

Electrospinning of Soy Protein Fibers and their Compatibility with Synthetic Polymers

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ABSTRACT

This paper critically reviews previous work in the field of electrospinning of biopolymers and antimicrobial polymeric materials, and investigates the potential of Soy Protein fibers in electrospinning. Biomaterials have since long been the popular choice for fabricating medical textiles or scaffolding materials owing to their biocompatibility, intrinsic anti-microbial activity and low immunogenicity. Even among biopolymers, plant-based protein fibers are more preferred than carbohydrates. Electrospinning of such biopolymers into nanofibers provides one with advantages of properties such as relatively very high porosity, pore interconnectivity, drug-carrying capacity and close similarity to the extra-cellular matrix in the body. Soy protein fibers are envisioned to be an excellent component in fiber spinning mixtures. Mixing of Soy protein with other spinnable polymers such as cellulose, PEO, PVA and chitosan is proposed. Wound-healing is an important application for these materials. The emphasis needs to be on the functionality of such materials, more than mechanical strength which is not a vital necessity for class II medical devices such as wound dressings. A comparatively uninvestigated area is the potential of soy protein fibers for such uses.

Keywords: Soy protein; Electrospinning; Biopolymers; Antimicrobial; Nanofibers

Importance of biomaterials in healing applications/medical textiles

Medical textile products are made from a wide range of materials of synthetic and natural origin. A few trust synthetic fibers for their consistence and strong mechanical properties. While, some prefer going the 'green' way by choosing bio-materials such as chitin, chitosan, collagen, and gelatin for their biodegradability and ability to mimic natural conditions. Several attempts have been and are being made to optimize the process parameters in fabrication of such

materials along with exploring the behavior of different combinations of materials.

Biopolymers are renewable resources and may also intrinsically exhibit biocompatibility, biodegradability and anti-bacterial activity [1], [2]. Nanofibers made out of biopolymers find applications in reinforcements in nano-composites, sutures, filters for metal recovery and protective clothing [1]. Recent developments in the electrospinning technology have produced scaffolds made of nanofibers. Electrospinning process parameters can be varied to achieve difference in pore size,

number of anchoring sites, degree of wetting and degradation rates. Porosity of the material is one of the factors that determine the efficiency of medical textiles, battery separators, filters and fiber composites [1].

Biopolymer nanofibrous mats have shown potential for applications within the medical field due to the aforementioned intrinsic properties of these renewable materials [1], [2]. Medical and pharmaceutical fields could use nanofibers to fabricate wound dressings, tissue engineering scaffolds for drug delivery, or other medical devices. The success rate of artificially recreating the extracellular matrix and other tissue engineering applications depends on the properties of the scaffolds, such as their biocompatibility, osteoconductivity, degradability, high surface-area-to-volume ratios, and mechanical properties [2].

While these various natural polymers are being explored for fabrication of biomaterials, plant proteins are preferred over carbohydrates because of their easy availability, low potential to be immunogenic and ability to be made into fibers, films, hydrogels and micro- and nano- particles for medical applications. Proteins are preferred over synthetic polymers (more importantly carbohydrate polymers) because they form a major part of the human body, they are 'bio'- and 'cyto-' compatible, and it is easier to maintain their functions in the extra-cellular matrix than with synthetic polymers. Plant proteins are co-products generated when cereal grains such as corn and wheat are processed for food or fuel. They have considerably less potential to be immunogenic as compared to traditional biopolymers such as bovine collagen. Proteins are most suited for especially drug-delivery mechanisms; their wide range of isoelectric points helps one choose specific proteins for the specific drug. Owing to the several advantages of plant proteins compared with collagen and silk, efforts have been made to explore the possibilities of utilizing plant proteins to develop hydrogels, films, fibers, nano- and

micro-particles for medical applications, mainly tissue engineering and drug delivery [3].

On the flip-side, these plant proteins have inferior mechanical properties, especially poor hydrolytic stability compared with silk but better than collagen-based biomaterials. Some other plant proteins that have remained fairly unexplored are zein from corn, soy proteins and wheat gluten. Thermoplastics made of soy proteins were found to have mechanical and degradation properties suitable for scaffolding. Soy protein promoted higher growth of cells than another natural polymer such as chitosan when it was used as part of the cross-linked blend [2], [3].

