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Asymmetric Electrospun Nanofiber Composed of Polycaprolactone, Chitosan, and Curcumin to Promote Chronic Wound Healing

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ABSTRACT

Chronic wounds are a growing medical issue around the world, with 2.4 - 4.5 million people afflicted in the United States alone (1). The condition currently makes up 2-3% of healthcare costs for developed countries (1), and this number is expected to increase as chronic wound-causing problems, like diabetes, grow in prevalence. Due to the lack of effective wound dressings, chronic wounds face a higher risk for bacterial infection and express high morbidity and mortality rates. Many current dressings are homogeneous and fail to account for the complexities of the different layers of skin. Alternatively, asymmetric electrospun nanofibers have a bilayer design that corresponds to the skin's dermis and epidermis. We intend to demonstrate that an asymmetric electrospun nanofiber composed of polycaprolactone (PCL), chitosan (CS), and curcumin (Cur) can be utilized as a chronic wound dressing. These three biodegradable and biocompatible components were applied in an asymmetric design to mimic the protective epidermis and porous dermis. Upon electrospinning our asymmetric nanofiber and running it through a scanning electron microscope, we found that our hydrophobic PCL layer had a low porosity (52.16%) and thin nanofibers (281.82 nm), while our hydrophilic PCL/CS/Cur layer had a high porosity (61.18%) and thick nanofibers (425.54 nm). Our asymmetric nanofiber was also more effective at resisting bacterial permeation than the homogeneous PCL nanofiber. Our results highlight the superiority of asymmetric membranes for resisting bacterial infection and suggest that with further experimentation, we can establish our nanofiber as a viable wound dressing.

Introduction

A wound is defined as any disruption of the epithelial lining of skin resulting from physical damage. The healing of a wound is complex. The typical process of wound healing is divided into four phases: hemostasis, inflammation, proliferation, and remodeling (1). The first stage of wound healing, hemostasis, occurs directly after injury. During it, vasoconstriction begins in an attempt to prevent the loss of blood from the human body (1). Nearby platelets activate, setting loose adhesive glycoproteins along with soluble mediators that recruit macrophages and neutrophils (1). The glycoproteins motion for platelets to stick to one another. Growth factors are released from the platelets, which in turn leads to the creation of a fibrin clump at the site of a wound. Here, the fibrin acts as a placeholder extracellular matrix (ECM), where aggregated platelets clot together (1). During the second part of the healing process, inflammation, the body's immune system works to clean the wound site. The previously recruited neutrophils and macrophages kill bacteria and other debris. These two immune system cells then release more soluble mediators that attract endothelial cells and fibroblasts (1). The third stage of healing, proliferation, consists of many different aspects. During this stage, the temporary fibrin ECM is replaced by a collagen matrix as a result of fibroblast migration (1). Simultaneously, the process of angiogenesis

is replacing the vasculature that had been damaged by the wound. Next, a sequence known as granulation occurs, which creates a replacement for the normal dermis. This replacement is filled with capillaries, collagen fibers, and immune system cells (1). In the last part of proliferation, epithelialization occurs. Wounds that are open are able to heal from contractions; in proliferation, new tissue moves toward the center of the wound. The last stage of wound healing is remodeling. In this stage, the tissues formed during granulation turn into scars (1). It is important to note that these phases do not directly occur one after another--they overlap.

There are two broad categories for the different types of wounds: acute and chronic. Acute wounds progress through the four phases of the wound healing process described above (1). Chronic wounds, on the other hand, do not abide by the same sequence (2). While they do begin the healing process in a similar fashion, chronic wounds are known to have longer inflammatory, proliferative, and remodeling phases (1). Additionally, they are known to cause bacterial infections, excessive inflammation, and the creation of biofilms within a wound microenvironment. Chronic wounds are ubiquitous in the United States, impacting 2.4-4.5 million people (3). Chronic ulcers, a specific chronic wound, have the ability to last over a year and frequently recur in patients. Chronic wounds are known to have a high mortality rate, mainly due to bacterial penetration of the wound microenvironment (1). This high mortality rate is correlated to a deficiency in efficient wound dressings (4). Current homogeneous wound dressings are unable to account for the complexities of the different layers of the skin; therefore, the development of a novel solution to treat these chronic wounds effectively is an important step in worldwide healthcare.

