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# Chitosan as a Biomaterial — Structure, Properties, and Electrospun Nanofibers

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## Abstract

Chitosan is a polysaccharide derived from chitin; chitin is the second most abundant polysaccharide in the world, after cellulose. Chitosan is biocompatible, biodegradable and non-toxic, so that it can be used in medical applications such as antimicrobial and wound healing biomaterials. It is also used as a chelating agent due to its ability to bind with cholesterol, fats, proteins and metal ions.

Chitosan is difficult to electrospin into a fibrous structure because it has a polycationic character in an acidic aqueous solution due to the many amino groups in its backbone. Fibrous structures were successfully formed by electrospinning chitosan solutions in 90 wt. % aqueous acetic acid solution or trifluoroacetic acid (TFA) or TFA/dichloromethane (DCM) in the presence of poly vinyl alcohol (PVA) or Poly ethylene oxide (PEO). Chitosan nanofibers are biocompatible and biodegradable, so they can be used as hemostatic and wound healing materials.

**Keywords:** Chitosan, Biocompatibility, Biodegradability, Antimicrobial activity, Functionality, Nanofibers, Electrospinning

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## 1. Introduction

Chitosan is a polysaccharide derived from chitin; chitin is the second most abundant polysaccharide in the world, after cellulose. The presence of amino groups in the chitosan structure might be protonated, providing solubility in diluted acidic aqueous solutions, several remarkable properties of chitosan offered unique opportunities to the development of biomedical applications. The elucidation of their mechanism will lead to a better understanding of chitosan's medical and pharmaceutical interest. The haemostatic activity of chitosan can also be related to the presence of positive charges on the chitosan backbone. Due to its positive charges, chitosan

can also interact with the negative part of cells membrane, which can lead to reorganization and an opening of the tight junction proteins, explaining the permeation enhancing property of this polysaccharide.

The polycationic nature of chitosan also allows explaining chitosan analgesic effects. Now, to explain chitosan biodegradability, it is important to remember that chitosan is not only a polymer bearing amino groups, but also a polysaccharide, which consequently contains breakable glycosidic bonds. Chitosan is actually degraded in vivo by several proteases, and mainly lysozyme.

Chitosan is biocompatible, biodegradable and non-toxic, so that it can be used as medical applications as antimicrobial and wound healing biomaterials. It used as chelating agent due to its ability to bind with cholesterol, fats, proteins and metal ions [1]

Due to chitosan's many attractive properties such as biodegradability, natural origin, abundance, reactivity, etc., it has many areas of application including medical, agricultural, food processing, nutritional enhancement, cosmetics, and waste and water treatment.

Chitosan is difficult to electrospun into a fibrous structure because it has a polycationic character in an acidic aqueous solution due to the many amino groups in its backbone. Fibrous structures were successfully formed by electrospinning chitosan solutions in 90 wt. % aqueous acetic acid solution or by using trifluoroacetic acid (TFA) or TFA/dichloromethane (DCM).

Since the electrospinning of chitosan itself proved to be difficult, chitosan was mixed with other synthetic or natural polymers, such as PEO or PVA.

Chitin and chitosan nanofibers with (50-500nm diameter) are biocompatible and biodegradable, so they can used as hemostatic and wound healing materials [2].

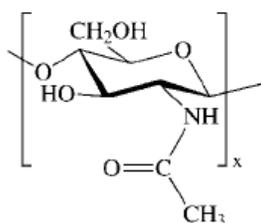
## 2. Chitosan

Chitosan is a polysaccharide derived from chitin. Its molecular weight is typically between 300-1000 kDa depending on the source of chitin. After cellulose, chitin is the second abundant natural polymer in the world. It is found in crustacean such as shrimps and crab [1, 3]

The main Sources of Chitin and Chitosan are : Insects (e.g. Cuticle, Ovipositors and Beetle cocoon), Crustaceans e.g. (Crab shell and Shrimp shell), Squid e.g. (*Ommastrephes* pen and Loligo stomach wall), Centric Diatoms e.g. (*Thalassiosira fluviatilis* and Algae) and Fungi e.g. (*Mucor rouxi* and *Aspergillus nidulans*).

Chemical structure of chitin made up of 1-4 linked 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose (Figure 1).

Although chitin is found naturally in large amounts through many sources, chitosan is only found in nature in limited quantities, such as in some fungi. The chitosan used in industrial or research applications is typically derived from chitin through the use of chemical or enzymatic treatments [4].



