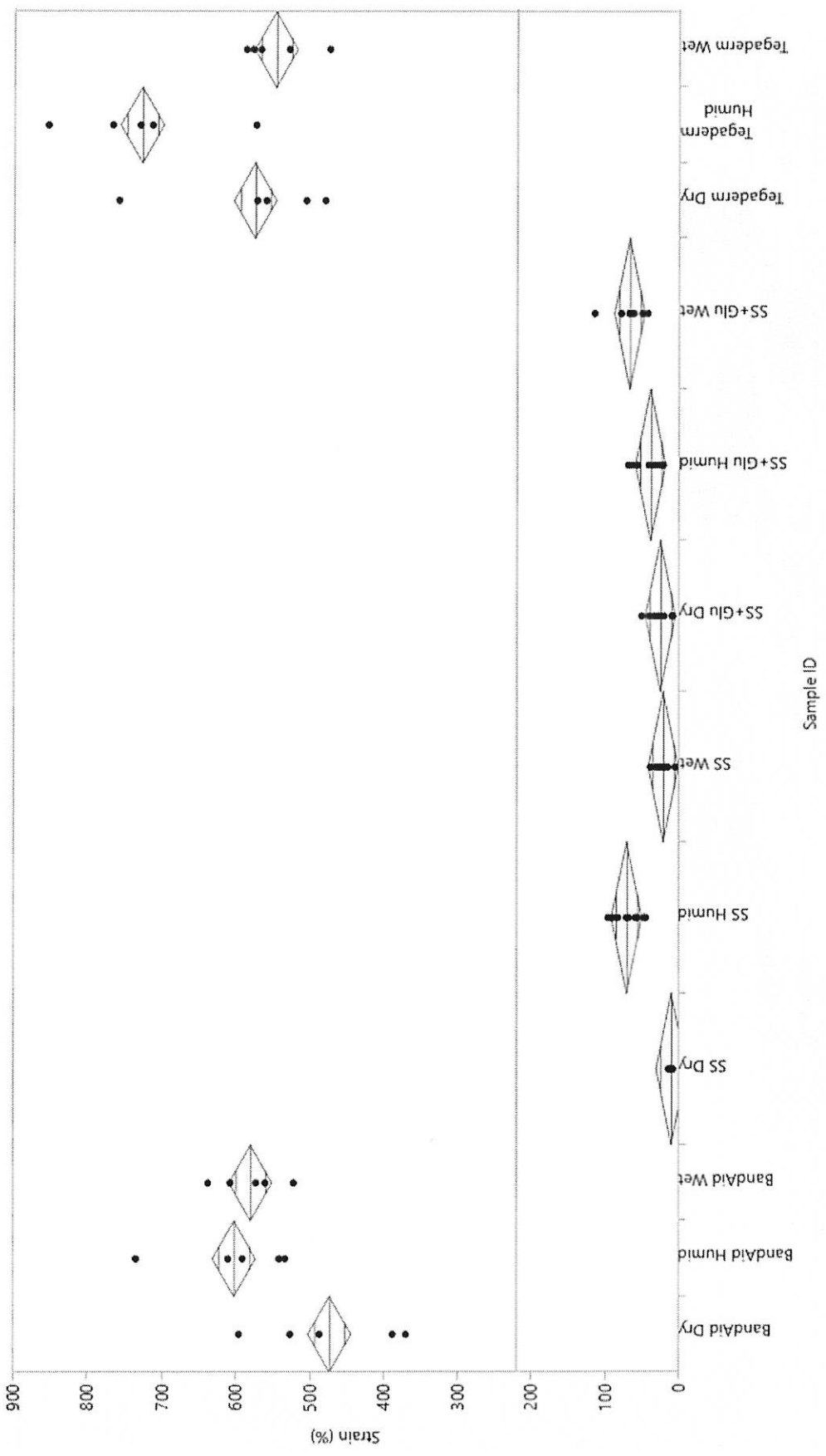
Figure 23. Comparison of strain at failure.

The highly elastic natures of Tegaderm and Band-Aid can be noted in above Figure 23. As has been observed previously, the rSSps bandage becomes more elastic in humid and wet conditions conditions. The samples are grouped by statistical significance in Figure 24.

⊿ **Connecting Letters Report**

Level					Mean
Tegaderm Humid	A				727.83927
BandAid Humid	B				601.73479
BandAid Wet	B				579.58185
Tegaderm Dry	B				575.19696
Tegaderm Wet	B				547.14594
BandAid Dry		C			472.90854
SS Humid			D		70.02188
SS+Glu Wet			D E		66.49676
SS+Glu Humid			D E F		38.18770
SS+Glu Dry			D E F		24.60110
SS Wet			E F		20.68700
SS Dry			F		9.56714

Figure 24. Strain at Failure Grouped by Statistical Significance.

As noted in the elastic moduli section, the rSSps bandage comples is not as elastic at the Tegaderm and Band-Aid controls. The rSSps/nylon bandages resulted in considerably lower strain at failure values than the Tegaderm or Band-Aid controls, however the values were much closer to the 25% strain typical of human skin. In dry conditions, spider silk underperformed the strain at failure of skin, but in a wound application, the bandage is more likely to be under high moisture condtions.

Ultimate Tensile Strength:

Ultimate tensile strength denotes the maximum resistance to elongation exhibited by the material at any point before failure. The average ultimate tensile strength of human skin was found to be 27.2 ± 9.3 MPa (Gallagher et al.). Optimal values would mimic the natural skin, as a higher value could lead to discomfort when the skin is able to stretch more than the bandage, and a lower value could lead to bandage inefficiency. The ultimate tensile strength of the rSSps bandaging structure and commercially-available products is shown in Figure 25.

Figure 25. Comparison of Ultimate Tensile Strength
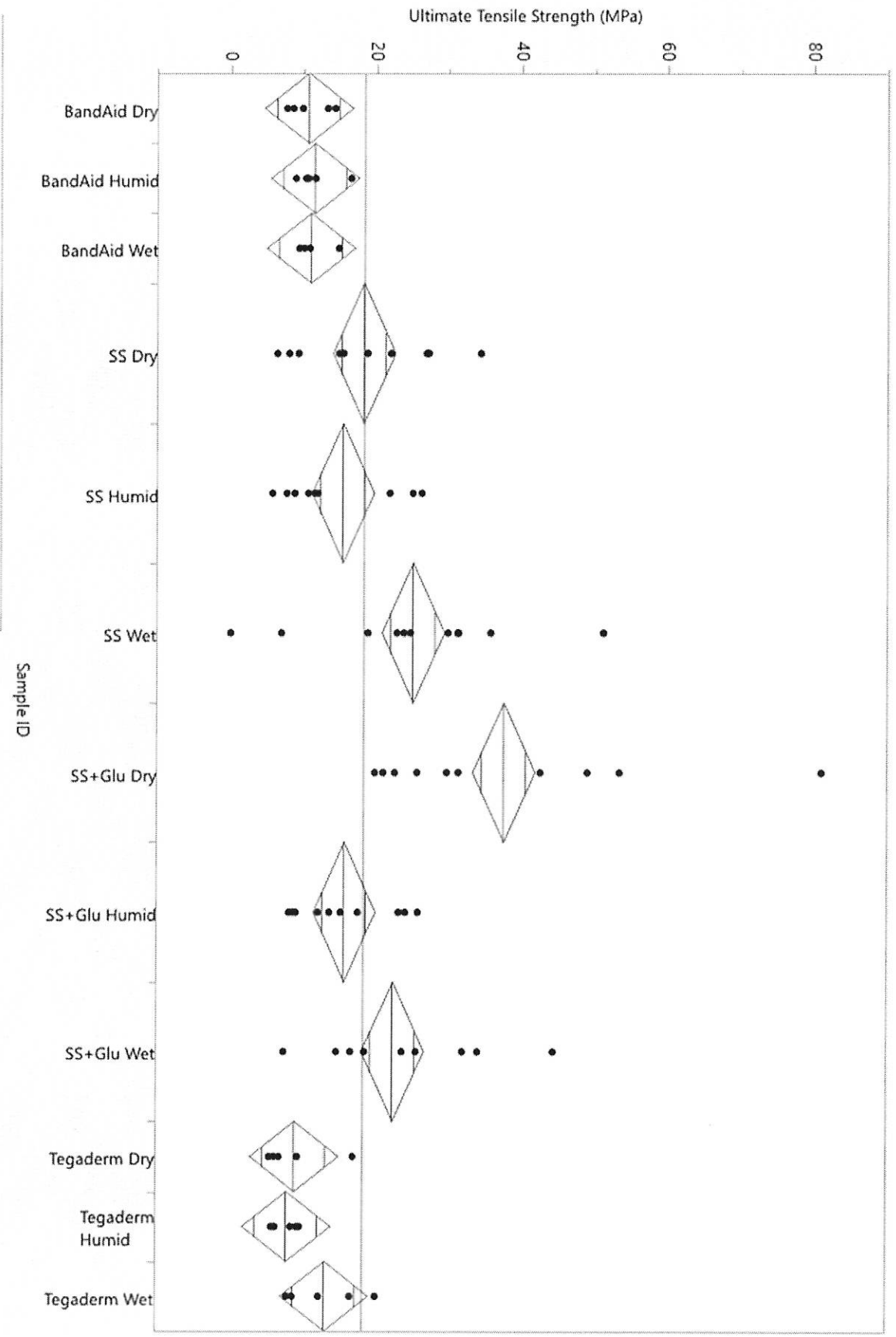
Ultimate tensile strength was the most similar of any tested parameter. On average, rSSps formulations exceeded the values of both Tegaderm and Band-Aid, however, as can be seen in Figure 26, these values were often insignificant.

⊿ **Connecting Letters Report**

Level					Mean
SS+Glu Dry	A				37.595118
SS Wet		B			25.081620
SS+Glu Wet		B	C		22.403089
SS Dry		B	C	D	18.252513
SS+Glu Humid		B	C	D	15.664019
SS Humid		B	C	D	15.401418
Tegaderm Wet		B	C	D	13.100770
BandAid Humid			C	D	11.449892
BandAid Wet			C	D	10.901183
BandAid Dry			C	D	10.591657
Tegaderm Dry				D	8.904093
Tegaderm Humid				D	7.841741

Figure 26. Ultimate tensile strength grouped by statistical significance.

The rSSps adhesive formulations were not significantly higher than Tegaderm or Band-Aid, however they were closer to the average ultimate tensile strength for human skin. The rSSps adhesives in wet environments resulted in the closest values to that of human skin.

Conclusion

The rSSps adhesives, both with and without glutaraldehyde, replicated the mechanical properties of human skin closer than either commercial product, Tegaderm or Band-Aid. The toughness of both rSSps adhesive/mat formulations were significantly less than that of the commercial alternatives, but more similar to the value of human skin. The elastic moduli of the rSSps adhesive/mat formulations were higher than Tegaderm or Band-Aid, but again closer to human skin. The bandage failed at similar values to human skin, which was much lower than the strain failure values found for Tegaderm and Band-Aid. The most similar property to human skin for the rSSps adhesive/mat complex was the ultimate tensile strength, in which very little statistical significance was found between groups, and most were below the strength of human skin.