This paper critically reviews the various attempts at electrospinning biopolymers, alone or in combination with other synthetic polymers and the results that were achieved thereafter. The concept of electrospinning a blend of Soy and Chitosan to make nanofibers is proposed, based on previous research where these were spun individually or with other polymers. It is anticipated that both materials would complement each other in mechanical and functional properties.

The Significance of Electrospinning

Electrospinning scores over other methods to generate nanofibers because of the simplicity of the process and uniformity of the fibers produced. Nanofibers have dimensions of the order of 1×10^{-9} and surface properties are different on that level from other lower magnitudes. Nanofibers are today widely used in tissue engineering applications as scaffolds and have the following properties that differentiate them from conventional fibers for bio-medical end-uses [4], [5], [6]: (1) Increased surface area as fiber diameter decreases, and hence unusually high surface energy, (2) Raised surface reactivity, (3) Fibrous mats can mimic the extra-cellular matrix (ECM), (4) Enhanced drug loading capacity, and (5) Increased strength even at low density

In the past decade, research has seen resurgence in electrospinning of nanofibers from natural polymers owing to vast possibilities for their enhanced use in bio-based materials. Many parameters like solution properties, processing and ambient conditions influence the transformation of polymer solution into nanofibers during the electrospinning process. With their distinctive properties, nanofibers have found applications in wound dressing, filtration, composites, tissue engineering and others. For most applications, it is always desirable to have smooth, ultrafine nanofibers with uniform diameter [6].

Through electrospinning, biocompatible polymers can be spun into a nano-sized mesh. This nanomesh would be composed of fibers with a high surface area, and a nano-sized diameter that can be used to mimic a natural extra-cellular matrix, which acts as scaffolding material that allows cells to attach, proliferate, differentiate, and develop essential functions within tissue [7].

Electrospun nonwoven type of biopolymers have a plethora of applications in medicine, aerospace, filtration and protective textiles and are being considered for additional research. Li and Younan reviewed the process of electrospinning and reported that what electrospun nanofibers form is a nonwoven web. For tissue engineering applications, several synthetic as well as biopolymers have been used as 3D scaffolds for tissue growth or as nonwoven membranes. For example, wound-dressings have been made from porous mats consisting of fibrous structures that can protect the wound from bacterial penetration via an aerosol particle capturing mechanism while having breathability. Thus these structures serve as drug carriers [4].

Wound healing is a process of regeneration of tissue after dermal or epidermal damage. Healing of deep cuts or burns takes a long time for complete re-epithelialization and scar tissue formation to take place. All wound repair materials must possess certain fundamental qualities such as: [8] (1)

Prevent and control infection in the wound and surrounding areas, (2) Make the environment moist, (3) Improve comfort and protect the wound from further trauma, (4) Absorb the exudates, if any, (5) Accelerate the process of wound-healing in general

Polymeric nanofibers have excellent pore densities and pore inter-connectivity for exuding fluid from the wound. It can be seen in *Figure 1* that fine fibers of biodegradable polymer can be sprayed directly onto the wound with the help of an electric field, to make a fibrous mat. Electrospinning, additionally provides a means to embed drugs into the nanofibers for any possible medical treatment and/or anti-microbial purposes. Electrospun polyurethane mats have shown to effectively exude fluid from the wound, without fluid stagnating under the wound cover and also no wound desiccation either. Dermal wound repair has been done effectively using electrospun nanofiber mats (scaffolds) that closely resemble the ECM. Besides, instead of impregnating polymeric nanofibers with antibacterial additives, polymers like chitin that are inherently antimicrobial have been explored [8], [9].



Figure 1. Nanofibers sprayed on the wound as ‘dressing’
(www.electrosols.com) [5]

Encapsulation is an important property of electrospun nonwoven webs. Enzymes

stored inside the pores of the web have shown better activity in the body of the organism. Fibers normally used in the past include polyurethane or collagen. Such structures can also be referred to as 'Antibiotic Impregnated Nanofiber Membranes' since they embed medicinal or healing materials in them [4]. Additionally, by electrospinning fibrous mats, a three-dimension malleable scaffold is fabricated. Thus, it could be molded around, spun directly onto, or into the pores of whatever size substrate that needs cell seeding.

Various biopolymers have been electrospun in the past. Cellulose, cellulose acetate, chitin, chitosan and chitosan with polyethylene oxide (PEO)/polyvinyl acetate (PVA)/polyethylene terephthalate (PET), proteins like collagen, gelatin and recently soybean are some of the natural polymers used in electrospinning. A few important ones are discussed below.