**Figure 1.** Chemical structure of chitin [4].

Chitosan is a copolymer of N-acetyl-D-glucose amine and D-glucose amine as shown in figure 2. It is a linear and semicrystalline polymer [5, 6] chitosan has a deacetylation degree of at least 60% of glucose amine residue (which corresponds to a deacetylation degree of 60). The deacetylation of chitin is conducted by chemical hydrolysis under severe alkaline conditions or by enzymatic hydrolysis in the presence of particular enzymes, among of chitin deacetylase [7, 8].

After cellulose, chitin is the second most abundant biopolymer [6] and is commonly found in invertebrates as crustacean shells or insect cuticles but also in some mushrooms envelopes, green algae cell walls, and yeasts [9-11]. At industrial scale, the two main sources of chitosan are crustaceans and fungal mycelia; the animal source shows however some drawbacks as seasonal, of limited supplies and with product variability which can lead to inconsistent physicochemical characteristics [12]. The mushroom source offers the advantage of a controlled production environment all year round that insures a better reproducibility of the resulting chitosan [13], chitosan is safe for both healthcare and biomedical application [5, 14]. The mushroom-extracted chitosan typically presents a narrower molecular mass distribution than the chitosan produced from seafood [14], and may also differ in terms of molecular mass, DD and distribution of deacetylated groups [13, 15]. Chitosan DD greatly varies between 60 and 100% while its molecular weight typically ranges from 300 to 1000 kDA [16], depending on the source and preparation. Chitosan oligomers can be prepared by degradation of chitosan using specific enzyme [5] or reagent as hydrogen peroxide [17].

After production, many different tools such as pH-potentiometric titration, IR-spectroscopy, viscosimetry, <sup>1</sup>H NMR spectroscopy, UV-spectroscopy, and enzymatic degradation can determine chitosan properties [5, 6, 18].

### 2.1. The relationship between structure and properties

Chitosan differs from chitin by the presence of amino groups which appears in its solubility in dilute acids (pH < 6), and forming complexes with metal ions so that it can be used for waste water treatment and purification. [5, 6]. In contrast, practical applications of chitin are extremely limited due to its poor solubility, if any [19]. Interestingly, the aqueous solubility of chitosan is pH dependent allowing processability under mild conditions [20].

Chitosan with protonated amino groups becomes a polycation that can subsequently form ionic complexes with a wide variety of natural or synthetic anionic species [20], such as lipids, proteins, DNA and some negatively charged synthetic polymers as poly (acrylic acid) [19-22]. As a matter of fact, chitosan is the only positively charged, naturally occurring polysaccharide [19].

Chitosan molecules have both amino and hydroxyl groups so that it can form stable covalent bonds via several reactions such as etherification, esterification and reductive amination reactions [5, 6].

Chitosan have remarkable antibacterial activity [5, 23, 24], along with antifungal [11], mucoadhesive [25], analgesic [11] and haemostatic properties [26]. It can be biodegraded into non-toxic residues [27, 28] the rate of its degradation being highly related to the molecular mass of the polymer and its deacetylation degree – and has proved to some extent biocompatibility with physiological medium [29, 30]. All these singular features make chitosan an outstanding candidate for biomedical applications.

Chitosan, deacetylated form of chitin to at least 50% of the free amine form, has a heterogeneous chemical structure made up of both 1-4 linked 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose as well as 2-amino-2-deoxy- $\beta$ -D-glucopyranose (Figure 2).

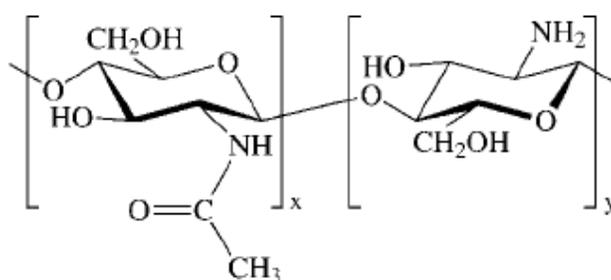


Figure 2. Chemical structure chitosan [4].

## 2.2. Chitin and chitosan production

Chitosan produced from crustacean shell such as crab and shrimp. These shells contains 30-40% proteins, 30-50% calcium carbonate and 20-30% chitin (etd.lsu.edu).