There are basic characteristics that are important for wound dressings to have in order to aid in the wound healing process. For one, the wound dressing must maintain a moist environment (2). A moist environment is crucial, as it promotes collagen creation and angiogenesis, along with preventing the dehydration of the wound (2). Wounds must also be able to protect against bacterial invasion; bacterial infection hinders the long-term health of not only the wound but the individual as well (2). Additionally, the dressing must allow for the gaseous exchange between the wound and the surrounding environment (2). Gas exchange is pivotal in wounds, as many of the processes occurring within the body require energy; for example, in wounds, there is a greater demand for oxygen because oxygen is required to create energy in order to repair tissue. Oxygenation of wounds can stimulate the healing process (2). Furthermore, wound dressings should be non-toxic.

There are thousands of current wound dressings available, each of them specialized for a specific type of wound. Among the most common wound dressings for skin tissue regeneration are skin grafts. However, the utilization of skin grafts is limited by the number of donor sites, patient morbidity, the extent of a wound, and the formation of scars (4). Additionally, skin grafts are expensive and hard to handle (5). The limitations of skin grafts paired with the high mortality rates of chronic wounds indicate that despite the large number, further research is required in order to develop a more effective treatment option for chronic wounds.

Over the past two decades, electro spun nanofibers emerged as an effective wound dressing. Electrospinning is the process of using an electric charge to synthesize fibers from a polymer solution or melt. To electro spin, an electric field is applied to a polymer solution located inside of a syringe with a metal nozzle. The solution is pushed through the nozzle via a pump, and the electric field applied forms a charged liquid stream. The stream lengthens and the polymer cools, creating a solid nanofiber (6). The distance between the syringe and the collector, along with the amount of voltage can alter the nanofiber product (7). A polymer solution consists of two main components: the polymer and the solvent. The polymer serves as the foundation for the future product, and different polymers have separate characteristics, like morphology, biodegradability, miscibility, and biocompatibility (8). When polymer fibers are shrunk to the nano-level, they have an increased surface area to volume ratio, flexibility, mechanical stiffness, and better resistance to traction. Many of these characteristics are why nanofibers are so attractive as wound dressings.

When a wound occurs, it is best that the skin is returned to its original function and structure as soon as possible (9). Nanofibers are morphologically similar to ECMs, which means that they can enable cell adhesion and proliferation in a way that is most similar to skin. Nanofibers have a high surface area-to-volume ratio

and porosity, which allows for the exchange of gases and better hemostasis (9). Furthermore, bioactive molecules can be loaded onto nanofibers. Biomolecules can be released from the membrane through desorption, diffusion, and degradation (10). The high surface area-to-volume ratios can increase the interactions between the wound microenvironment and the biological agents loaded onto a nanofiber, which ultimately makes the administration of the agent more effective (4). Moreover, the increased ratio means greater area available for cell proliferation, enhancing the speed of wound regeneration (11). Many nanofibers are also absorbent, which means that they would be able to absorb wound exudate, which is the mass of fluid and cells that escape from a wound (9).

The success of nanofibers for wound dressings is not solely determined by a specific polymer, solvent, or bioactive molecule. Considering that there are so many variables that are a part of the electrospinning process, it is the specific combinations of variables that yield the most effective results. With nanofibers, specific polymers, solvents, and bioactive molecules, components of the nanofiber are chosen in order to combine their best properties to form a singular, better wound dressing.

The optimal specifications for wound matrix materials include the matrix's ability to avoid dehydration, prevent infection, elicit minimal inflammation, have mechanical properties such as durability and flexibility, and mimic the function of the dermis and epidermis (12). The Restrata Wound Matrix (RWM) has a fibrous structure with high porosity (large pore size). Polylactic co-glycolic acid (PLGA) and polydioxanone are co-spun into nonwoven sheets. The polymer combination has mechanical properties, healing properties, and mimics the native skin's extracellular matrix. The RWM possesses the combined advantages of synthetic construction and biological materials, which leads to rapid and complete healing of chronic wounds. When compared with the Integra Bilayer Wound Matrix (IBWM), both wound matrices supported wound healing but the area of the wound decreased faster with RWM. RWM also demonstrated greater biocompatibility and wound healing than the IBWM. The RWM and IBWM are examples of current matrices that possess the optimal mechanical and antibacterial properties for a nanofiber wound healing mesh.