Cellulose: Being the most renewable, abundant and versatile polymer, cellulose has been one of the longest studied materials for electrospinning. Problems were faced when cellulose would not dissolve in conventional solvents dimethylsulfoxide/paraformaldehyde or sulfur dioxide due to its strong inter- and intra-molecular bonds. However, these solvents were not suitable for electrospinning. Due to the problems associated with dissolving cellulose, it is common to use cellulose derivatives (cellulose acetate and propionyl cellulose), which do dissolve in common solvents. In some cases, the disadvantage of cellulose derivatives, is the reduced stability and degradation of the cellulose structure. Oxidized cellulose was also explored as it degrades under physiological conditions and is bioresorbable; it has been used as a resorbable homeostatic dressing, in cosmetic preparations, fibrin formation catalysis-agents, and adhesion barriers. Cellulose composites made with biocompatible, non-toxic, and chemically resistant polymers like polyvinyl alcohol (PVA) and polyethylene

oxide (PEO) provides better fiber-forming properties. Moreover, changing the ratio of cellulose acetate/PVA altered the mechanical properties as well. Guanfushou and Liu reinforced soy protein isolate films with cellulose nanofibers. Cellulose nanofibers were made by hydrolyzing electrospun nanofibrous mats made from cellulose acetate solution [1], [6], [10].

Chitin: Second to cellulose, chitin is the most abundant organic material produced by biosynthesis. However, use of chitin in many applications has been limited due to its insolubility in most organic solvents. The process parameters for the electrospinning of chitin and chitin-containing solutions, including: MW, degree of deceleration (DD), solvent used, special polymer processing, electrospinning parameters. Chitin electrospun mats were also blended with other polymers such as poly (glycolic acid) PGA; the blended fibers degraded faster than pure PGA fibers since PGA is both biodegradable and biocompatible [1], [6], [11].

Chitosan: It is the N-deacetylated derivative of chitin and derived from the shells of crabs and other crustaceans which contain about 85% chitosan. The solvent choice is one of the most important optimizing parameters in electrospinning. For chitosan there is a wider range of solvents to choose from than for chitin as it is soluble in many. It has successfully been electrospun with trifluoro acetic acid (TFA) and acetic acid (AA). However, chitosan needs to be made chemically stable before being applied in end-uses. It is cross-linked in a two-step process with gluteraldehyde in a vaporization chamber. Chitosan blended with other polymers like PVA showed that higher concentration of chitosan improved the morphology of the electrospun fibers. Chitosan/PVA nonwovens were observed to have high water-uptake abilities and hence suitable for use in wound-dressings. Due to the polyelectrolytic nature of chitosan, it has a high viscosity in dilute aqueous solutions. Therefore, it can be desirable to use chitosan

as a thickener, especially since it is compatible with other biocompatible polymers such as PVA and PEO. Sometimes beads are observed when electrospinning. To counter this, additives such as salts or surfactants can be used. Similarly, cationic and anionic polyelectrolytes could increase the conductivity of a solution and thus decrease fiber diameter. Similar successful attempts were made with chitosan and PET or chitosan and PEO [1], [6], [10].

Collagen and gelatin: Proteins are the key component in scaffolding, protection, growth etc. Bio-proteins such as collagen and gelatin derived from animal tissues have been extensively researched despite their complicated structure and chemistry [1], [4], [6].

Collagen: is available in different grades based on the source; from calfskin, human placenta or bovine ligament. Collagen generally is cross-linked with other chemicals or is incorporated in a composite nonwoven mat (containing PEO, (polycaprolactone) PCL, chitosan, etc.) for better chemical and mechanical properties.

Gelatin: Similar to collagen in composition and biological properties from which it is derived, it is highly polar and electrolytic in character.

Silk: Silk is also a very common fiber after collagen used in tissue engineering applications. It has excellent mechanical properties although, only fibroin in silk is biocompatible, sericin, the gummy substance, is toxic to the cells. Moreover, among other plant proteins collagen and silk are more readily available and can be easily fabricated into scaffolds such as fibers or films having good mechanical properties [1].

With nanofibers made from protein that are actually amphiphilic biopolymers, they are ideal because they interact equally with both the drug and solvent. So far, drug-loaded nanoparticles have been synthesized from

insoluble proteins (zein, gliadin) as well as soluble proteins (bovine collagen) [12].