Production of chitosan involves four steps: demineralization, (DM), deproteinization (DP), decolorization (DC), and deacetylation (DA), as shown in Figure 3 (etd.lsu.edu).

The process for the deacetylation of chitin, obtained from crab or shrimp shells, to form chitosan is described in Figure 5 and generally produces a chitosan with 70% to 95% deacetylation [4].

## 3. Chitosan as biomaterial

Chitosan have several properties to be used in biomedical applications. It has positive charges in acidic medium, due to protonation of amino groups, and it can bind with negative residues in the mucin, that lead to improve mucoadhesive properties [5, 15].

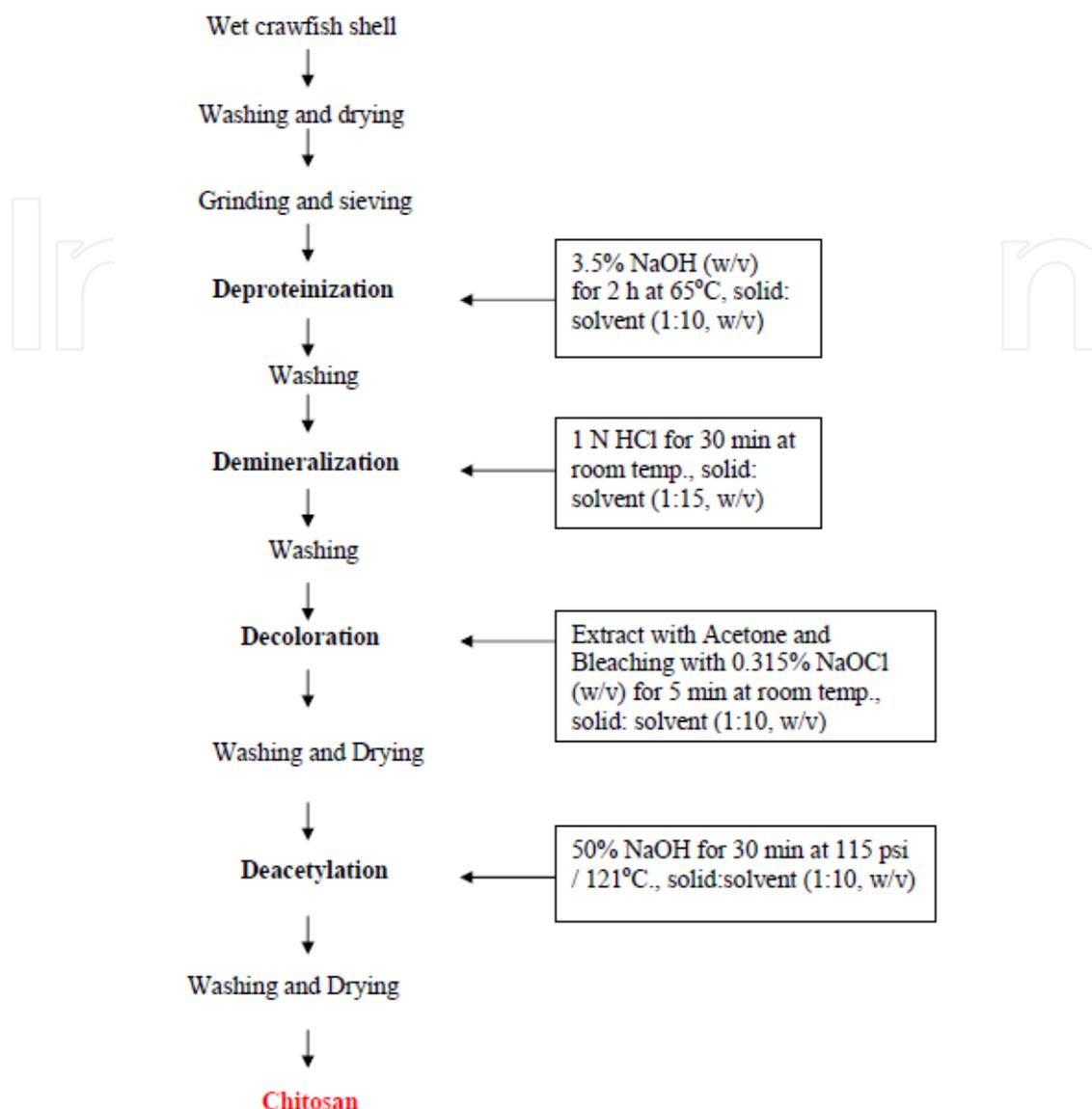


Figure 3. Chitosan production flow scheme [4, 31].