From the data that were collected, it was suggested that a "second skin" made from an rSSps adhesive a nylon/rSSps electrospun mat could match the mechanical properties of skin. In contrast to Tegaderm and Band-Aid, the rSSps adhesive has the potential to be loaded with growth-inducing and/or anti-microbial additives. An application of this loading will be demonstrated hereafter.

H. Chlorhexidine Gluconate Release

Rationale

Chlorhexidine gluconate is an antimicrobial compound useful in preventing infection. This testing was to evaluate the chlorhexidine release when incorporated into the bandage through the rSSps adhesive. This simultaneously tested the strength of the interaction between adhesive and mat complex and whether the addition of glutaraldehyde increases this interaction.

Decisions
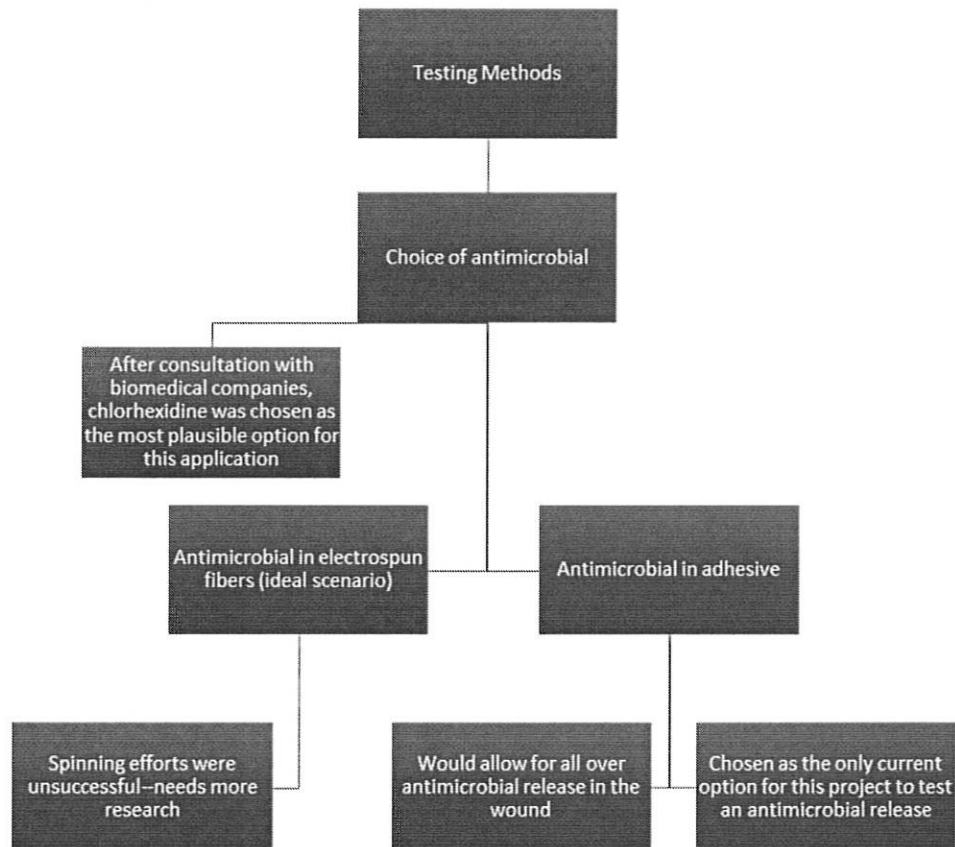
Figure 27. Decision tree for chlorhexidine gluconate release.

After the decision was made to only include the antimicrobial in the adhesive for this particular test, it was decided to test the mat/adhesive complex for antimicrobial release under total immersion in phosphate-buffered saline. These testing conditions would illustrate the fastest release possible for the bandaging material and allow for the best opportunity for a controlled experiment. However, a wound environment will have varying levels of moisture and the bandaging material would likely never be completely submerged as was done in this experiment.

Materials

- UPLC Chromatographic System

- Acquity UPLC BEH C18 Column

- Centrifugal Dryer

- Phosphate-buffered saline

- 6 well cell culture plate

10% Nylon + 10% rMaSp1 dope:

- 3 mL Formic Acid 97%

- 0.3 g Nylon (Rio 20# Monofilament)

- 0.3 g rMaSp1

12% 50/50 rMaSp1/rMaSp2 rSSps adhesive:

- 3 mL distilled water

- 0.18 g rMaSp1

- 0.18 g rMaSp2

- 0.03 µl Glutaraldehyde (50% stock solution)

12% 50/50 rMaSp1/rMaSp2 + 25% Chlorhexidine gluconate-loaded adhesive:

- 1 mL 24% 50/50 rMaSp1/rMaSp2 adhesive

- 1 mL 50% chlorhexidine stock solution

Methods

In this study, three sets of experimental samples (n = 2) were prepared. These three experimental sets were one set of nylon/rMaSp1 electrospun mats with no adhesive and two sets of nylon/rMaSp1 electrospun mats coated with the two different adhesives: 50/50 rMaSp1:rMaSp2 and 50/50 rMaSp1:rMaSp2 with 25% glutaraldehyde. These two sets including the rSSps adhesive included chlorhexidine gluconate to a final concentration of 25%. Analysis of the release was completed with ultra performance liquid chromatography (UPLC) and results were analyzed using a previously prepared standard release curve for chlorhexidine gluconate.

The electrospun mats and adhesives were prepared according to the methods detailed in Appendices A and B. Once these samples were prepared, they were each placed in 5 mL of 1x phosphate buffered saline in individual wells of a six-well cell culture plate. Initial 1 mL samples from each well were taken. The well plate was then placed in a 37 °C incubator. 1 mL samples were taken from each well at 24-hour intervals for 14 days. Each day, all of the phosphate-buffered saline was replaced, and the samples were placed in a dry six-well plate with a fresh 5 mL of phosphate-buffered saline.

Results

The analysis of chlorhexidine release showed a release for both SpiderSkin formulations up to four days. The rSSPs adhesive with glutaraldehyde exhibited a secondary peak at about three days, indicating that the cross-linking agent aided in the interaction between nanofiber and rSSPs adhesive. The release profiles can be seen in Figure 28.

Chlorhexidine Gluconate Release Profiles
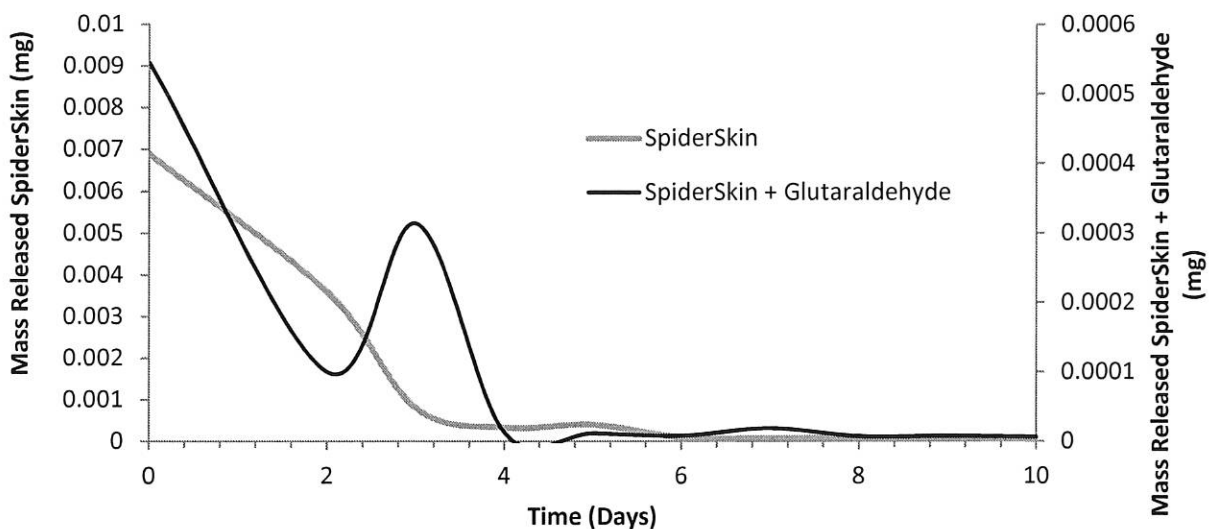
Figure 28. Chlorhexidine gluconate release profile from standard rSSps mat/adhesive complex with no cross-linking.

Conclusion

The chlorhexidine gluconate released much more rapidly than expected for each of the samples. This may be due to the majority of the chlorhexidine being around the bandage instead of intercollated in between the mat fibers. Because of this, the majority of the chlorhexidine was released in the first two to three days and, in the case of the standard bandage with no cross-linking, the drug was almost entirely eluted from the bandage by day four (Figure 28). However, the mat/adhesive interaction is evident in that not all of the antimicrobial was immediately released into the soluble phase. Because the adhesive is aqueous, it is a reliable assumption that the drug remained "stuck" in the nanofibers through an adhesive/fibrous mat interaction. These interactions seemed to be increased with the addition of glutaraldehyde as a cross-

linking agent. In this case, a large burst of chlorhexidine release was seen as late as day three (Figure 28). The glutaraldehyde also had a slight release after day five and up to day eight.

I. Cytotoxicity Evaluation

Rationale

Toxicity testing of the bandage was important to the project to demonstrate the ability of SpiderSkin to promote healing without causing further damage to the epidermis. Any toxic materials which are able to leach out of the bandage would pose a threat to the healing ability of the wound. There are generally three types of cytotoxicity tests that were available to choose from. First, the direct contact method involves growing a culture of cells to confluency, placing the biomaterial on the surface and evaluating cytotoxicity using live/dead cell counts. Second, agar diffusion works by minimizing the influence of physical contact between cells and biomaterial. Agar is placed between the cells and the material and the leachables from the biomaterial are allowed to travel through the agar. The final method is the elution method. In this method the biomaterial is soaked in media, allowing leachables to travel from the biomaterial into the media. This same media is then used to grow cells. Advantages and disadvantages of each of these methods are outlined in Figure 29.

	Direct contact	Agar diffusion	Elution
Advantages	Eliminate extraction preparation	Eliminate extraction preparation	Separate extraction from testing
	Zone of diffusion	Zone of diffusion	Dose response effect
	Target cell contact with material	Better concentration gradient of toxicant	Extend exposure time
	Mimic physiological conditions	Can test one side of a material	Choice of extract conditions
	Standardize amount of test material or test indeterminate shapes	Independent of material density	Choice of solvents
	Can extend exposure time by adding fresh media	Use filter paper disk to test liquids or extracts	
Disadvantages	Cellular trauma if material moves	Requires flat surface	Additional time and steps
	Cellular trauma with high density materials	Solubility of toxicant in agar	
	Decreased cell population with highly soluble toxicants	Risk of thermal shock when preparing agar overlay	
		Limited exposure time	
		Risk of absorbing water from agar	

Figure 29. Description and Comparison of Cytotoxicity Testing Methods.