Electrospun Soybean protein fibers: A potential wound care material

Soybean is an important source of protein and its production in North America continues to increase as the yield and per-acre income continue to increase steadily. It is a seasonal crop and is planted usually in the months May-June and harvested during October-November. Majority of the soy produced is used for oil production, textiles, or fiber formation than for direct consumption. Soybean contains approximately 40% protein (highest among legumes) on a dry weight basis. Soy-proteins are available in 3 forms namely [16]: (1) Soy-protein isolate (SPI) - contains 93 % soy protein fiber, (2) Defatted Soy flour (SF)- contains 74% soy protein fiber, (3) Soy-protein concentrate (SPC)- contains 53% soy protein fiber

SPI being the purest form of the protein, is used for fiber extrusion. It is made from defatted soy flakes during the production of oil and contains a minimum of 90% protein. Soy-protein is abundant, biodegradable, biocompatible and inexpensive, all of which make it a suitable polymer for addition in fiber spinning solutions [15]. Our target applications are in medical textiles, which require polymers that resist bacterial contamination and that do not generate a toxic response in the body tissues, while being cheap.

Soy protein, the major component of the soybean, has the advantages of being economically competitive and presents good water resistance as well as storage stability. The combination of these properties with a similarity to tissue constituents and a reduced susceptibility to thermal degradation makes soy an ideal biomaterial [14]. Soy protein has functional groups such as $-NH_2$, $-OH$, $-SH$ that are susceptible to chemical and physical modifications. Some studies have reported that the combination

of soy with other proteins like casein, and polysaccharides such as cellulose and chitosan in film form may trigger surface interactions which could improve certain properties [15].

Soy protein fibers could be found in various forms namely, as Textiles (Soy fibers could

be woven or knitted into apparel such as socks with antimicrobial properties), as Composites (they could be the reinforcing fibers in a synthetic or biopolymer matrix), as Nonwoven webs (electro-spun soybean is gaining popularity as it has the surface potential to immobilize enzymes [4], [13].

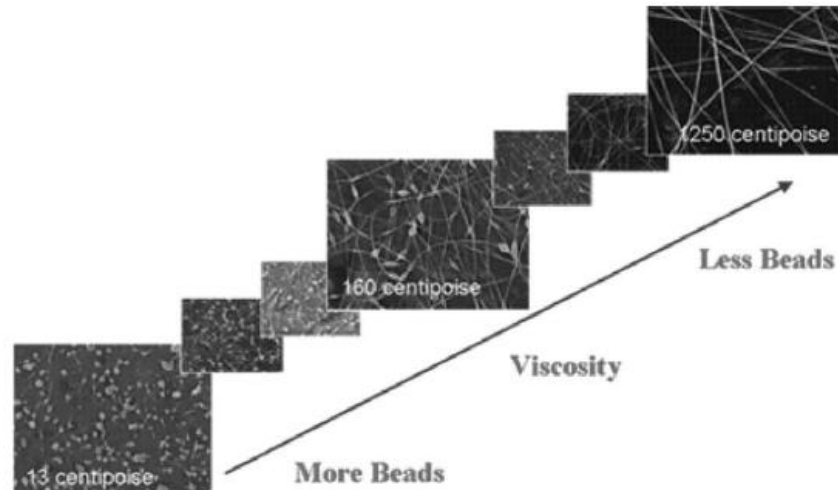


Figure 2. A schematic showing that as viscosity increases formation of beads reduces [5]

To date, electrospun 100% soy fibers have not been reported in the literature. Unlike fibrous proteins such as collagen and gelatin which have been extensively and easily electrospun in the past, the globular structure of soy-proteins must be unfolded by denaturation treatment to enable electrospinning. Soy alone is not a strong enough fiber owing to poor mechanical properties and other disadvantages that come with using natural polymers. Studies done in the past have proved that initially due to

high surface tension of water and low solution viscosity there was formation of dough and beads in the fibers electrospun from an aqueous polymers solution of 100% soy. It was then blended with PVA (or some other biodegradable polymer) to hold or bind the soy together [2], [15], [16]. *Figure 2* and *Figure 3* show how an increase in the polymer concentration, or solution viscosity, the resultant morphology changes goes from beads to uniform fibers.