Also positive charges on chitosan can bind to negative charges on red blood cells (RBC) so that chitosan used as haemostatic agent [5, 32, 33].

Chitosan has two mechanisms to explain its antimicrobial activity. The first mechanism proposed that positive charges on chitosan could bind with negative charges at the bacterial cell surface, which alter permeability and leaks solutes outside the cells. The second one proposed that it could bind with bacterial DNA cell, which inhibit RNA synthesis [5].

The polycationic nature of chitosan also allows explaining chitosan analgesic effects. Indeed, the amino groups of the D-glucosamine residues can protonate in the presence of proton ions that are released in the inflammatory area, resulting in an analgesic effect [34].

Now, to explain chitosan biodegradability, it is important to remember that chitosan is not only a polymer bearing amino groups, but also a polysaccharide, which consequently contains breakable glycosidic bonds. Chitosan is actually degraded *in vivo* by several proteases, and mainly lysozyme [11, 35, 36]. Till now, eight human chitinases have been identified, three of them possessing enzymatic activity on chitosan [37]. The biodegradation of chitosan leads to the formation of non-toxic oligosaccharides of variable length. These oligosaccharides can be incorporated in metabolic pathways or be further excreted [38]. The degradation rate of chitosan is mainly related to its degree of deacetylation, but also to the distribution of N-acetyl D-glucosamine residues and the molecular mass of chitosan [39-41].

Chitosan shows biocompatibility in biomedical applications such as sutures and artificial skins [5, 6, 10, 34] and was notably approved by the Food and Drug Administration (FDA) for use in wound dressings [42]. However, the compatibility of chitosan with physiological medium depends on the preparation method (residual proteins could indeed cause allergic reactions) and on the DD – biocompatibility increases with DD increase. Chitosan actually proved to be more cytocompatible *in vitro* than chitin. Indeed, while the number of positive charges increases the interaction between cells and chitosan increases as well, which tends to improve biocompatibility [43].

Besides, some chemical modifications of chitosan structure could induce toxicity [35].

Production process of chitosan has great effect on chitosan properties because these processes control the degree of acetylation of chitosan, i.e. free amino groups that allow it to bind with negatively charged molecules [1, 4, 44].

Chitosan has several biological properties that make it an attractive material for use in medical applications. These properties include: biodegradability, lack of toxicity, anti-fungal effects, wound healing acceleration and immune system stimulation [4, 44-46].

## **4. Applications of chitosan and chitosan derivatives**

Due to chitosan's many attractive properties such as biodegradability, natural origin, abundance, reactivity, etc., it has many areas of application including: medical, agricultural, food processing, nutritional enhancement, cosmetics, and waste and water treatment [4, 44].

### **4.1. Agricultural applications**

The abundance, biodegradability, nontoxic, and natural origin of chitosan allow it to be safely used in agricultural applications because it can be used without concerns of pollution, disposal, or harm to consumers if ingested. Seed coating, leaf coating, fertilizer, and time released drug or fertilizer responses are some of the applications within agricultural where chitosan is utilized. The use of chitosan in these areas has shown to increase the amount of crops produced by improving germination, rooting, leaf growth, seed yield, and soil moisture retention, while reducing the occurrence of fungal infections and diseases [47].

## 4.2. Wastewater treatment applications

Chitosan's functional groups and natural chelating properties make chitosan useful in wastewater treatment by allowing for the binding and removal of metal ions such as copper, lead, mercury, and uranium from wastewater [4]. It can also be utilized to breakdown food particles that contain protein and remove dyes and other negatively charged solids from wastewater streams and processing outlets [47].

## 4.3. Food industry applications

Chitosan's chelating properties and high functionality make it valuable in several applications within the food industry such as binding with and removing certain elements, particles, and materials such as dyes and fats from foods. The antibacterial and antifungal properties found in chitosan can also be used during the storage and preservation of food [4, 46, 47].

## 4.4. Medical applications

Due to chitosan's ability to function in many forms it has many areas of interest within the medical industry including orthopedic and Periodontal Applications [44, 46]. Tissue engineering [44, 45, 47-49], Wound Healing [44, 45, 50, 51] and Drug Delivery [52, 53].