A widely used polymer for nanofibers acting as wound dressings is polycaprolactone (PCL). PCL is characterized as being incredibly spinnable, mechanically strong, and also possesses slow degradation (5). Yin and Xu (13) combined PCL, chitosan, and aloe vera in order to create a novel wound dressing. They used PCL due to its good mechanical properties, non-toxicity, and its biodegradability. The researchers noted that the PCL supplied the homogeneous nanofiber with a strong framework, allowing the chitosan and aloe vera to be incorporated and contribute to the wound healing process. Ghaee et al. (11) followed a similar line of reasoning, using PCL as the mechanical framework for a nanocomposite scaffold containing curcumin, chitosan, and gelatin. Overall, present research suggests that the usage of PCL in the creation of nanofiber wound dressings is encouraged, due to its inherent mechanical properties.

Chitosan is an inexpensive and available polysaccharide polymer that has also been used extensively in wound dressings. Miguel et al. (4), when creating an asymmetric PCL/chitosan/aloe vera membrane, found that chitosan promoted the synthesis of collagen, an important protein in the structure that comprises the ECM. Additionally, Miguel et al. (4) found that the addition of chitosan in their nanofiber led to increased hemostatic and bactericidal processes. Moreover, Mousavi et al. (2020) observed from other studies that the incorporation of chitosan in wound dressings can enhance the membrane's performance. Ghaee et al. (11) included chitosan in their composite scaffold, finding that the presence of chitosan promoted fibroblast attachment and proliferation, ultimately leading to enhanced tissue regeneration. The promotion of fibroblast attachment points to the use of chitosan as the basis for an ECM that can increase the speed of the wound healing process. One of the downfalls of chitosan, however, is that it is not very spinnable. Yin and Xu (13) addressed this, using a combination of chitosan and PCL in order to improve the spinnability of their polymer solution. Chitosan must also be deacetylated in order to improve its biological activity, solubility, and flocculation ability (13).

A promising candidate for an agent within a nanofiber wound dressing is curcumin, a natural compound derived from turmeric. Past research has shown that curcumin is antibacterial, anti-inflammatory, an

antioxidant, while it also promotes the formation of granulation tissue, collagen deposition, fibroblast proliferation, and epithelial regeneration (14). Inherently, this makes curcumin a viable and attractive option for wound healing. Ghaee et al. (11) saw that the combination scaffold containing PCL, chitosan, and curcumin was successfully able to enhance skin regeneration. In terms of cell viability, Ghaee et al. (11) also found that fibroblasts were able to spread and proliferate at better rates on scaffolds containing curcumin as opposed to scaffolds without it. Additionally, Kohal et al. (15) found that with their composite nanofiber scaffolds, curcumin had an enormous influence on cell attachment and proliferation because of its ability to increase cell signaling pathways among local cells. With present research in mind, it is apparent that curcumin is an agent that can be incorporated in order to create a more efficient wound dressing.

The creation of asymmetric nanofibers that mimic the structure and functions of skin has grown in popularity in recent years. Miguel et al. (4) attribute this popularity to the fact that it is important to return damaged skin to its original structure and function as quickly as possible in order to enhance the wound healing process. By specializing certain layers of the nanofiber to mimic the skin's natural dermis and epidermis, the wound dressing has the ability to expedite the wound healing process. Miguel et al. (4) find that, in general, asymmetric nanofiber wound dressings consist of a dense exterior layer that defends against the outside environment, fending off physical damage and invading bacteria. Furthermore, the wound dressings also have an absorbent inner layer that more closely resembles the skin's natural ECM, allowing for cell growth in an aseptic environment (4). It is important that the dense exterior of the asymmetric nanofiber mesh is impermeable to bacteria, while also being porous enough to allow the exchange of gases (5). Miguel et al. (4) created an asymmetric nanofiber mesh with PCL, chitosan, and aloe vera. Using a transwell system, the researchers found that the upper dense layer prevented the bacteria from entering and the inner layer created an environment ideal for wound healing. Furthermore, the interior aspect of the nanofiber mesh (consisting of polyethylene oxide) (PEO), chitosan, and aloe vera) prevented fluid accumulation and helped cell adhesion and proliferation. Overall, the creation of wound dressings that more closely replicate the native structure and function of skin pose as a way to enhance the wound healing process.