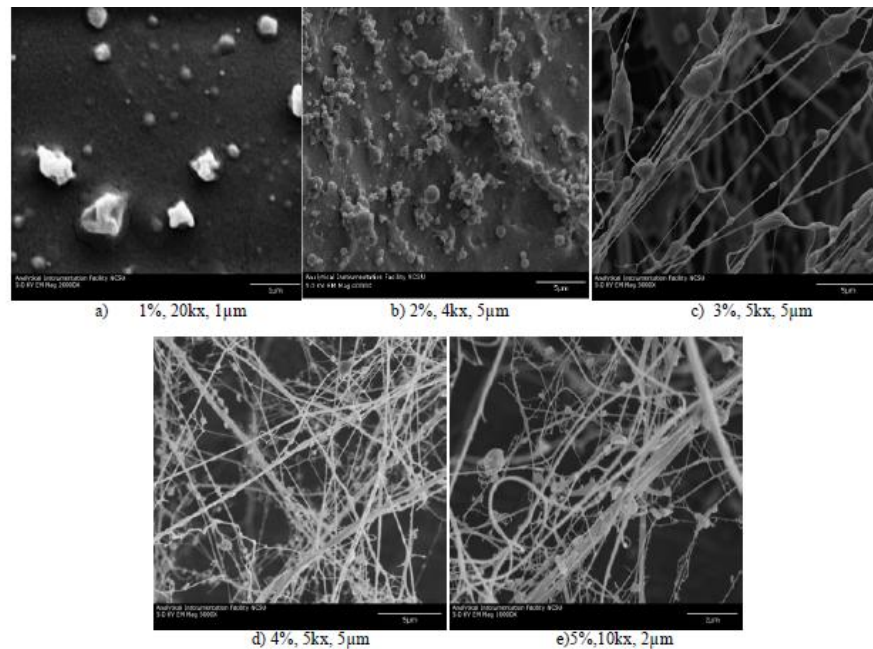


Figure 3. An example of how morphology of electrospun fibers changes from beads/film to continuous fibers as polymer concentration increases in the solution from 1% - 5% [25]

Another recent study built on a previous study suggesting that agro-wastes from oil palm and soybean (*okra*) could be spun into nanofibers using electrospinning technology, by developing and evaluating an agrowaste-based nanofiber encapsulating lactic acid bacteria [17].

Soy-proteins contain fewer proteins and hence the range of solvents to electrospin them is limited. In the past they have been developed for ‘hydrogels’ for controlled release of the drug embedded in these gel structures. The other forms of soy-proteins are fibers and films. Fibers are stronger than films. They are mainly involved in tissue engineering applications as they mimic the extra-cellular matrix. Moreover, these tissue engineering applications use nanofibers form of soy (with other polymers). Protein fiber from soy have certain specific advantages like water stability, biocompatibility, strength required for medical textile applications, without having to cross-link them [2], [15], [16].

Several studies related to spinning soy protein fibers either as a whole or in

combination with other polymers have been done until now. A comparative account of a few is discussed in the following sections.

100% soy-protein fibers by wet-spinning:

Reddy and Yang investigated the possibility of producing 100% soy fibers with water stability and mechanical properties suitable for tissue engineering applications without using cross-linking agents. The fibers were prepared using wet-spinning with solvents such as urea and sodium sulfite. The spun fibers were tested for their mechanical properties on the Instron Tensile tester, physical structure, water stability and scaffolding ability. The diffraction pattern showed that soy fibers had low crystallinity and poor orientation. The tensile strength of soy was seen to be higher than other cereal proteins which could have been largely due to the optimum aging time and temperature in the solution. The water stability of soy fibers was better than polylactic Acid (PLA) fibers in the 3-11 pH range, 90⁰C for one hour. As regards the morphology, the fibers were 50-150 μm with a uniform longitudinal surface with striations that help in cell

attachment. Overall, it was concluded that soy proteins are novel biomaterials for the medical textile applications [2].

Soy protein isolate and Polyethylene oxide fibers:

Soy protein when synergized with PEO fibers was found to enhance the electrospinning of the protein solution by increasing solution viscosity and reducing electrical conductivity. Vega-Lugo et al mixed 100% Soy protein isolate with different concentrations of PEO to achieve various morphologies of fiber (fibers, beads, combination of both). 100% SPI when denatured in combined thermal and alkaline treatment could not be spun into a fiber readily. However, when PEO solution was added SPI could be readily spun resulting in fibers of diameters 200-260nm. Non-ionic surfactant added to improve the surface tension of the spinning solution, helped facilitate the dispersion of the SPI powder for making homogeneous polymer solutions [15].