Some examples of biomedical applications of are artificial skin, surgical sutures, artificial blood vessels, controlled drug release, contact lens, eye humor fluid, bandages, sponges, burn dressings, blood cholesterol control, anti-inflammatory, tumor inhibition, anti-viral, dental plaque inhibition, bone healing treatment, wound healing accelerator, hemostatic agent, antibacterial agent, antifungal agent, weight loss effect [44].

# 5. Electrospinning of chitosan

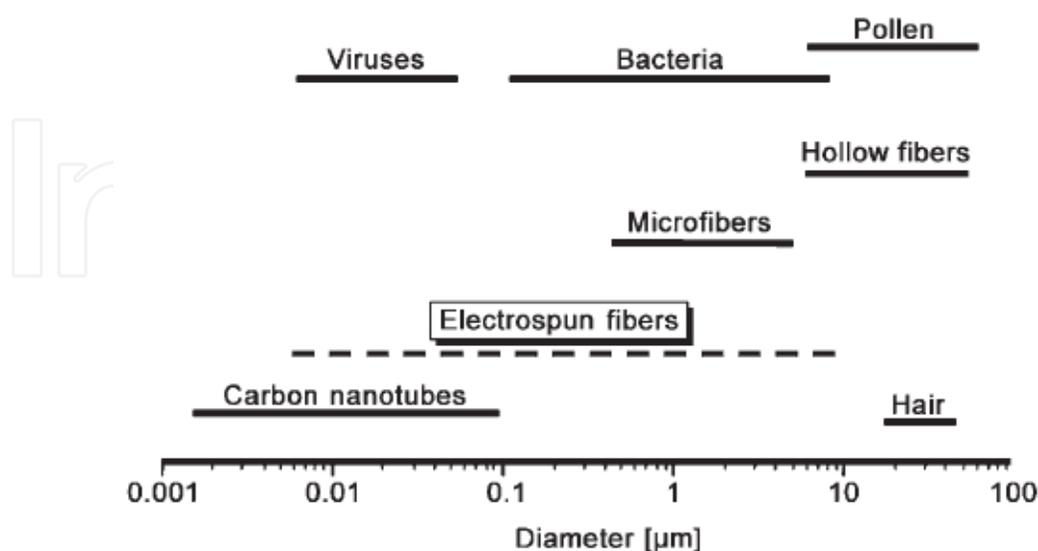
Electrospinning is a process that utilizes a strong electrostatic field to obtain ultrafine fibers from a polymer solution accelerated towards the grounded collector due to the motion of charge carriers present in the solution in order to complete the electrical circuit. Electrospun fibers with their high surface area to volume ratio and small pores, are drawing interest in vast variety of applications, some being, filtration products, scaffolds for tissue engineering, wound dressings, drug release materials, fiber reinforcement composites, protective clothing [54-56].

## 5.1. History of electrospinning

In 1700s, influence of electrostatics was observed on water behavior and an electric charge influenced the excitation of dielectric liquid. This probably led to the invention of electrospinning to produce fibers in the early 1900s by Cooley and Morton. Cooley added rotatory electrode to the electrospinning jet. Formhals, in 1930, produce yarns by using electrospinning without spinneret [57] and patented his invention relating to the process and the apparatus. In 1940, Formhals patented another method for producing composite fiber webs from multiple

polymer substrates by electrostatically spinning polymer fibers on a moving base substrate. In 1969, Taylor studied the shape of the polymer droplet produced at the tip of the needle when an electric field was applied and showed that it was a cone and the jets ejected from the vortices of the cone. This cone was later referred to as the 'Taylor cone'. The effects of electric field, experimental conditions and the factors affecting the atomization and fiber stability were studied [58]. For the fiber industries, one important consideration is the rate of fiber production. Electrospinning, compared to the popular industrial fiber spinning processes, has very low production rates [57]. Industrial dry spinning has a yarn take-up rate of 200–1500 m min<sup>-1</sup> while yarn fabricated from electrospinning has a take-up speed of 30 m min<sup>-1</sup>. Thus, before 1990, there was very little industrially oriented research interest found on electrospinning. Melt spinning being the preferred method for producing synthetic fibers, efforts were made to electrospin fibers using polymer melts, but difficulties were encountered in fabricating fibers with nanometer diameters and, therefore, little progress was made in this specific approach. Nevertheless, Dalton et al [59] recently succeeded in depositing electrospun polymer melt fibers directly on to cells to form layered tissue constructs for tissue engineering. This eliminated the introduction of cytotoxic solvents into the cell culture when the fibers were deposited. While there have been patents filed for various electrospinning set-ups since the 1900s, it is only in the last decade that academia got heavily involved in using electrospinning to fabricate various nano-fibrous assemblies for a range of potential applications.