Decisions

The method chosen was most similar to the elution method. This method was decided upon because it was a quick and simple method to determine the cytotoxicity of leachable chemicals from the bandaging material. Other possible methods could have included animal testing models, sample submersion cell culture, or chemical testing of leachables into solution. The animal testing model was decided against due to the complexity of testing and the high variability and interfering variables. Sample submersion cell culture was decided against due to lack of materials and the added complexity of the testing model. Chemical testing of leachables into solution was not used because it would be difficult to tie these results to cytotoxicity in vivo. MRC-5 cells were used because they are commonly used in cytotoxicity studies and could be obtained from GE Healthcare Hyclone. The design decision tree can be seen in Figure 30.
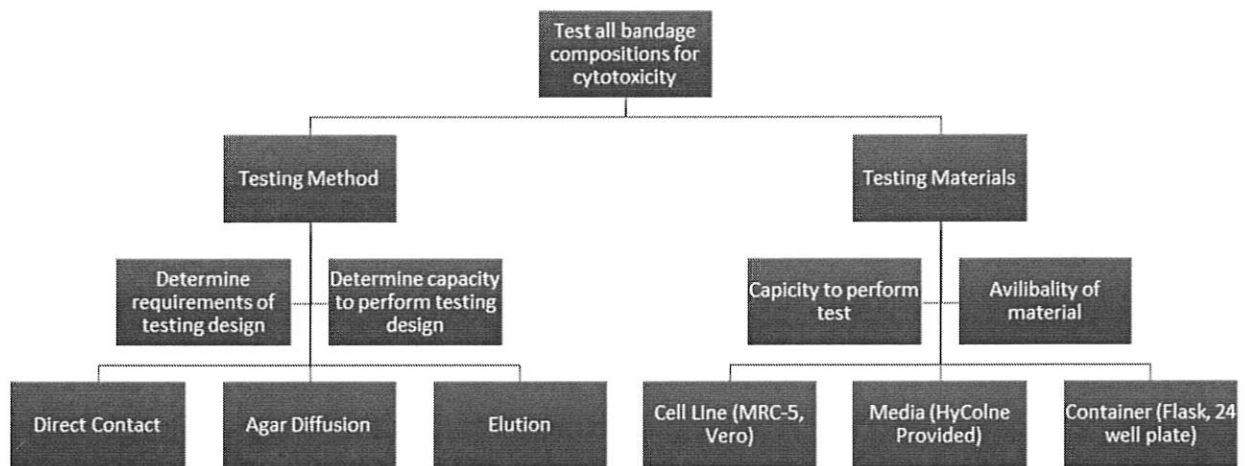
Figure 30. Decision tree for cytotoxicity evaluation.

Materials

- 8 mL screw cap tubes with a flat base
- Nunclon 24 well plates
- MRC-5 Lung cells
- DMEM + 10%FBS media
- 50% glutaraldehyde in solution
- Trypsin
- ViCell

10% Nylon + 10% rMaSp1 dope:

- 3 mL Formic Acid 97%
- 0.3 g Nylon (Rio 20# Monofilament)
- 0.3 g rMaSp1

12% 50/50 rMaSp1/rMaSp2 rSSps adhesive:

- 3 mL distilled water
- 0.18 g rMaSp1
- 0.18 g rMaSp2
- 0.03 μl Glutaraldehyde (50% stock solution)

      o   Bovine Serum Albumin (BSA)

12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde rSSps adhesive:

- 1 mL 12% 50/50 rMaSp1/rMaSp2 rSSps adhesive
- 20 µl 50% glutaraldehyde stock

Methods

The desired amount of electrospun mat/rSSps adhesive was weighed out using an analytical scale (weights within the range of 10-30 mg). Weighed out mats were placed in petri dishes and exposed to UV light in a sterile hood for 20 minutes on each side. Sterilized mat was then added to 5 mL of DMEM + 10% FBS media in 8 mL tubes. The tubes were then allowed to shake in an incubator for 20-24 hours, allowing the mats to leach into the media. The 8 mL tubes were then placed under a sterile hood and the mat material was removed using tweezers. The media was then run through a 22 um syringe filter to remove any mat particulate remaining in the media. 4 mL of each media treatment was then placed in a new 8 mL tube, and the remainder was discarded. The amount of inoculum required to get 10,000 cells/cm^2 was then calculated, and each of the 8 mL tubes was inoculated with the same volume of inoculum. 1 mL of this inoculated media was then placed in the 4 wells of a column of a 24 well plate. The plate was covered and placed in an incubator. Fresh media, which had not been exposed to any mat sample, was used as the negative control, while a concentration range of 50% glutaraldehyde was used as the positive control. The desired volume of glutaraldehyde was added to separate wells in the 24 well plate.

To measure the cell growth of each media treatment, the cells were pulled into suspension using standard adherent cell passaging techniques. 300 µL of trypsin was used and once the cells had released 600 µL DMEM + 10% FBS was added. The cell densities were then determined on the ViCell Cell Counter.

Results

Initial Trial:

The initial trial was conducted with the mats and concentrations expressed in Table 4. Each tube was inoculated at 5000 cells/cm^2. As can be observed from Figure 31 there was a significant change in coloration of the media during the 24-hour incubation period. This phenomenon was likely due to contamination of the mats when they were immersed in the media. Samples were eventually discarded due to lack of cell growth and contamination issues.

Table 4. Initial Trial Cytotoxicity media treatments

Media Treatment	Concentration
Fresh Media (No Treatment)	N/A
Mat with SS + Glut. adhesive	10 mg

Mat with SS + Glut. adhesive	20 mg
Mat with SS + Glut. adhesive	30 mg
Mat with SS adhesive	10 mg
Mat with SS adhesive	20 mg
Mat with SS adhesive	30 mg
SS Mat with No Adhesive	40 mg
50% Glutaraldehyde solution	10, 20, 30, 40, 100, 200, 300, and 400 ul

(A)

(B)

(C)

(D)

Figure 31. Cytotoxicity testing. (A) media samples with treatment before incubation (B and C) media samples after 24-hour incubation. (D) 24-well plates with post-incubation media.

UV Trial 1:

The initial trial was conducted with the mats and concentrations expressed in Table 5. Vials were inoculated at 10,000 cells/cm^2. Prior to immersion in the media, each mat sample was sterilized using UV radiation as can be seen in Figure 32. Cell counts were taken on day 5 after inoculation and results

can be seen in Figure 33. It was observed that viable cell density readings were outside the recommended range of the ViCell cell counter.

Table 5. UV trial 1 cytotoxicity media treatments

Media Treatment	Concentration
Fresh Media (No Treatment)	N/A
Mat with SS + Glut. adhesive	10 mg
Mat with SS + Glut. adhesive	30 mg
Mat with SS adhesive	10 mg
Mat with SS adhesive	30 mg
50% Glutaraldehyde solution	5, 10, 15, and 20 ul
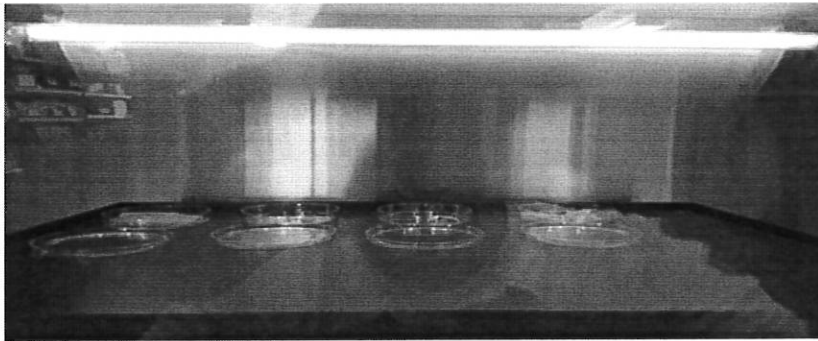
Figure 32. UV techniques and setup for sample sterilization

Figure 33. Cytotoxicity testing. (A) media samples with treatment before incubation (B) media samples with treatment after 24-hour incubation.

UV Trial 2:

The initial trial was conducted with the mat formulations and concentrations expressed in Table 6. Vials were inoculated at 10,000 cells/cm^2. Prior to immersion in the media, each mat sample was sterilized using UV radiation. After inoculation, the media was extruded through a 22 um syringe filter to remove mat particulate matter. As can be observed in Figure 34 there was once again some discoloration of the media indicating contamination during the incubation period. Cell counts were taken on day 6 after inoculation and results can be seen in Figure 35.