Soy protein isolate and polyvinyl alcohol (PVA) fibers:

PVA is a common choice among synthetic polymer blends with soybean due to its biodegradability and water-solubility. It dissolves at similar conditions as proteins and when added to proteins, it increases the

fibers strength. PVA is also biodegradable in the soil. Moreover, both soybean protein and PVA exhibit hydrogen bonding. The experimental soybean protein fibers were made from two macromolecular components combined together into [19]: (1) Bi-constituent fibers, where a spinning dope was prepared from a homogeneous mixture of two solutions – a soybean protein-water solution and a water solution of synthetic polymer (polyvinyl alcohol. Single fibers made from such spinning dopes had homogenous structure, (2) Bi-component fibers, where the fibers core was made from a soybean protein and the fibers sheath from polyvinyl alcohol.

Cho et al explored the rheological behavior and spinnability of biomaterials based on soyprotein and PVA for the production of electrospun fibers. pH level, processing temperature and heating time were the parameters optimized on the experiments. Continuous production of uniform fibers was not possible when pure SPI solution was electrospun. On addition of PVA, the mixture could be fully denatured by adopting correct temperature and pH. These process parameters were adjusted to achieve the desired morphology of the fibers; for example, increasing pH of the solution reduced the dough and bead formation during spinning [16]. SEM images from these experiments in *Figure 4* show the effect of increasing pH on morphology.

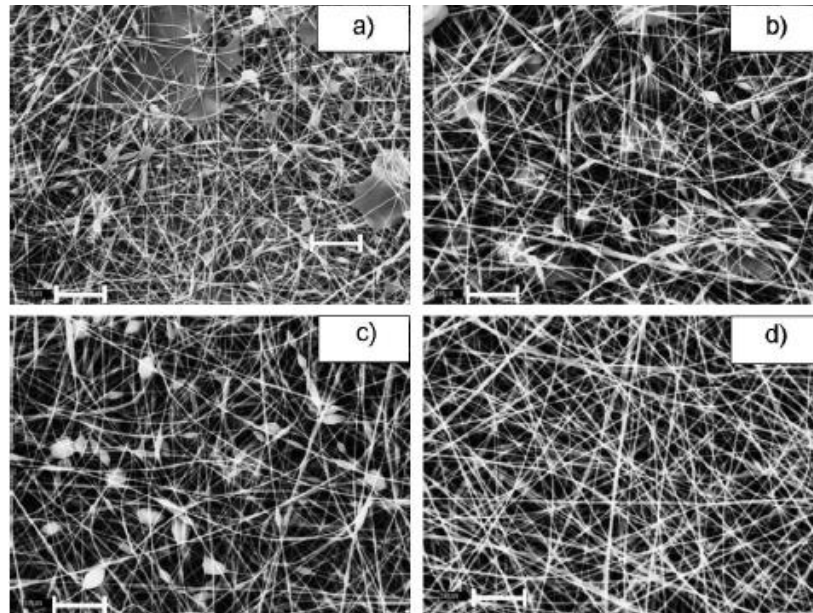


Figure 4. Electrospun nanofibers of Soy and PVA blended together in 50:50 ratio; a) at pH=7, b) at pH=9, c) at pH=12, d) at pH=12 with surfactant [16]

Zhang et al produced soy-PVA fibers using the wet-spinning technique, with thermal denaturation of the protein solution. Testing of the dynamic mechanical properties and scanning electron microscopy (SEM) studies suggest compatibility between soybean and PVA fibers. Also, the fiber morphology depended upon the amount of PVA in the blend. For only soybean or less than 40% PVA blends were amorphous, whereas the ones containing more than 40% PVA showed crystalline regions of PVA [20]. The mechanical properties were enhanced when PVA was blended in. Denaturation of soy may cause solution viscosity to drop below that required for spinnability. Increasing the amount of PVA increases the proportion of smooth, continuous fibers in the web.

Denaturing is an important procedure to prevent the protein molecules from forming more complex structures. The disulfide, hydrogen, and ionic bonds and even steric and hydrophobic interactions in the native state of the protein, which are responsible for forming higher order structures, have to be broken down to achieve a spinnable solution. Sodium sulfite and urea have been

known to be effective for breaking disulfide bonds and, to some extent, to prevent the gel formation of the solution. In addition, protein denaturation using either high pH condition or heat is needed to obtain a suitable dope for spinning [18].

Su et al prepared 'green' SPI films with PVA blended in. The blend films showed better tensile strength than what soybean individually would have, and soybean/PVA proved to be compatible in terms of similar properties and processing requirements indicated by SEM analysis [21].