Figure 4 shows a comparison of diameters between nanofibers, proteins, viruses and bacterial cells [60].



**Figure 4.** Comparison of the Diameters of Electrospun Fibers to those of Biological and Technological Objects [60].

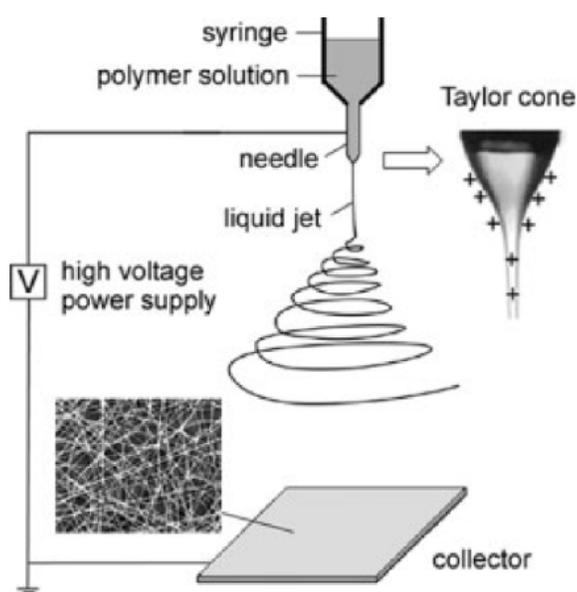
## 5.2. Electrospinning process

Electrospinning as the production of fine fibers (either nano or micro) from polymer solutions by using high voltage electric field (kV) at room temperature and atmospheric conditions. There are two electrospinning setups, vertical and horizontal [1, 61, 62].

Electrospinning devices, Figure 5, consists of three main components: high voltage electric field, spinneret and collecting electrode. [1, 63, 64].

Through electrospinning process, polymer solutions subjected to high voltage electric field that induce electric charge on its surface. At critical electric field, the repulsive electrical forces can overcome the surface tension and eject unstable charge jet from Taylor cone tip, which evaporate the solvent and leave the polymer [1, 65-68]. The jet is only stable at the tip of the spinneret and after that, instability starts. Thus, the electrospinning process offers a simplified technique for fiber formation.

Due to the critical voltage, applied potential reaches a critical value and the repulsive force within the charged solution exceeds surface tension and a jet erupts from the tip of the cone. These charged ions in the polymer jet move in response to the applied electric field towards the electrode of opposite polarity, thereby transferring tensile forces to the polymer jet making the latter undergo a chaotic motion or bending instability with whipping action. The jet moves towards the opposite charged collector, which collects the charged fibers. The jet ejected from the apex of the cone continues to thin down along the path of its travel towards the collector. As the jet travels through the atmosphere, the solvent evaporates, leaving behind a dry fiber on the collecting device. The structure formation happens on a millisecond scale [69]. An important step within production of the fibers is the elongation taking place within the jet with a strain rate as high as  $10^4 \text{ sec}^{-1}$  [55, 58, 70, 71].



**Figure 5.** Schematic illustration of electrospinning setup [56].

A typical electrospinning setup only requires a high voltage power supply, a syringe, a flat tip needle and a conducting collector as shown in Figure 7. Electrospinning is able to produce continuous nanofibers from a wide range of materials. Nevertheless, there are many parameters, which affect the fiber morphology and properties in electrospinning. The main parameters are polymer parameters and processing conditions [54, 56, 72].

### 5.3. Effects of various parameters on electrospinning

There are several parameters affect the electrospinning process. These parameters are solution, process and ambient parameters [1, 56, 73]. In Table 1, there are summary of various parameters and their effects on fiber morphology[1].