Table 6. UV trial 2 cytotoxicity media treatments

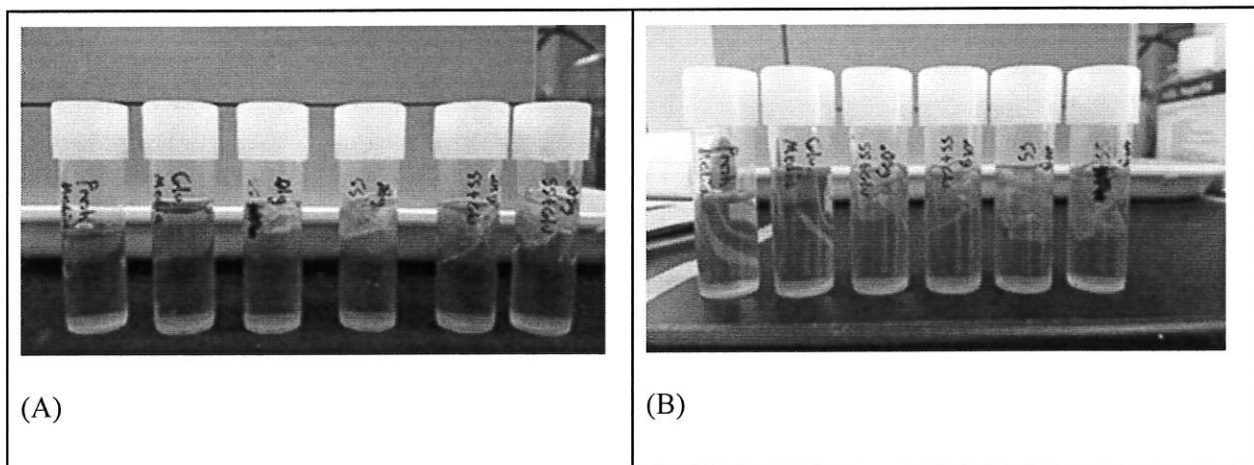| Media Treatment	Concentration
Fresh Media (No Treatment)	N/A
Mat with SS + Glut. adhesive	10 mg
Mat with SS + Glut. adhesive	20 mg
Mat with SS + Glut. adhesive	30 mg
Mat with SS adhesive	10 mg
Mat with SS adhesive	20 mg
SS Mat with No Adhesive	30 mg
50% Glutaraldehyde solution	1, 2, 3, and 4 ul
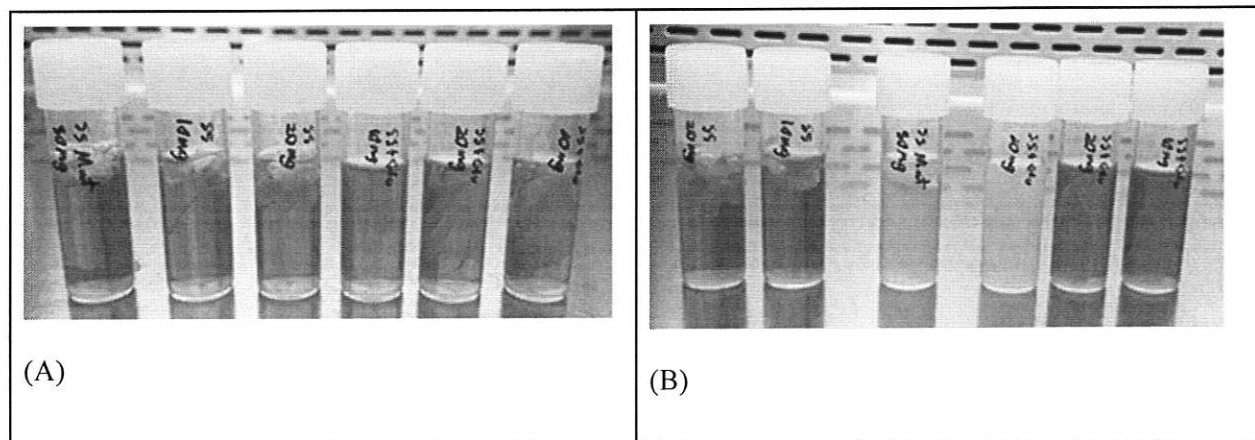
Figure 34. Cytotoxicity testing. (A) media samples with treatment before incubation (B) media samples with treatment after 24-hour incubation.
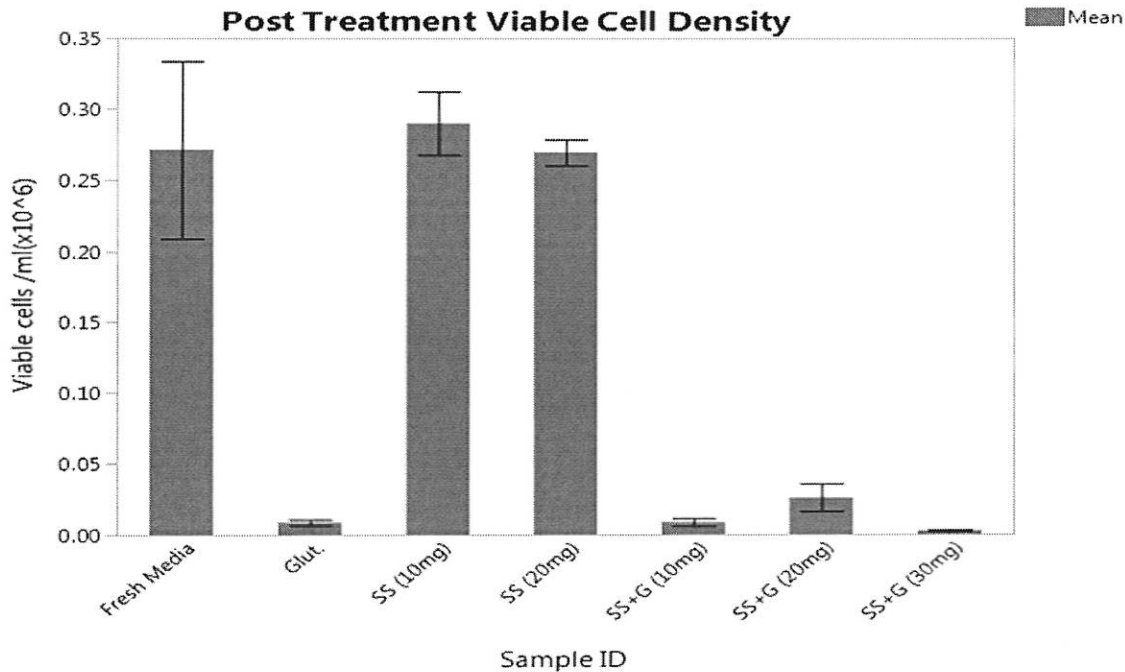
Figure 35. Average Viable Cell Density under various treatments. Error bars constructed using one standard error from the mean.

Conclusion

The purpose of these tests was to determine the plausibility of using these electrospun mat/adhesive formulations as bandages. Bandages which leach cytotoxic by-products would not meet the overall design criteria of creating a non-cytotoxic bandage. As can be observed in Figure 35, all SpiderSkin bandages including glutaraldehyde had significantly lower viability. Due to these results, there will need to be caution and further research done for the glutaraldehyde treated bandages. However, it is important to note some of the testing parameters in reference to these results. MRC-5 is a notoriously difficult cell line to work with. It would make the results stronger to have more replications of each condition grown in larger volumes. This would ensure that a lack of growth was in fact due to bandage properties and not bad growth populations. That being said, it does appear very clear from the data obtained that glutaraldehyde-leaching caused cytotoxicity (seen in Table 7). It has been shown in the literature (Bhamidipati, Coselli, & LeMaire, 2012) that glutaraldehyde can be used for indwelling medical devices without causing a cytotoxic response. These applications are made possible when the glutaraldehyde cross-links completely and there is no excess glutaraldehyde to crosslink with unintended tissues. This excess glutaraldehyde was likely the cause for the cytotoxic response seen in the SpiderSkin bandage material. It would be beneficial to do more testing on the required concentrations of glutaraldehyde needed to ensure complete cross-linking, without compromising the biocompatibility of the bandaging material.

Table 7. Score chart for cytotoxicity assay

	rSSps + Gluteraldehyde adhesive/rSSps + Nylon Mat	rSSps adhesive/rSSps+Nylon Mat
Score (1-5 based on cell growth)	1	5

Final Design Review

The decision progression throughout this design process can be seen in Figure 36. The areas highlighted in blue show the paths followed that led to the final product, SpiderSkin. While there are still many areas left to be explored in the design of an interactive and tunable bandaging system, the final product of this project serves as a prototype.
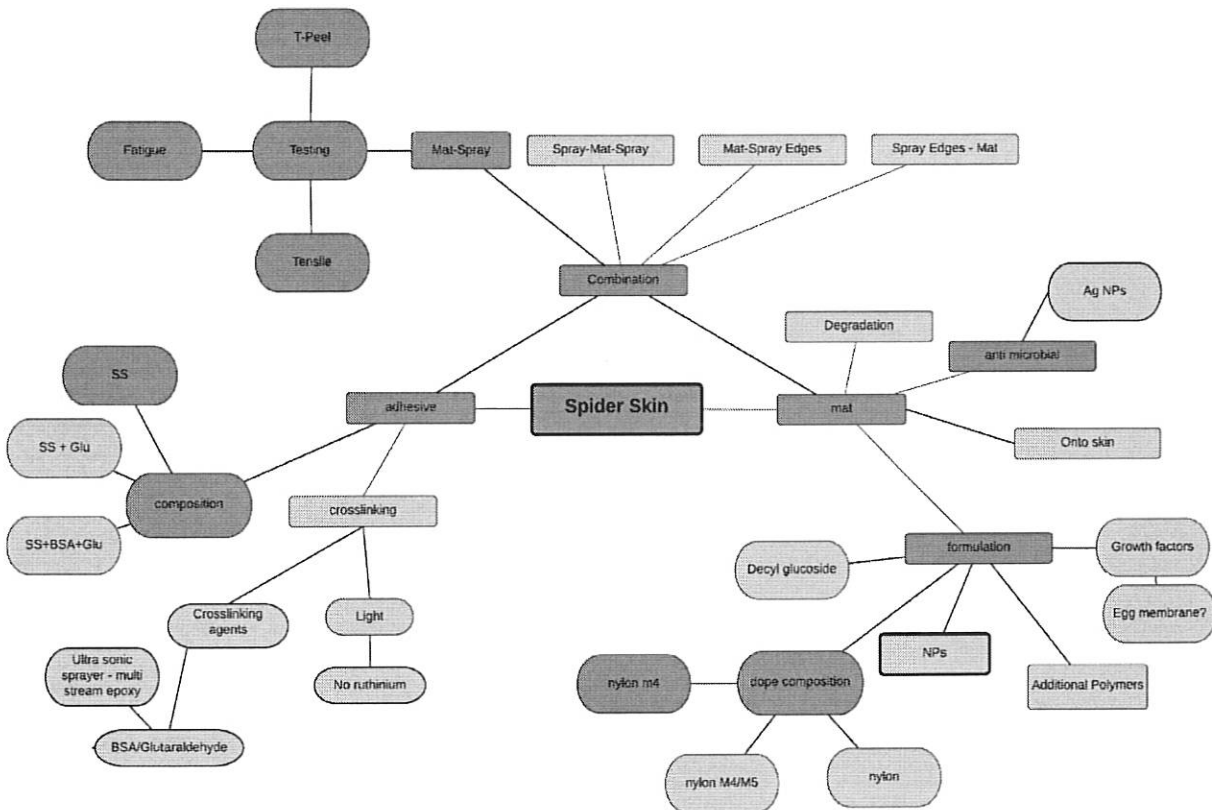
Figure 36. Final flow diagram. Aspects incorporated into the final design have blue fill.

The final formulation of the mat and adhesive for SpiderSkin was chosen to be the Nylon/rMaSp1 and 12% 50/50 rMaSp1/rMaSp2, respectively. This formulation resulted in similar mechanical properties to the Nylon/rMaSp1 and 12% 50/50 rMaSp1/rMaSp2 + 1% Glutaraldehyde formulation, however in cytotoxicity testing glutaraldehyde at 1% concentration was causing cytotoxicity. Without sacrificing mechanical stability, the chosen formulation maintains biocompatibility with mammalian cells.

In mechanical properties, the chosen formulation, deemed "SpiderSkin," is more comparable to skin than to Tegaderm or water-proof Band-Aid. While providing protection similar to these commercial products, the product is also able to provide support similar to that of skin for larger epithelial injuries.

As was expected from the biocompatibility properties of spider silk, SpiderSkin does not decrease the viability of cells. This leaves room for further exploration of the possibilities of SpiderSkin as a cell

scaffold. Antimicrobial incorporation into the adhesive produced a strong initial burst of chlorhexidine, however the release was not sustained over a desired period of seven to ten days.