In our research, we combined PCL, chitosan, and curcumin to form an asymmetric nanofiber mesh that will be used for chronic wound healing. Each component of our nanofiber serves a specific purpose: PCL is a strong mechanical backbone that prohibits the transmission of bacteria to the wound, chitosan is biocompatible and a great replacement for the skin's natural ECM, and curcumin is an agent that can help reduce inflammation and bacterial infection within the wound, while also aiding in other parts of the wound healing process. Our research is unique as it is the first time that PCL, chitosan, and curcumin have been combined to form an asymmetric nanofiber mesh. As such, the independent variable for our research will be the symmetry of the nanofiber mesh. By creating an asymmetrical nanofiber mesh composed of some of the most effective nanofiber wound dressing components, we can more closely mimic the native structure and function of skin, which can ultimately lead to a more effective wound dressing. We utilized a transwell system, similar to Miguel et al. (4), and determined if our asymmetric nanofiber composed of polycaprolactone, chitosan, and curcumin prevents bacterial penetration of *Staphylococcus epidermidis*. *S. epidermidis* is the most common cause of infections on the skin and promotes diseases on the surface of the skin. We hypothesized that due to the asymmetric nanofiber's design and components, our experimental nanofiber will be more effective by preventing bacterial infection and protecting the wound environment. Through our research, we hope to exhibit the viability of our novel asymmetric nanofiber for wound healing and display the benefits of a multi-layered wound dressing design.

Chronic wounds, despite the work that researchers have put in to limit their impact on society, are still a growing problem. Our research has the opportunity to yield an effective wound dressing and act as a foothold for researchers who wish to create a nanofiber with similar components.

Chronic wounds are defined by their tendency not to follow the four standard parts of the wound healing process: hemostasis, inflammation, proliferation, and remodeling. Excessive inflammation, persistent

infections, and the formation of drug-resistant biofilms are all common characteristics of chronic wounds. Chronic wounds are prevalent in developing countries, affecting anywhere between 2.4 and 4.5 million people in the United States alone (16). These numbers will only continue to increase, as the rise of obesity and diabetes within the United States contributes to the formation of chronic wounds (1). Chronic wounds are also known to have high morbidity and mortality rates, and these high rates are associated with a lack of effective wound dressings (4). The creation of an effective wound dressing that can limit the common characteristics of chronic wounds and best mimic the dermis and epidermis of the human skin is important for the future of healthcare.

Methods

For all procedures, goggles, gloves, close-toed shoes, and lab coats were worn. Long hair was tied back. Additionally, the fume hood was utilized when working with flammable substances. A large beaker (AOS) with stir bar was filled with water (SDS) and placed onto a magnetic stirrer and set to 50 degrees Celsius and 315 rounds per minute. 500 mg of Chitosan (Sigma-Aldrich #448869, 50g = $63.80) (SDS) was combined with 10 ml of 1M NaOH (SDS) in a Florence flask. A small stir bar was added to the same flask. Once the magnetic stirrer reached 50 degrees Celsius, using the clamp apparatus (AOS), the flask was positioned in the water bath such that it out of the way of the large stir bar. The combination was mixed for 4 hours at 50 degrees celsius. This process deacetylated the chitosan. The mixture was filtered using 0.22µm filter paper (AOS) to collect the solid deacetylated chitosan and washed with distilled water until sample pH reached 7.4. The samples were dried at 40 degrees celsius overnight. The optimal deacetylation was expected to be 85%-95%. To measure the degree of deacetylation, 0,.15 g of Chitosan (SDS) was dissolved in 25 mL aqueous solution of 0.1M hydrochloric acid (SDS) and stirred continuously for 30 minutes. When chitosan was completely dissolved, the solution was titrated with a 0.1 M NaOH solution (SDS). A methyl orange indicator was used to determine the completion of the reaction. NH_2% was calculated. $NH_2\% = (((C_1V_1 - C_2V_2) * 0.016) / (G * (100 - W)) * 100$. C1 – HCl concentration (mol·dm-3) (also known as M). C2 – NaOH concentration (mol·dm-3) (also known as M). V1 – volume of HCl solution (mL). V2 – volume of NaOH solution (mL). 0.016 – molecular weight of NH_2 in 1 mL 0.1 M HCl (g). G – the sample weight (g). W – the water percentage of sample (%). The Degree of Deacetylation (DD) was calculated. $DD(\%) = (NH_2\% / 9.94\%) * 100\%$. 9.94% is the theoretical NH_2%.