All previous attempts have managed to fabricate soyprotein fibers either by electrospinning or some other technique resulting fibers of the range of 200-300 nm or even 50-150 microns. However, there has till date been not one systematic approach on fabricating nanofibers using 100% soyprotein isolate. In recent times, Teng et al showed that SPI nanoparticles could be successfully fabricated from the method of desolvation. The nanoparticles were used for controlled release of curcumin as the drug by encapsulation of the drug within the

spheres with an average size of 220 to 286 nm. These nanoparticles exhibited satisfactory encapsulation capabilities and a desirable stability at high concentrations owing to the right balance of amino acids [12].

Soyprotein has been incorporated into hydrogels and films successfully in the past. As sustained drug-release materials these films had excellent binding capacity and release of up to 80% in 6 hours [3].

Chitosan-Soy based materials:

Chitosan is a relatively more established polymer in the biomedical field than Soy. Chitosan has been successful in skin-wound healing applications. It may not directly be involved in the resurrection of the extracellular matrix (ECM), but it has the capabilities to trigger the growth factors that enhance the production of the ECM. Therefore, the combination of chitosan with soybean protein is foreseen as a suitable approach to control the biological behavior of soy-based biomaterials. This combination has been researched before, but in forms different from fibers. Tircia C. Santos et al went on to prove that chitosan improves the biological activity of soy protein. They compared the in vivo performance of the SPI films to that of the Chitosan-soy films when they were injected subcutaneously into rats. As expected, the infusion of chitosan into soy improved the host response, which showed features of a typical inflammatory response [6], [22].

Silva et al fabricated chitosan/soy protein isolate blended membranes by solvent casting methodology. The objective of the work was to test the influence of chemical cross-linking in the water uptake, degradation rate and biocompatibility of the blend system composed by chitosan and soy protein. The membranes exhibited different degradation pattern and, improved cell spreading with respect to pure chitosan. The results, the incorporation of chitosan associated to network formation by cross-

linking promoted a slight decrease of water absorption and a slower degradability of the membranes. The biological studies performed suggested that the crosslinking with low glutaraldehyde concentration changed the membrane surfaces, promoting a better cell adhesion of the membranes [14].

The above mentioned research projects have been aimed at making film or membranes from chitosan-soy blends. Also, these films were used in fabricating scaffolds which would be implanted inside the body. However, most wound-dressing materials require fibrous structures that have dense, interconnecting pores and that would hold a drug in them. Hence, the advent of electrospinning for wound-dressing materials has gained much attention in the recent past. Nawalakhe et al [24] electrospun a derivative of chitosan, imminochitosan in combination with cellulose acetate with the aim of making better wound dressings. Electrospinning of chitosan as a homopolymer is not possible. Being a polyelectrolyte, it can be electrospun, but cannot be assembled layer-by-layer as it would dissolve when placed in the counter ion surface. Hence, a derivative of chitosan was used with Trifluoroacetic acid (TFA), a common solvent for both polymers. These bicomponent nanofibers were made with imminochitosan as the sheath and cellulose acetate in the core. The nanofibers produced had a wide range of diameters but showed good mechanical integrity and antimicrobial activity. The antimicrobial activity was tested with different micro-organisms and the contact kill performance was assessed with respect to the size of the inhibition zone formed in the disc diffusion tests.

It would be futile to compare the strength of fibers made by conventional spinning techniques such as melt or wet spinning with that made by electrospinning. Electrospun nanofibers are not distinguished for their mechanical properties, but for their morphology. It is therefore, reasonable to not overvalue the strength of the nanofibers.

Our aim here, is to achieve a suitable recipe that assures us of a product that forms continuous fibers, resists microbial invasion and/or encapsulates drugs. In a subsequent study by Nawalakhe et al [25], instead of biocomponent fibers, iminochitosan alone was electrospun, with TFA as the solvent. Wound dressings require properties of biocompatibility and better antimicrobial activity more than mechanical strength. Hence, perhaps, the need for the cellulose-based core was lost. Moreover, chitosan has better antimicrobial properties as compared to cellulose. Iminochitosan on hydrolysis produces salicylaldehyde and this was expected to have better antimicrobial properties as compared to chitosan. They were successful in producing barbed nanofibers (See *Figure 5*), of the range of 70-200nm. Seyam et al produced electrospun nanofibers using yet another derivative of chitosan namely, cyanoethyl chitosan using the same solvent of TFA. The above studies have established that chitosan-based electrospun nanofibers have great potential as wound-dressing materials, but the limiting factors are the cost of chitosan and the need to first derive a non-poly-electrolytic form of chitosan.