The initial goals of this project were to create a bandaging product that could compete with existing bandaging products, and add more factors to promote wound healing. Although most of the objectives were achieved, the ones that were not have been noted for future work. Figure 37 shows the simplified design process, while Table 8 illustrates the initial objectives compared to the results that this design was able to achieve.
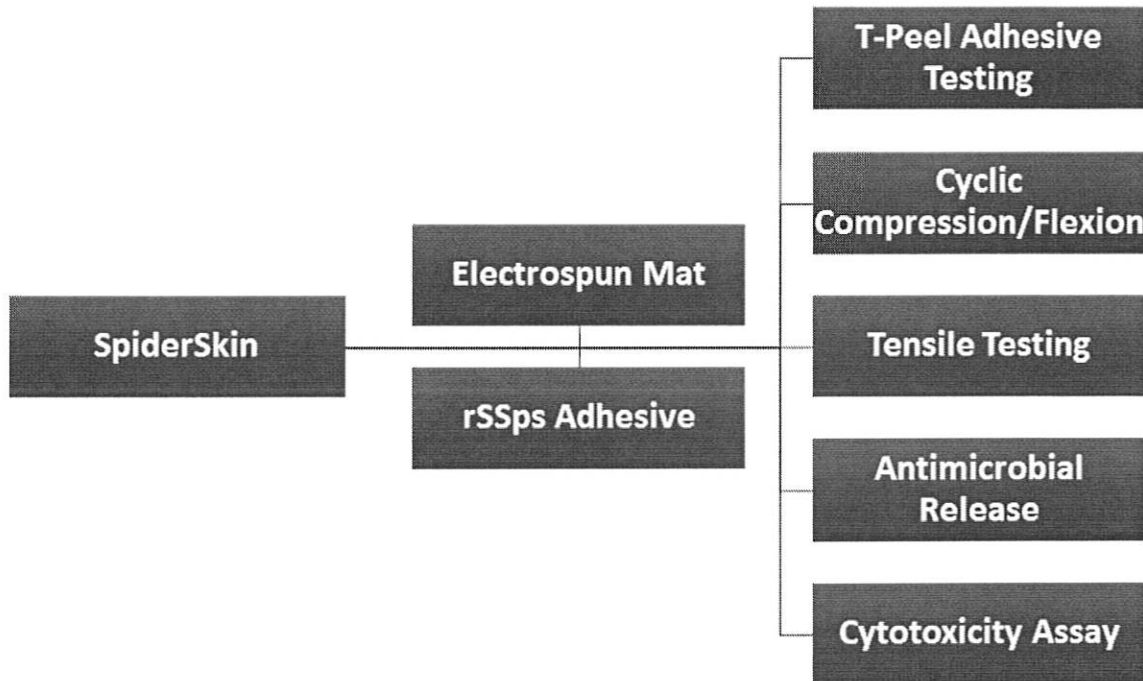
Figure 37. Overview of the design process for the final product.

Table 8. Comparison between initial objectives and final results.

Initial Objectives	Results Achieved
Use glutaraldehyde as a cross-linking agent to strengthen the mechanical properties of the adhesive.	In some cases, glutaraldehyde offered statistically significant increases in mechanical properties under different environmental conditions. However, these increases were not drastic.
Create a mat/adhesive complex with and without cross-linking that stretches without removal in cyclic flexion and compression on pigskin.	Under cyclic compression the rSSps and rSSps + glutaraldehyde samples produced similar results to the Tegaderm and Band-Aid controls, with minimal from the pigskin. Under cyclic flexion, the rSSps and rSSps + glutaraldehyde samples had similar results to the Band-Aid samples, and outperformed the Tegaderm samples. The SpiderSkin adherence was not affected by wetness and humidity compared to the controls.
Ensure that the bandage does not exibit cytotoxicity, with paticurlar focus glutaraldehyde treated bandages.	Excess glutaraldehyde that was not cross-linked in the SpiderSkin bandage was shown to be cytotoxic. The SpiderSkin bandage without glutaraldehyde did not exhibit any cytotoxicity.
Create an rSSps antimicrobial adhesive using chlorhexidine and exhibit a release of the drug over time.	A release profile of chlorhexidine was created from the rSSps and rSSPs + glutaraldehyde adhesives. The adhesive with the glutaraldehyde showed a slightly longer release profile, and a secondary peak around day three.
Design a mat/adhesive complex that adheres to the skin as well as or better than conventional skin adhesives.	SpiderSkin showed ability to maintain more adherence under dry wet and humid conditions when compared to Tegaderm and Band-Aid controls.

Discussion

Selection of Mat/rSSps Adhesive

By doing a criteria comparison test using the criteria: ease of spinning, material availability, and the pressure sensitive tape test results it was possible to obtain an rSSps adhesive/mat formulation that would work well for this design process. Using these criteria as guidelines helped create a product that would be easy to work with as well as easy to test. Of course, in the future much more research can be done on altering these formulations to achieve a wide variety of mechanical properties depending on the desired application/area of the body that bandaging material is needed. Nylon was not the ideal polymer to blend with the rSSps to make the final electrospun mat mostly due to the fact that it is not biodegradable. However, other polymers proved to be too hard to spin and combine with the rSSps in order to complete their testing. Without further investigation into the electrospinning methods using these polymers, it is not possible to tell whether nylon was the ideal choice for this product. However, adequate mechanical properties were achieved using nylon and the design objectives were met.

T-Peel Testing

Using the T-Peel testing method was the best way to quantify the adhesive properties of the SpiderSkin product. This testing method is commonly used for tissue adherence testing and allowed for a quantitative evaluation. The SpiderSkin adhesive without glutaraldehyde did not exhibit a decreased adherence when exposed to different environmental conditions like moisture and complete submersion. Although this adherence did not quite match the adherence of the water-proof Band-Aids, it did outperform Tegaderm in every environmental condition. Furthermore, the cross-linked rSSps adhesives exhibited a stronger adherence to pigskin than their counterparts especially in the dry and wet testing categories. With these results, it is reasonable to conclude that the cross-linking did allow for the adhesive to bond more strongly with the skin tissue. This finding was statistically significant, however it was not as drastic of a change as was expected. This could be due to the fact that the concentration of glutaraldehyde to optimize cross-linking was not studied. There may be a better method to include this component to obtain the best mechanical properties possible.

Cyclic Fatigue Testing with Bandages in Compression

Cyclic fatigue testing was a valuable parameter for the quantification of the durability of SpiderSkin. As bandages are constantly being subjected to compression/extension, cyclic testing is a necessity in evaluating the viability of the product in real-world application. SpiderSkin matched the compression durability of Tegaderm and Band-Aid, and exceeded the result of Band-Aid under wet conditions.

As SpiderSkin, Tegaderm, and Band-Aid all yielded high results, further testing should be conducted to evaluate the bandaging systems under a greater number of cyclic compressions. In this testing, glutaraldehyde did not seem to exhibit a significant impact on the durability of the product, although that impact may be observed under more extreme cyclic testing.

Cyclic Fatigue Testing with Bandages in Flexion

Similar to cyclic testing in compression, cyclic flexion testing is valuable for quantifying the durability of the SpiderSkin. An inability to flex with the needs of the patient could cause the bandage to come off prematurely, increase pain, or aggravate the wound in its recovery stages. More variability was seen in flexion than compression, and SpiderSkin yielded higher results than commercial products in almost every condition.

In flexion testing, glutaraldehyde seemed to decrease the durability of the bandage. This is likely due to an increased number of crosslinked proteins, inhibiting the ability of the bandage to stretch with the skin. Band-Aids were most able to compete with SpiderSkin, and Tegaderm was the least durable. In summary, SpiderSkin matched or exceeded the durability of its commercial counterparts in every condition.

Tensile Testing of Materials

Tensile testing is a valuable metric for describing the mechanical properties of the bandage itself. As previous T-Peel and fatigue tests are valuable for comparing the adhesive properties of the bandage, tensile testing describes the entire bandaging system, encompassing both the mat and adhesive. Individual parameters will be discussed in their respective spheres, followed by an integrative discussion of the properties and their implications to the product as a whole.

Toughness

SpiderSkin was considerably less tough than Tegaderm and Band-Aid. This phenomenon will be explained further in subsequent parameters obtained via tensile tests, however, the lower resultant values for toughness are due to the rigidity of the SpiderSkin bandage relative to the elastic natures of both Tegaderm and Band-Aid.

Elastic Modulus

SpiderSkin had a much higher elastic modulus than Tegaderm and Band-Aid. Higher values of elastic moduli indicate that greater pressure must be exerted before deformation in the product will occur. As was expected, the dry condition resulted in higher elastic moduli than in wet/humid conditions, as water permeating the bandage allows the bandage to relax and become more elastic.

SpiderSkin more closely mimicked the elastic modulus of human skin than did either commercial product. This can be advantageous as the body seeks to repair the wound. Beyond providing protection for the wound, spidersilk can give support similar to that of skin. As underlying repair mechanisms and newly formed tissue are very delicate and sensitive to mechanical perturbations, the relative inelasticity of SpiderSkin can prevent excessive stress from disturbing sensitive tissue.

Strain

Tegaderm and Band-Aid were much more elastic than SpiderSkin. Although a higher elasticity would be preferable if the elastic modulus and were able to be maintained, SpiderSkin resulted in similar/higher values of strain as compared to human skin. Whereas Tegaderm and Band-Aid stretch to many times their original size, Spiderskin averaged about 1.5 times its original length.

Ultimate Tensile Strength

As is often correlated with a higher modulus of elasticity, SpiderSkin resulted in higher ultimate tensile strength values than did Tegaderm or Band-Aid. As was discussed previously, this can be advantageous for providing support to underlying tissue as it is very sensitive during the repair process. Once again, SpiderSkin more closely mimicked the properties of human skin as compared to the commercial products Tegaderm and Band-Aid.

Integrative Discussion

Spiderskin has favorable mechanical properties for use in severe epithelial injury. While providing needed protection, having similar strength and elasticity allows the bandage to provide support beyond what commercial products provide. Thus, instead of mechanical stresses being applied to delicate tissue, the perturbations will be dispersed along the bandage and applied to healthy surrounding tissue.

Chlorhexidine Release

The ability of this bandaging product to be functional and promote healing is integral to the design product and it creates the possibility to set this product apart from other products on the market. Using chlorhexidine in the rSSps adhesive instead of incorporating it into the electrospun mat fibers was not ideal, however with further research it is proposed that this would be plausible in the future by altering the concentration of chlorhexidine in the electrospinning dope. Without this antimicrobial in the fibers, a longer and sustained release was not expected, however in this scenario it was possible to test the physical entanglement interaction between the rSSps adhesive and the electrospun mat nanofibers. Because the rSSps is aqueous, much of it was expected to release early, however the addition of glutaraldhyde allowed for more chlorehexidine gluconate to stay in the bandage upon submersion. This is evident by the high amount of mass released as late as day 3 (Figure 29). This level of mass release is comparable to the same amount that was released almost immediately upon submersion from the standard bandage. Even without the gluataraldehyde, however, it is evident that the physical entanglement of the rSSps adhesive/fibrous mat complex can hold in at least some of the antimicrobial. As you can see in Figure 28, even the standard bandage had a slight release of antimicrobial as late as day 4 submerged in phosphate-buffered saline. With these results in mind, it has to be noted that it would be rare in real-life application for this "second skin" material to be fully submerged for long amounts of time. Therefore, these results represent the fastest release possible. It is probably that the release may be more sustained over longer periods of time in conditions that have varying levels of moisture. For further research, it may be most beneficial to create this product with an antimicrobial both in the rSSps adhesive and in the fibers of the electrospun mat. Of course, the different concentrations of antimicrobial in the complex may need to be tuned for optimal effectiveness.