Next, a Polycaprolactone(PCL) nanofiber layer was created. A 15 wt % PCL 80,000 Da (Sigma-Aldrich #440744, 250g = $131) (SDS) solution in 70:30 combination of formic acid (AOS) and acetic acid (v/v) (AOS) was made using a magnetic stirrer for 3 hours. The PCL solution was placed into a syringe and electro spun (Electrospinning Apparatus (Gamma High Voltage Research ES30P-60W/DAM) (AOS), at a constant flow rate of 1mL/h, applying a voltage of 20kV while having a distance of 12.5 cm between the needle tip and the collector.

A PCL/CS/Cur (Polycaprolactone, Chitosan, Curcumin) Nanofiber was created next. A solution of acetic acid (AOS) and acetone (SDS) was created at a ratio of 3:1. PCL was added at a concentration of 9 wt % and stirred for 30 minutes at room temperature. Chitosan (deacetylated from previous method) was added at a concentration of 1 wt % to the polymer solution and stirred for two hours at room temperature. A 5 wt % of curcumin was added to the PCL/CS solution and stirred for 2 hours at room temperature. The solution was sonicated for 2 hours. The PCL/CS/Cur solution was placed into a syringe and electro spun at a constant flow rate of 0.3mL/h, applying a voltage of 15kV while having a distance of 8 cm between the needle tip and the collector. The mesh was dried at room temperature for 48 hours to remove left over formic acid and acetone. The curcumin concentration was varied as necessary in the PCL/CS solution.

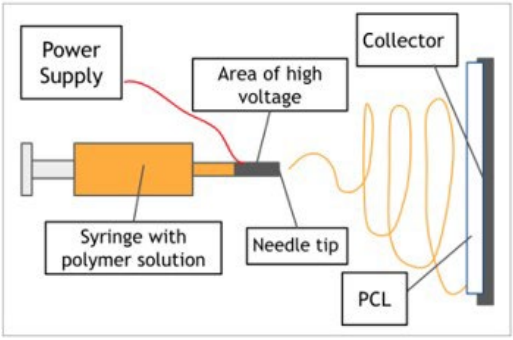


Figure 2. Diagram of the electrospinning apparatus for the asymmetric nanofiber. With the addition of a high voltage, the polymer solution in the syringe is drawn out onto the collector forming a thin layer of nanofibers.

Next, the Nanofiber was characterized with SEM (Phenom G5 Scanning Electron Microscope AOS). Two circular samples were cut with a radius of 1.5 cm (chosen haphazardly) from the PCL/CS/Cur nanofiber mesh and the PCL nanofiber in order to get a sample with an evident cross section. A gold sputter coater (Cressington 108 Gold Sputter Coater AOS) was used to coat the nanofiber samples. The nanofiber mesh samples were oriented such that the SEM was able to scan the samples' cross section. The samples were scanned with the SEM at a resolution of 300 µm. The resulting photos were collected to compare the homogeneous nanofiber to the asymmetrical nanofiber. Computer w/ Image J (AOS) was used to measure the nanofiber diameter using the line tool in Image J after being adjusted according to the scale shown in each SEM image. 25 nanofibers per SEM image were evaluated.

To measure the porosity percentage, the same 3 SEM images were converted to 32-bit images. The threshold in ImageJ was set such that nanofibers on the upper layer of the image were differentiated from all nanofibers in the background. The ratio of background space to the total area of the image as porosity percentage was interpreted.