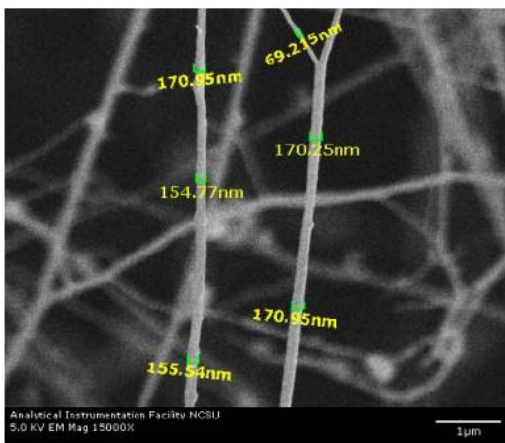


Figure 5. Barbed structure of fiber web observed at a specific concentration of iminochitosan [25]

Instead of working on the solution parameters for electrospinning which are

slow and are costly, various new processes have deviated from electrospinning to more sophisticated ones. Khansari et al [26] produced soy-protein (core) and nylon 6 (shell) nanofibers by solution blowing (subjecting the polymer solution to a coaxial turbulent air-jet streams). Formic acid was used as solvent for both polymers and separate core and shell solutions were prepared. The fibers were collected on a rotating drum covered with aluminum foil. The nano-fibrous mat was 0.15-0.30 mm thick made of oriented fibers and layered in cross-section. Although these structures were aimed to be used in nonwoven applications, the process of solution blowing produces an interesting morphology and high tensile strength. Recently, by combining a sol-gel and freeze-drying process it was possible to develop cross-linked porous structures based on chitosan and soy protein. The thus developed porous structures demonstrated adequate interconnectivity and porosity. It was demonstrated that these structures have immense potential for tissue engineering for cartilage. [23]

Chitosan's unique biological properties promote healing in the ECM. It is biodegradable, biocompatible, non-toxic, a haemostatic, and a natural antibacterial agent. The antibacterial behavior of chitosan is hypothesized by the interaction between the positively charged chitosan and the negatively charged residues at the cell surface of the microbes, which causes surface alterations and facilitates the release of the antimicrobial components to inhibit the normal functioning of the microbe. This finally leads to the death of the cells [7], [11], [14], [22].

Chitosan in the form of films/membranes is excellent for wound-healing and curing burns. But alone it is very expensive and possesses delicate film properties. Chitosan derived from the shell of crustaceans requires fishing, harvesting and extraction of chitosan from the shell which is all a labor intensive process. Also, it is seasonal. To

overcome such problems chitosan has been synergized with other polymers such as cellulose, PEO, or PVA. However, soybean and chitosan as a combination is a fairly unexplored area. Soy has been combined with other proteins such as wheat gluten or, casein, in the past. It may promote physical and chemical interactions, which improve some properties. Moreover, it also makes the process economical, since chitosan is costly. Chitosan cannot be easily electrospun, due to its polyelectrolyte nature as well. Hence, using the core/sheath technique blending of chitosan with another polymer had been investigated [7], [11], [14], [22].

Producing nanofibers out of a homogenous mix of Soy and Chitosan is a new concept. The biocompatibility of this combination has been well established by various attempts to analyze the in vivo behavior of chitosan combined with soy. Chitosan being a biopolymer has its inherent disadvantages, like any other of its kind, of having variability and poor mechanical properties. Also, being a more expensive material blending it with other materials economizes on the process. Soy alone promotes higher cell growth as compared to chitosan alone. With soy, a relatively less expensive biopolymer, efforts could be made to investigate the possibility of producing fibers for tissue engineering purposes made of soy and chitosan by electrospinning. Optimization of the process or solution parameters to electrospun the blended fibers could be a potential area of research.

Systematic research investigations should address finding the right recipe of the blend of the two polymers with respect to stir time, pH, temperature along with optimization of the electrospinning parameters such as voltage, distance between tip and collector, drum speed, and flow rate of the solution. This should be followed by a series of characterization experiments to determine solution viscosity, morphology of fibers by microscopic analysis and testing for antimicrobial activity. Our team is aiming at undertaking extensive research in this field.

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