Cytotoxicity testing

The biocompatibility of the bandage is significant as it will be applied to compromised tissue. The focus of the cytotoxicity tests was to determine initial toxicity of spider silk bandage with spider silk based

adhesive and the effect of the addition of the cross-linking agent glutaraldehyde. The tests showed that the addition of glutaraldehyde to the adhesive did cause cytotoxicity. It is hypothesized that this was due to excess glutaraldehyde in our adhesive formulation which was not crosslinked. This non-crosslinked glutaraldehyde was free to interact with the cells and cause cell death or inhibit cell growth. Due to this finding, our final design product will be created without the addition of glutaraldehyde. The mechanical properties of the rssp's adhesive were satisfiability close to those of skin to accomplish our initial design criteria without the need for a crosslinking agent. It was, however, observed that the addition of the glutaraldehyde adhesive improved the release profile for chlorohexidine. It is recommended that further studies be done to determine the correct concentration of glutaraldehyde which will prevent cytotoxicity but still allow for crosslinking benefits. It has been shown in the literature (Bhamidipati, Coselli, & LeMaire, 2012) that glutaraldehyde can be used for indwelling medical devices without causing a cytotoxic response. Cell adherence studies would also be worthwhile to show bandage benefit to tissue regrowth.

Conclusions

Through an intensive design process, a prototype of the final product has been created. The final bandaging system has been called SpiderSkin.

Tensile testing results showed that SpiderSkin has mechanical properties more similar to human skin than commercial bandaging products such as Tegaderm Tape and water-proof Band-Aids. Further mechanical testing of the adhesive showed the ability of the adhesive (compared to the Tegaderm and Band-Aid controls) to stay on the skin as it underwent cyclic fatigue under wet, humid and dry conditions. Thus, while providing superficial protection similar to a bandage, SpiderSkin can also provide mechanical support to injuries in variable conditions. Furthermore, SpiderSkin has the potential to be loaded with antimicrobials and/or growth factors to further protect a wound and encourage healing.

Although the design objectives were achieved within the scope of this project, this design was only able to cover the initial investigation into an exciting new product. Further research should be considered to continue improving the SpiderSkin bandaging system. The following is a list of potential branches for further research and these areas are detailed in the next section:

- The concentration of glutaraldehyde and other cross-linking investigations should be studied to prevent cytotoxicity
- Incorporate antimicrobial into electrospun mat nanofibers to prolong the antimicrobial release
- Incorporation of growth factors
- Combination of rSSps with other biodegradable polymers to improve mechanical properties
- Testing of cell adhesion to electrospun mat as potential application for a cell scaffold

Recommendations for Future Work

Alternate Polymers/Mat Formulations

Due to the time constraints of this project, it was not possible to explore all of the different polymer/mat formulations that could be used to create this bandaging product. Nylon was the main polymer chosen for testing due to its availability to us and the previous research on electrospinning with recombinant spider silk that had already been done with nylon. However, we believe that using a degradable polymer may be best for specific applications. The use of alternate polymers would require much more research on their use in electrospinning and how they may integrate with recombinant spider silk proteins. Fortunately, much research is currently being done on the production of biodegradable polymers, both synthetic and natural. Possible choices of synthetic biodegradable polymers include starch-based polymer blends and polylactic acid blends, while natural biodegradable polymers include cellulose and soy proteins. Furthermore, it may be possible to create a product made of only recombinant spider silk protein, since this can be tuned for degradability, however pure spider silk is hard to electrospin and more research would need to be done to produce a purely rSSps electrospun mat. Films, foams, and gels made of recombinant spider silk protein may turn out to be better for some of the bandaging and drug delivery applications as well.

Alternate Antimicrobial Constituents

Chlorhexidine was the antimicrobial chosen due to recommendations from health care professionals and biomedical companies. Antibiotics are not a good choice for this product because of the high risk of super bugs and antibiotic resistant infections occurring. However, electrospinning using zinc oxide nanoparticles or silver may be good alternatives to an antimicrobial like chlorhexidine. From the results we obtained, it seems that to have a sustained release of an antimicrobial, it would be best if the fibers themselves contained the antimicrobial constituent within them, instead of trying to incorporate the antimicrobial within the adhesive. Again, this would depend on the desired application/type of injury.

Cell Attachment/ Capability as a Scaffold for Epidermal Recovery

Preliminary research has shown that rSSps materials have potential for cell scaffolding and cell attachment. With further research, it may be possible to incorporate epithelial cells onto the surface of the fibrous bandage. This would allow for a porous, integrative structure for cell growth and attachment. As this is applied to the skin, it may be possible to encourage epidermal recovery.

Cross-linking

In this study, glutaraldehyde was used as the cross-linking agent due to the fact that it is commonly used as a tissue cross-linking agent. However, excess glutaraldehyde is a proven cytotoxin. Further research needs to be done to deactivate the remaining uncross-linked glutaraldehyde or to find the exact glutaraldehyde concentration needed to maximize cross-linking, while having very little excess at the end of the process causing toxicity.

Timetable
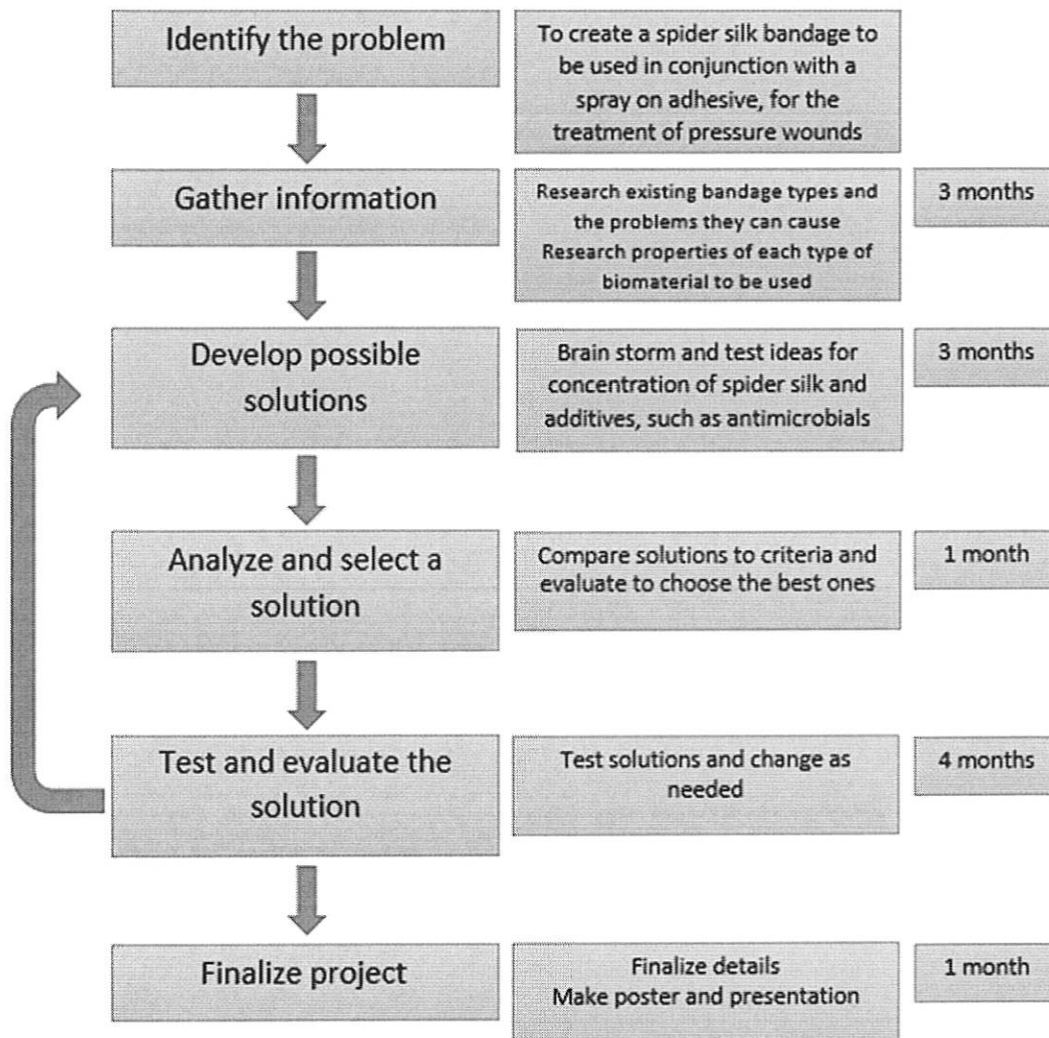
Figure 38. Timetable for completion of this project.

Background, Literature Review, Project Aims and Objectives

Personnel: All members

Completed: March 2016

Pressure-sensitive Tape Test

Personnel: Ana Laura Licon, Danielle Gaztambide

Completed: May 19, 2016

SEM Imaging of Electrospun Mat/Adhesive Formulations

Personnel: Danielle Gaztambide, Ana Laura Licon, Thomas Harris

Completed: May 2016

T-Peel Adhesion Test On Pigskin

Personnel: Danielle Gaztambide, Ana Laura Licon, Sam Briggs

Completed: Trial 1-May 2016 and Trial 2-July 2016

Electrospun Mat/Adhesive Tensile Testing

Personnel: Danielle Gaztambide, Sam Briggs, Ana Laura Licon

Completed: July 2016

Fatigue Testing On Pigskin and Analysis

Personnel: Danielle Gaztambide, Ana Laura Licon, Michael Paskett

Completed: End of July 2016

Chlorhexidine Elution and Analysis

Personnel: Danielle Gaztambide, Thomas Harris

Completed: November 2016

Cyclic Stretching Test on Pigskin

Personnel: Danielle Gaztambide, Ana Laura Licon

Completed: October 2016

Analysis of Tensile Testing Data and Statistical Analysis

Personnel: Michael Paskett

Completed: October 2016

Analysis of T-Peel Data

Personnel: Ana Laura Licon, Michael Paskett

Completed: October 2016

Toxicity Testing

Personnel: Sam Briggs, Ana Laura Licon, and Danielle Gaztambide

Completed: October 2016

Analysis of Toxicity Testing

Personnel: Sam Briggs

Completed: October 2016

Statistical Analysis on all Other Tests

Personnel: Michael Paskett

Completed: October 2016

Write-Up and Final Report/Poster

Personnel: Sam Briggs, Danielle Gaztambide, Ana Laura Licon, and Michael Paskett
Completed: December 2016

Reflective Writing

For my senior capstone project, I have been working in Dr. Randy Lewis Spider Silk Laboratory, designing a "second skin" from recombinant spider silk proteins. With a group of four undergraduates we have worked this project from conceptualization stages to a final product. Through this project, I have learned about the complete spectrum of research and design. It has been a valuable project and the related experience will benefit me greatly as I pursue graduate education.

Our project began with a brainstorm. As our group was working in the Spider Silk Laboratory, we had many potential applications to choose from. After hearing the experience of a postdoctoral fellow at a conference, we realized that there is a big market for bed sore/burn victims. We started to hypothesize different methods of formulating our bandage and adhesive, and drew up a proposal which was later approved by the department.