The water contact angle was measured next. The nanofiber scaffolds were laid on a flat even surface. 4µL of water was pipetted using a micropipette onto the nanofiber scaffold. A USB camera (AOS) that is also on a flat even surface was used to take a picture of the water droplet on the scaffold. The camera was oriented to see the angle at which the water interacts with the nanofiber. The photos were uploaded and Image J software was used to determine the water contact angle and recorded. Repeat trials were performed in order to get 3 trials for the nanofiber meshes of varying symmetries.

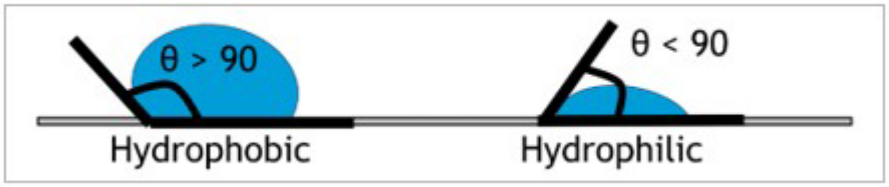


Figure 3. Modified from Baltatu et al., 2018. Water contact angle test. If the contact angle between the water droplet and the surface is greater than 90 degrees, then the surface is considered hydrophobic. If the water contact angle is less than 90 degrees, the surface is hydrophilic.

The porosity was measured with SEM (Phenom G5 Scanning Electron Microscope AOS). The nanofiber samples were cut to match the dimensions necessary for the SEM. The gold sputter coater was used to coat the nanofiber mesh samples. 3 different PCL nanofiber meshes were scanned with the SEM zoomed into a resolution of 2µm (20 nanometers). The ImageJ program was applied to the photos gathered from the SEM. Measurements were made of the pore size of 50 different pores for each sample.

Next an Asymmetrical nanofiber was created as follows. A parchment paper (AOS) was taped onto the collector of the electrospinning apparatus. The PCL nanofiber was electro spun on parchment paper using the same parameters. The PCL nanofiber and parchment paper were separated using tweezers. The PCL nanofiber was attached to the collector in the electrospinning apparatus. The composite nanofiber was electro spun on top of the PCL nanofiber using the same parameters as previous. The nanofiber was dried at room temperature for 48 hours to remove any removing solvent.

Bacterial permeation was recorded as follows. 3 grams of tryptic soy powder (AOS) was mixed with 100 ml of distilled water in an Erlenmeyer flask using a magnetic stirrer. The flask was sealed tightly with aluminum foil. A piece of autoclave tape was placed on top of the foil, labelled and autoclaved at 121 degrees celsius for 15 minutes. The broth was refrigerated for later use.

S.epidermidis bacterial cells (Staphylococcus epidermidis (ATCC) (https://www.atcc.org/products/14990) were cultured next. All added materials including the broth flask, test tube rack, inoculation loop and pipette were sterilized. 6 mL of the broth were added to 4 test tubes using a pipette. A sterile inoculation loop was used to add *S.epidermidis* bacteria to the tubes one by one. Since the bacteria were on a slanted agar, the inoculation loop was dragged across the agar to collect the bacteria. The inoculation loop was added to the test tube with broth and swirled around and stored at 37 degrees celsius.

The transwell system (Corning Cell Culture Inserts, $96) was modified by removing 6 transwell inserts from the well plate. The filter paper membrane was cut carefully for each insert using a scalpel. The PCL and asymmetric nano fibers were peeled off the foil. Three 2cm x 2.5cm samples for each nanofiber were cut out and pasted onto the transwell inserts using adhesive caulk to act as the membrane between the upper and lower chamber. This transwell system was sprayed with 70% ethanol and let to dry.

Bacterial permeation test was performed as follows. After about hours, a 0.6mL of tryptic soy broth was added to each of the wells. The surface of the nanofibers in the upper well was inoculated with *S.epidermidis*. This process was completed for each of the three wells (3 for PCL modification,3 for asymmetric modification and 3 for control filter paper) and stored at 37 degree celsius for approximately 48 hours. After 48 hours, the tryptic soy broth in the lower chamber was removed and placed into a micro cuvette. A Spectro-Vis spectrophotometer was calibrated using a blank sample of tryptic soy broth. Following this, each experimental sample was run through the spectrophotometer and the absorbance was measured at a wavelength of 595 nanometers. The spectrophotometer was calibrated using blank cuvette between trials.