Parameters	Effect on fiber morphology
<i>Solution parameters</i>	
Viscosity ( $\eta$ )	Low ( $\eta$ ) generate beads, high ( $\eta$ ) causes increase in fibre diameter and disappearance of beads
concentration	Fibre diameter increase with increasing of polymer concentration.
Molecular weight of polymer	The number of beads decrease, with increasing of molecular weight.
Conductivity	Decrease in fiber diameter with increase in conductivity.
Surface tension	Jets. Instability appears with high surface tension
<i>Processing parameters</i>	
Applied voltage	Decrease in fiber diameter with increase in voltage.
Distance between tip and collector	Generation of beads with too small and too large distance, minimum distance required for uniform fibers.
Feed rate/Flow rate	Decrease in fiber diameter with decrease in flow rate, generation of beads with too high flow rate.
<i>Ambient parameters</i>	
Humidity	High humidity results in circular pores on the fibers.
Temperature	Increase in temperature results in decrease in fiber diameter.

**Table 1.** Electrospinning Parameters (Solution, Processing and Ambient) and their Effects on Fiber Morphology [1].

### 5.4. Solvents used for electrospinning

Solvents play an important role in electrospinning to the dissolution of polymer in soluble solvent is the first step in the electrospinning process. These solvents should be volatile and have low boiling point such as chloroform, ethanol, dimethylformamide (DMF), trifluoroacetic acid (TEA), dichloromethane (DCM) [1, 74-76].

Solution properties such as viscosity and surface tension have great effect on the morphology of nanofibers [1, 77].

Basically, a solvent performs two crucial roles in electrospinning: Firstly, to dissolve the polymer molecules for forming the electrified jet. Secondly to carry the dissolved polymer molecules towards the collector [78], e.g. dimethylformamide, a dipolar aprotic solvent, has been successfully used as a solvent for electrospinning of poly (acrylonitrile) and its addition enhances the solution conductivity which is a prerequisite for the formation of bead free uniform fibers [79]. It was found that by increasing the concentration, there was a gradual decrease in surface tension of the solution, which favoured production of thinner fibers [80].

## 6. Electrospinning of chitosan solutions

Chitosan cannot form nanofibers through electrospinning process because its poly cationic nature due to the presence of many amino groups in its structure which increase solution surface tension [2, 81].

Fibrous structures were successfully formed by electrospinning chitosan solutions in 90wt% aqueous acetic acid solution [80] or by using trifluoroacetic acid (TFA) or TFA/dichloromethane (DCM) [78]. However, electrospinning conditions are relatively limited in terms of concentration, molecular weight, and degree of deacetylation [82]. The resultant chitosan fibers need to be cross-linked to maintain their structural integrity, as they can readily swell in aqueous solution [83].

Since the electrospinning of chitosan itself proved to be difficult. Chitosan was mixed with other synthetic or natural polymers, such as PEO in aqueous acid, PVA, aqueous acetic acid solution or aqueous acrylic acid solution, poly (lactic acid) (PLA) in trifluoroacetic acid/methylene chloride mixture solvents or its copolymers in aqueous acetic acid solution, silk fibroin (SF) in formic acid, and collagen in 1,1,1,3,3,3-hexafluoro-2-propanol/trifluoroacetic acid mixture solvent [2]. Several chitosan derivatives such as hexanoyl chitosan in Chloroform as a solvent, quaternized chitosan aqueous acetic acid solution or Water, N-carboxyethylchitosan in aqueous acrylic acid solution or water as solvent, and chitosan grafts with L-lactide or PEG oligomer in dimethylformamide/tetrahydrofuran solvent [2] were synthesized and electrospun with or without polymer additives, to improve the solubility and electrospinnability of chitosan

Chitin and chitosan nanofibers are biocompatible, biodegradable and nono-toxic so that it is used in biomedical applications such as antithrombogenic, hemostatic, and wound healing. The use of nanofibrous chitosan matrices is thus expected to mimic the natural ECM, in which cells attach, proliferate, and differentiate [2, 84, 85]. The use of nanofibrous chitosan matrices is thus expected to mimic the natural ECM, in which cells attach, proliferate, and differentiate.

An organic/inorganic composite scaffold of hydroxyapatite (HAp) and electrospun nanofibrous matrix was prepared by using chitosan/poly(vinyl alcohol) (CS/PVA) and N-carboxyethylchitosan/PVA (CECS/PVA) electrospun membranes, and HAp was formed in supersaturated  $\text{CaCl}_2$  and  $\text{KH}_2\text{PO}_4$  solution [86].

Xu et al was successful to prepare chitosan nanofibers used for enzyme immobilization by mixing its solution with polyvinyl alcohol (PVA) then added to sodium hydroxide solution for remaining the PVA and stabilizing chitosan nanofibers [87].