We produced several formulations for the mat for our bandage and performed mechanical testing on the mat to ensure we had the properties we needed in a bandage. After making a decision on which formulation of mat to use in the final product, we moved on to adhesive formulations.

Similar to choosing a mat formulation, we tested out several spider silk adhesives to use with our mat. We performed several tests on the adhesive in combination with the chosen mat. We harvested pig skin, and tested the formulation's adhesion to pig skin. Pig skin gave us a good representation of how the product would perform in vivo.

After the selection of a mat and adhesive, we began testing an antimicrobial compound, chlorhexidine, and its ability to prevent bacterial growth when incorporated into the bandage. Through our testing we found that there was no growth, however the inhibition was likely due to our antimicrobial and an additive in the adhesive. Finally, we evaluated our product's biocompatibility by growing mammalian cells in the presence of our bandage.

Overall, my experience in the spider silk laboratory has been very good. I have been able to learn much more about the research process. I have been able to interact with very intelligent people who have given me great advice for pursuing my goals in both the academic and professional world. I have been exposed to many different thinking and working styles and those have influenced my own personal styles of work and thinking.

My experience with this senior capstone project will benefit me greatly. I have gained a much deeper foundation for the research process. I have learned better how to work with groups and manage dates and deadlines. As I am soon to begin research in a graduate setting, my background from the capstone project adds a major experience component that will be unlike many peers.

My time in Dr. Lewis' Spider Silk Lab has been a great experience for multiple reasons. I have been able to understand and apply practical information about proteins and purification better. As I have increased my scientific understanding of what goes on in the laboratory, I have also become more familiar with the managerial aspects of laboratory work. I am excited to continue my work in the laboratory.

As a mentor, Dr. Lewis has been very helpful. He has been hands-off, allowing us to perform the research in our own way, while providing valuable input and feedback whenever we need it. Our relationship is very similar to what I will look for in a graduate setting as well. I couldn't really say that there were any difficulties.

Lastly, I want to share some advice I have for future capstone projects:

1.   Realize the importance of your team.

2.   Don't bite off more than you can chew.

3.   Utilize resources within and without the laboratory.

1. Your team will (hopefully) become your best friends for the duration of the project. You will spend more time thinking about your project and working on that than you want to. The importance of trust and hard work from all members is critical to your success. I was lucky to have honest, hard-working teammates who didn't put off any work that needed done. With that trust for each other, we were able to complete a large amount of testing in a relatively short amount of time. It is more important to like your team than the research you will be conducting. Research, by nature and of necessity, can be repetitive and get boring. There will be times when none of you want to perform testing, data analysis, literature-searches, writing, and the wide array of responsibilities with taking on a senior project. If you and your team get along well, people won't be shirking duties out of laziness. It is important to have a team with a variety of skills. Although we had all worked in the same lab, each member brought specific skillsets and knowledge that were absolutely necessary to completing a project from such diverse concepts. In summary, don't choose a project and then a team. Choose a team and then a project. Reversing the chronology of these events can cause problems down the road.

2. I don't know exactly what other majors are like, but for Biological Engineering we had about 3 semesters to formulate, carry out, and report on a project. It seems like a lot of time. We had a lot of ideas. That time proved to be far insufficient for the number of ideas we had. Realize that on top of your project, you are still going to have class, homework, work, and (if you really budget your time) some form of a social life. Decide early what the fundamental needs are for your project and finish those. If you have any extra time, pursue some of the additional ideas your team has had along the way. Don't get distracted along the way and forget about your fundamental requirements. We had lots of ideas. Some of which we began to pursue before it was appropriate. Don't make the same mistake we did. It's very easy to overlook the additional work beyond the pursuit of the ideas. We didn't think about finding more literature, analyzing data, and writing when we began to pursue some of our little ideas.

3. Think critically about the resources you have and use them. You are still a student. This is a learning project. You aren't expected to begin as an expert on every method you encounter. You probably don't even know some of the methods exist which could be very beneficial to evaluating your product. You have graduate students as an easy resource. If they don't know, reach out to post-doctoral researchers. Your principal investigator will know if they don't. If within your lab you don't have enough of those

resources, look to other labs. The research community is a pretty friendly one. Don't be afraid to reach out to another professor's lab. If you read a paper and want some more information about it, contact the author. You'll be surprised how nice people actually are, even if they don't know you.

I hope this helps some of you on your endeavor. Senior design projects are a lot of work, but it's experience that can really help you to stand out as a student if you do it the right way. Good luck!

References

American Burn Association. (n.d.). Retrieved September 29, 2016, from

http://www.ameriburn.org/resources_factsheet.php

Arenbergerova, M., Arenberger, P., Bednar, M., Kubat, P., & Mosinger, J. (2012). Light-activated

nanofibre textiles exert antibacterial effects in the setting of chronic wound healing. *Experimental*

Dermatology, *21*(8), 619–624. https://doi.org/10.1111/j.1600-0625.2012.01536.x

Bauer, F., Wohlrab, S., & Scheibel, T. (2013). Controllable cell adhesion, growth and orientation on

layered silk protein films. *Biomaterials Science*, *1*(12), 1244. https://doi.org/10.1039/c3bm60114e

Burkatovskaya, M., Castano, A. P., Demidova-Rice, T. N., Tegos, G. P., & Hamblin, M. R. (2008). Effect

of chitosan acetate bandage on wound healing in infected and noninfected wounds in mice. *Wound Repair*

and Regeneration : Official Publication of the Wound Healing Society [and] the European Tissue Repair

Society, *16*(3), 425–431. https://doi.org/10.1111/j.1524-475X.2008.00382.x

Gallagher, A., Anniadh, A., Bruyere, K., Ottenio, M., Xie, H., & Gilchrist, M. (n.d.). Dynamic Tensile

Properties of Human Skin. *IRCOBI Conference 2012, 12-14 September 2012, Dublin (Ireland)*,

(International Research Council on the Biomechanics of Injury, 2012).

Jones, J. A., Harris, T. I., Tucker, C. L., Berg, K. R., Christy, S. Y., Day, B. A., … Lewis, R. V. (2015).

More Than Just Fibers: An Aqueous Method for the Production of Innovative Recombinant Spider Silk

Protein Materials. *Biomacromolecules*. https://doi.org/10.1021/acs.biomac.5b00226

Karami, Z., Rezaeian, I., Zahedi, P., & Abdollahi, M. (2013). Preparation and performance evaluations of

electrospun poly(epsilon-caprolactone), poly(lactic acid), and their hybrid (50/50) nanofibrous mats

containing thymol as an herbal drug for effective wound healing. *Journal of Applied Polymer Science*,

129(2), 756–766. https://doi.org/10.1002/app.38683

Lee, K. Y., Jeong, L., Kang, Y. O., Lee, S. J., & Park, W. H. (2009). Electrospinning of polysaccharides for regenerative medicine. *Advanced Drug Delivery Reviews*, *61*(12), 1020–1032. https://doi.org/10.1016/j.addr.2009.07.006

Lewis, R. V. (2006). Spider Silk: Ancient Ideas for New Biomaterials. *Chemical Reviews*, *106*(9), 3762–3774. https://doi.org/10.1021/cr010194g

Maleki, M., Latifi, M., Amani-Tehran, M., & Mathur, S. (2013). Electrospun core-shell nanofibers for drug encapsulation and sustained release. *Polymer Engineering and Science*, *53*(8), 1770–1779. https://doi.org/10.1002/pen.23426

Mone, G. (n.d.). The Hyper-Intelligent Bandage. Retrieved November 7, 2016, from http://cacm.acm.org/magazines/2015/12/194635-the-hyper-intelligent-bandage/fulltext

Moore, Z. E., & Cowman, S. (2013). Wound cleansing for pressure ulcers. In *Cochrane Database of Systematic Reviews*. John Wiley & Sons, Ltd. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD004983.pub3/abstract

O'Neil, C. K. (2004). Prevention and Treatment of Pressure Ulcers. *Journal of Pharmacy Practice*, *17*(2), 137–148. https://doi.org/10.1177/0897190004263217

Padol, A. R., Jayakumar, K., Shridhar, N. B., Narayana Swamy, H. D., Narayana Swamy, M., & Mohan, K. (2011). Safety evaluation of silk protein film (a novel wound healing agent) in terms of acute dermal toxicity, acute dermal irritation and skin sensitization. *Toxicology International*, *18*(1), 17–21. https://doi.org/10.4103/0971-6580.75847

Panilaitis, B., Altman, G. H., Chen, J., Jin, H.-J., Karageorgiou, V., & Kaplan, D. L. (2003). Macrophage responses to silk. *Biomaterials*, *24*(18), 3079–3085. https://doi.org/10.1016/S0142-9612(03)00158-3

Pressure-Sensitive Medical Technology: Challenges and Considerations. (n.d.). Retrieved November 7, 2016, from http://www.adhesivesmag.com/articles/94731-optimizing-adhesives-for-tankless-dispensing-systems

Rising, A., Widhe, M., Johansson, J., & Hedhammar, M. (2011). Spider silk proteins: recent advances in recombinant production, structure–function relationships and biomedical applications. *Cellular and Molecular Life Sciences*, *68*(2), 169–184. https://doi.org/10.1007/s00018-010-0462-z

Rowan, M. P., Cancio, L. C., Elster, E. A., Burmeister, D. M., Rose, L. F., Natesan, S., … Chung, K. K. (2015). Burn wound healing and treatment: review and advancements. *Critical Care*, *19*, 243. https://doi.org/10.1186/s13054-015-0961-2

Unnithan, A. R., Nejad, A. G., Sasikala, A. R. K., Thomas, R. G., Jeong, Y. Y., Murugesan, P., … Kim, C. S. (2016). Electrospun zwitterionic nanofibers with in situ decelerated epithelialization property for non-adherent and easy removable wound dressing application. *Chemical Engineering Journal*, *287*, 640–648. https://doi.org/10.1016/j.cej.2015.11.086

Vepari, C., & Kaplan, D. L. (2007). Silk as a Biomaterial. *Progress in Polymer Science*, *32*(8–9), 991–1007. https://doi.org/10.1016/j.progpolymsci.2007.05.013

Wendorff, J. H., Agarwal, S., & Greiner, A. (2012). Nanofiber Properties. In *Electrospinning* (pp. 69–104). Wiley-VCH Verlag GmbH & Co. KGaA. Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/9783527647705.ch3/summary

Wendt, H., Hillmer, A., Reimers, K., Kuhbier, J. W., Schäfer-Nolte, F., Allmeling, C., … Vogt, P. M. (2011). Artificial Skin – Culturing of Different Skin Cell Lines for Generating an Artificial Skin Substitute on Cross-Weaved Spider Silk Fibres. *PLoS ONE*, *6*(7), e21833. https://doi.org/10.1371/journal.pone.0021833

Xia, X.-X., Qian, Z.-G., Ki, C. S., Park, Y. H., Kaplan, D. L., & Lee, S. Y. (2010). Native-sized recombinant spider silk protein produced in metabolically engineered Escherichia coli results in a strong fiber. *Proceedings of the National Academy of Sciences*, *107*(32), 14059–14063. https://doi.org/10.1073/pnas.1003366107

Zahedi, P., Rezaeian, I., Ranaei-Siadat, S.-O., Jafari, S.-H., & Supaphol, P. (2010). A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polymers for Advanced Technologies*, *21*(2), 77–95. https://doi.org/10.1002/pat.1625

Appendix A: Mat Formulations

Electrospun mats were used as the base for the scaffold complex made. Three different initial mat formulations tested: 20% nylon, 10% nylon 10% rMaSp1, and 10% nylon 10% 80/20 rMaSp1/rMaSp2, all of which were dissolved in formic acid. To make the 20% nylon solution 0.6 g of nylon were placed in a glass vial with 3.04 mL of 97% formic acid. To make the nylon rMaSp1/rMaSp2 solution 0.3 g of nylon, 0.24 g rMaSp1 and 0.06 g of rMaSp2 were added to a glass vial with 3.04 mL of 97% formic acid. To make the nylon rMaSp1 mats 0.3 g of nylon and 0.3 g of were added to a glass vial with 3.04 mL of 97% formic acid. As each solution was made, it was left on an end-over-end plate mixer plate overnight for the and nylon to dissolve into a homogenous solution.