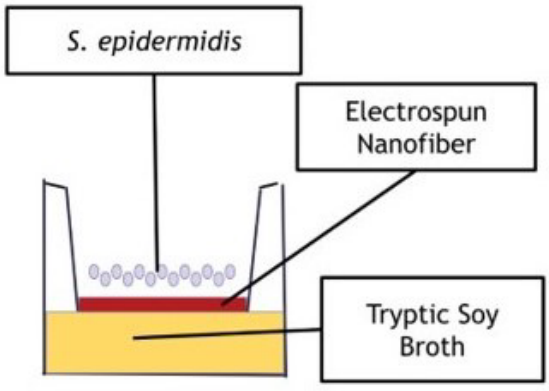


Figure 4. Modified transwell insert. The regular transwell inserts were cut out and replaced with 2.5 cm x 2 cm samples of the PCL or asymmetric nanofiber, attached with adhesive caulking.

Results

Our research was split into two sections: one that analyzed the difference in physical characteristics between the PCL/CS/Cur nanofiber and the PCL nanofiber, and then one that compared our asymmetric nanofiber design to a homogeneous PCL nanofiber. To start, chitosan was deacetylated. A strictly PCL polymer solution was created and electro spun. The deacetylated chitosan was used to form a PCL/CS/Cur polymer solution, which was then electro spun. These nanofibers were then characterized for nanofiber diameter and porosity percentage by using the scanning electron microscope (SEM) and ImageJ. After this step, the nanofibers went through a water contact angle test to determine hydrophobicity. As such, the independent variable for this section of the experiment was the nanofiber tested, while the dependent variables were nanofiber diameter, porosity percentage, and hydrophobicity. The constants for this step included the temperature and humidity of polymer solution preparation and the SEM used for data observation. Following the completion of initial characterization, an asymmetric nanofiber composed of one layer of PCL/CS/Cur and one layer of PCL was created, along with a homogeneous nanofiber composed of solely PCL. *S. epidermidis* was cultured and a transwell system was modified such that each nanofiber served as the membrane between the lower and upper well. After being seeded on top of the nanofiber and allowed to incubate for 40 hours, the absorbance of the broth in the lower chamber was recorded. For this section of the experiment, the independent variable was the symmetry of the nanofiber, while the dependent variable was bacterial infiltration. The constants included the method of transwell modification, the relative amount of bacteria seeded per well, and the spectrophotometer used for data collection.

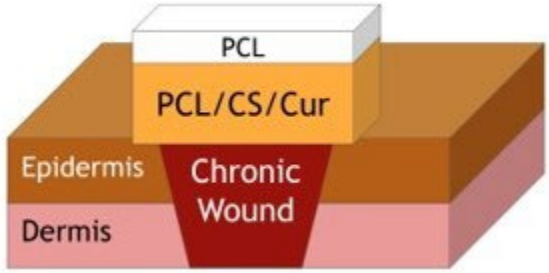


Figure 1. The SEM Image comparison shows that the PCL layer was less porous and more hydrophobic than the PCL/CS/Cur layer. The SEM images and water contact angle images were analyzed using ImageJ.

Table 1. Full scale nanofibers, SEM image and hydrophobicity test

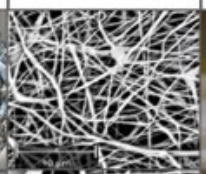
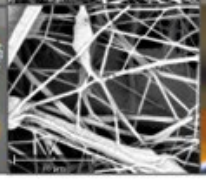
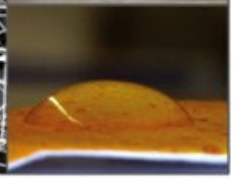
Nanofiber Layer	Image of Nanofiber	SEM Image at 10 µm	Water Contact Angle
PCL			
PCL/CS/Cur			

Table 2. Absorbance measures of broth after bacterial permeation of *S. epidermidis*

Row	Nanofiber	Absorbance at 595 nm
1	PCL (n=1)	0.103
2	Asymmetric Nanofiber (n=1)	0.047
3	Control Filter (n=1)	0.01

The absorbance value for the asymmetric nanofiber was less than that for the PCL nanofiber, demonstrating that the lower layer of the asymmetric nanofiber composed of PCL, chitosan, and curcumin may possess antibiotic qualities. However, the asymmetric wound dressing was not able to eliminate bacterial infiltration.

Table 3: Nanofiber Characteristics from the SEM and Water Contact Angle Tests

Nanofiber (n=3)	Average Nanofiber Diameter (nm)	Average Porosity (%)	Average Water Contact Angle
PCL	281.82	52.16	133.57
PCL/CS/Cur	425.54	61.18	19.085

The PCL nanofiber had a smaller average nanofiber diameter and average porosity than the composite layer of the asymmetric nanofiber. Additionally, based on the average water contact angle, the PCL nanofiber was more hydrophobic than the composite layer. The layers retained their distinct nanofiber diameter and porosity when incorporated into the asymmetric design.

Discussion

Our results indicate that we were able to create a nanofiber wound dressing composed of two distinct layers. These layers are different in their average nanofiber diameter (281.82 nm vs. 425.54 nm), average porosity (52.16% vs. 61.18%), and hydrophobicity (hydrophobic vs. hydrophilic). These quantities agree with our expected results, as the characteristics mimic the differences in structure displayed in the skin's epidermis and dermis.

Our nanofiber also abides by wound dressing standards. MacEwan et al. (12) established that wound dressings should have fiber diameters between 50-500 nm and porosities between 60-90% in order to ensure enough space for cell migration and proliferation. Both layers of our nanofiber fall within the acceptable nanofiber diameter range, and our PCL/CS/Cur layer falls within the acceptable porosity range. Since the PCL/CS/Cur layer is the part of our nanofiber that is directly interacting with the wound (and is therefore the component that is responsible for allowing cell migration/proliferation), the PCL layer's relatively low porosity has little effect on the viability of our wound dressing. In fact, the porosity of our PCL layer (52.16%) matches very closely with the porosity of PCL layers from past research (55%) (4).

Our asymmetric nanofiber outperformed a homogeneous PCL nanofiber at resisting bacterial infiltration. The addition of the PCL/CS/Cur layer decreased the absorbance reading from 0.103 to 0.047. Given the large porosity of the PCL/CS/Cur layer, the change in bacterial infiltration can be attributed to the antibacterial properties from both the chitosan and curcumin. Our asymmetric nanofiber did not perform as well as the control filter. This difference may be attributed to the method of modification we used for the transwell system, but

it may also be because our nanofiber layers are too thin to effectively reduce the bacteria crossing the membrane. Future bacterial infiltration tests are necessary to determine the cause of this discrepancy. As our sample sizes were too small and only represented 1 or 2 nanofibers, we only reported a simple average of the data. Our results are promising but will require generating many more nanofibers such that we could have sample sizes large enough to conduct statistical analyses.

By creating an asymmetric wound dressing composed of PCL, chitosan, and curcumin with distinct characteristics similar to the skin's layers, we demonstrated the importance of the asymmetric design and our nanofiber design's potential as a viable wound dressing that prevents bacterial infection. Our results can be used as important evidence in support of the use of asymmetric nanofibers with antibacterial properties as wound dressings. The conclusions drawn are consistent with those from Miguel et al. (4) and can provide significant background information for electro spun nanofiber wound dressing research in the biomedical field.

Future experimentation can determine the most successful components, dimensions of each layer, and concentrations to encourage chronic wound healing. Additionally, we can test cell proliferation and migration to build on the work we have provided on asymmetric nanofiber wound dressings and fully establish our nanofiber as a viable wound dressing.

Awards

This research project was awarded the First place at the Loudoun County,Virginia Regional Science and Engineering Fair (RSEF) on March 17, 2022 and was a Finalist at the Regeneron International Science and Engineering Fair (ISEF) in Atlanta, Georgia, May 7-13,2022. The research project was also selected for a joint international collaboration with Hwa Chong Instituition in Singapore.

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