Each polymer solution was placed into 1 mL syringes fitted with a 0.5 inch 27-gauge flat tip disposable needle. To make a mat a needle filled with one of the solutions was placed into the electrospinner. The electrospinner was set to an extrusion rate between 0.25-0.3 mL/h and the voltage kept between 24-29 kV. The drum was wrapped with a sheet of nonstick aluminum foil and set to rotate at 1000 rpm. The positive wire on the electrospinner was attached to the syringe and the rotating drum was connected to the negative wire. Each mat was spun for about 3.5 hours until the solution in the syringe had been used, and a complete mat had been formed.

Appendix B: Adhesive Formulations

Three different adhesives were initially tested on the electrospun mats: a 12% 50/50 rMaSp1/rMaSp2 adhesive, a 12% 50/50 rMaSp1/rMaSp2 with 1% glutaraldehyde, and a 12% 50/50 rMaSp1/rMaSp2 with 10% bovine serum albumin (BSA) and 1% glutaraldehyde.

To make the 12% 50/50 rMaSp1/rMaSp2 adhesive 0.18 g of rMaSp1 and 0.18 g of rMaSp2, and 3 mL of distilled water were added to a small glass vial with a screw on lid. A sonicator was used with a microtip to create vibrations to break down the larger rSSp agglomerates, and increase protein solubilization. The vial was microwaved in 3-second intervals until it reached 250°C and the protein was completely dissolved. The 12% 50/50 rMaSp1/rMaSp2 with 1% glutaraldehyde was made in the same way as the 12% 50/50 rMaSp1/rMaSp2 adhesive, but 0.02 mL of a 50% glutaraldehyde stock was added per 1 mL of the rSSps adhesive to make it a 1% glutaraldehyde solution. The 12% 50/50 rMaSp1/rMaSp2 with 10% BSA and 1% glutaraldehyde was made in the same way as the 12% 50/50 rMaSp1/rMaSp2 adhesive, with an additional 0.02 mL of a 50% glutaraldehyde stock per mL of the rSSps adhesive and 0.3 g of BSA.

Appendix C: Pigskin Cleaning

Pigskin was used to set the mat/adhesive formulations for all of the tests except the toxicity assay. Strips of fresh pigskin were acquired from the Utah State University South Farm. Hair clippers were used to remove the bulk of the hair and then disposable razors were used to remove the final hair left after clipping. The skin was washed in baths of 30% isopropyl alcohol and water, cut into smaller pieces, sealed into bags, and kept in a 4°C refrigerator to keep the skin fresh for as long as possible (approximately 3 weeks). The skin was cut into smaller pieces as necessary for each test.

Appendix D. Conditions Created for Mechanical Testing

Three different conditions were chosen for all of the mechanical testing: dry, wet, and humid. The dry samples were tested as they were on the pigskin after the adhesive had dried. The wet samples were submerged in water for 1 minute, dried with a paper towel and tested immediately. The humid samples were tested under 100% humidity conditions by using a humidifier attached to a plexiglass box with a hose to saturate that space with water vapor. The humidity set up can be seen in Figure 39:

Figure 39. Setup for humidity testing

Appendix E. Recombinant Spider Silk Proteins Used

All of the rSSps used in this experiment were derived from the milk of transgenic goats. The sequences of the two proteins used are shown below.

rMaSp1:

(QGAGAAAAAAGGAGQGGYGGLGGQGAGQGGYGGLGGQGAGQGA
GAAAAAAAGGAGQGGYGGLGSQGAGRGGQGAGAAAAAAGGAGQG
GYGGLGSQGAGRGGLGGQGAGAAAAAAAGGAGQGGYGGLGNQGA
GRGGQGAAAAAAGGAGQGGYGGLGSQGAGRGGLGGQGAGAAAAA
AGGAGQGGYGGLGGQGAGQGGYGGLGSQGAGRGGLGGQGAGAA
AAAAAGGAGQGGLGGQGAGQGAGASAAAAGGAGQGGYGGLGSQG
AGRGGEGAGAAAAAAGGAGQGGYGGLGGQGAGQGGYGGLGSQGA
GRGGLGGQGAGAAAAGGAGQGGLGGQGAGQGAGAAAAAAGGAG
QGGYGGLGSQGAGRGGLGGQGAGAVAAAAGGAGQGGYGGLGSQ
GAGRGGQGAGAAAAAAGGAGQRGYGGLGNQGAGRGGLGGQGAG
AAAAAAAGGAGQGGYGGLGNQGAGRGGQGAAAAAGGAGQGGYG
GLGSQGAGRGGQGAGAAAAAAVGAGQEGIRGQGAGQGGYGGLGS
QGSGRGGLGGQGAGAAAAAAGGAGQGGLGGQGAGQGAGAAAAAA
GGVRQGGYGGLGSQGAGRGGQGAGAAAAAAGGAGQGGYGGLGG
QGVGRGGLGGQGAGAAAAGGAGQGGYGGVGSGASAASAAASRLSS
PQASSRLSSAVSNLVATGPTNSAALSSTISNVVSQIGASNPGLSGCDVLI
QALLEVVSALIQILGSSSIGQVNYGSAGQATQIVGQSVYQALG)

rMaSp2:

(PGGYGPGQQGPGGYGPGQQGPSGPGSAAAAAAAAAAAGPGGYGPGQQG
PGGYGPGQQGPGRYGPGQQGPSGPGSAAAAAAGSGQQGPGGYGPRQQG
PGGYGQGQQGPSGPGSAAAASAAASAESGQQGPGGYGPGQQGPGGYGP
GQQGPGGYGPGQQGPSGPGSAAAAAAAASGPGQQGPGGYGPGQQGPGG
YGPGQQGPSGPGSAAAAAAAASGPGQQGPGGYGPGQQGPGGYGPGQQG
LSGPGSAAAAAAAGPGQQGPGGYGPGQQGPSGPGSAAAAAAAAAGPGG
YGPGQQGPGGYGPGQQGPSGAGSAAAAAAAGPGQQGLGGYGPGQQGP
GGYGPGQQGPGGYGPGSASAAAAAAGPGQQGPGGYGPGQQGPSGPGSA
SAAAAAAAGPGGYGPGQQGPGGYAPGQQGPSGPGSASAAAAAAAAGP
GGYGPGQQGPGGYAPGQQGPSGPGSAAAAAAAAAGPGGYGPAQQGPSG
PGIAASAASAGPGGYGPAQQGPAGYGPGSAVAASAGAGSAGYGPGSQAS
AAASRLASPDSGARVASAVSNLVSSGPTSSAALSSVISNAVSQIGASNPGL
SGCDVLIQALLEIVSACVTILSSSSIGQVNYGAASQFAQVVGQSVLSAF)

Appendix F. Understanding JMP® visual outputs

From the JMP® Tutorial:

Categorical plots:
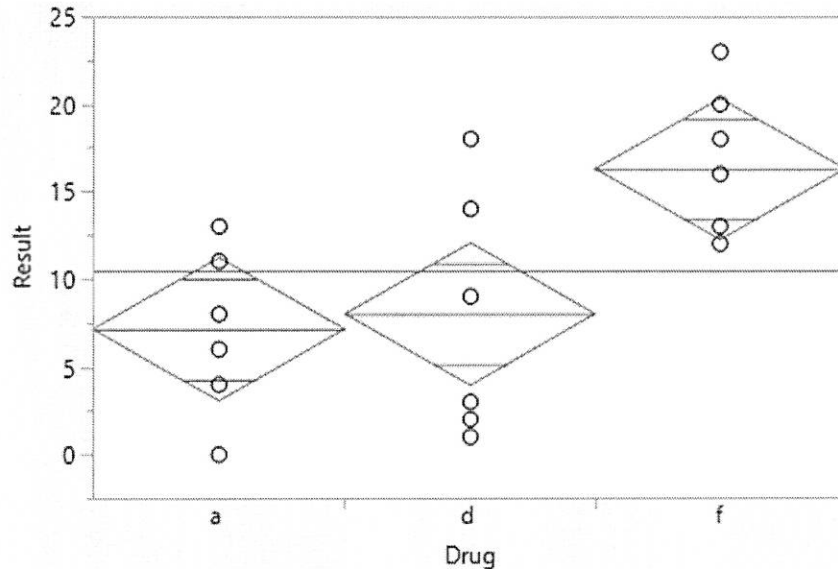
A black dot represents an individual datum point. In the green diamond, the center line represents the mean of the data. The top and bottom points represent upper and lower confidence points of each group. The lines that slice the top and bottom of the diamonds are called overlap marks. If there is horizontal separation between the top overlap of one group and the bottom overlap of another, the means of those two groups could be significantly different. Group 'f' appears to be different than group 'a' and 'd' in this example.

Connecting Letters Report:

⊿ **Connecting Letters Report**

Level		Mean
f	A	16.285714
d	B	8.000000
a	B	7.142857

The letter table lists the group means with letters to the right of them. Means that have the same letter are not significantly different. Those with different letters are different. Groups 'd' and a' are both denoted with the letter 'B' -- they are not different. Group 'f' has the letter 'A', different than both 'd' and 'a'.

Professional Author Bio

Michael Paskett studied Biological Engineering as an undergraduate at Utah State University. Michael is an outdoor enthusiast who loves hiking, skiing, and climbing. Michael played rugby for the USU rugby team and spent 2 years conducting research in Randy Lewis' Spider Silk Laboratory. With primary interest in neural engineering, specifically neuroprosthetics, Michael had a summer internship at Washington University in St. Louis in which he performed research involving neural interfaces and wireless nerve stimulation. Michael intends to pursue a Doctor of Philosophy in Bioengineering from the University of Utah following graduation from Utah State. Following graduation from the University of Utah, Michael intends to pursue a career in an industrial setting.