Chitosan nanofibers used for wound healing because it shows antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [87, 88]. In addition, chitosan nanofibers with (40-290 nm diameters) were prepared by electrospinning of its solution with polyethylene oxide (PEO) blended solution [89].

Quaternized chitosan (QCS) form nanofibers in the presence of polyvinyl alcohol (PVA) and poly vinyl pyrrolidone (PVP) as fiber aiding materials [90, 91].

Ultrafine fibers could be generated by controlling the addition of PEO in 2:1 or 1:1 mass ratios of CS to PEO from 4–6 wt% CS/PEO solutions to be used for cartilage tissue repair [92, 93].

Chitosan electrospun nanofibers are cellular biocompatible. Chitosan molecular and solvent used control the beads formation such as hexanoyl chitosan/poly lactide nanofibers in chloroform formed without beads. Also in chitosan/PVA nanofibers, beads formation decreased by increased the content of PVA repair [92, 94].

PVA are non-toxic, non-ionogenic and water-soluble polymer. Therefore, the nanofibrous materials prepared by electrospinning of CECS/PVA aqueous solutions, dissolved when put in contact with water. CECS / PVA mats stabilized by heating at 100°C. it is used in tissue scaffolds applications [92, 94]. FTIR, XRD, and DSC studies demonstrated that there were strong intermolecular hydrogen bonds between the molecules of CS and PVA in the PVA/CS blend nanofibrous membranes [95]. SEM images showed that the morphology and diameter of the nanofibers were mainly affected by concentration of the blend solution (weight ratio of the blend) respectively [95]. It appears that electrospinning may emerge as a versatile method to manufacture CS fibers.

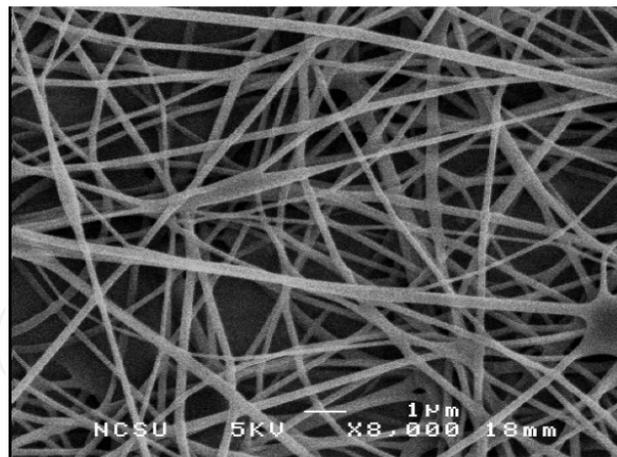
## 7. Nanofibers produced by electrospinning

### 7.1. Nanofiber morphology

Nanofibers produced by electrospinning have gained popularity in research in part due to their morphological characteristics. These nano-diameter fibers have high surface areas, small pore sizes and are able to be produced in three dimensional forms (Figure 6). Because the above mentioned characteristics can be modified through process parameters to suit individual applications and needs, electrospinning has become a growing topic among researchers [71].

### 7.2. Nanofiber properties

The properties associated with nanofibers can be traced back to both process parameters and morphological characteristics. For example electrospun fibers have small pores that are a result of the evaporation of the solvent used during the electrospinning process and these pores affect mechanical properties of the fibers such as tensile strength and Young's modulus [58]. Other

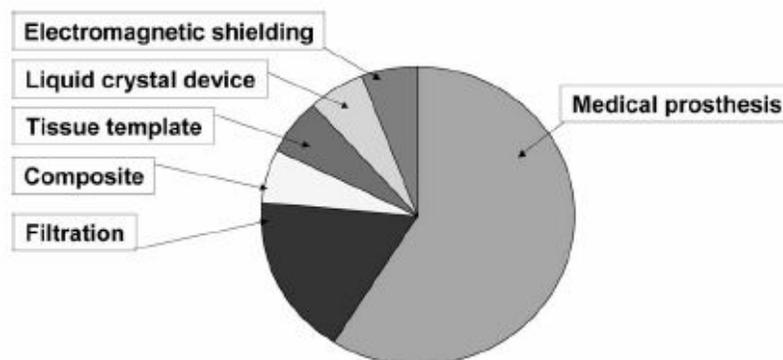


**Figure 6.** Scanning electron microscopy (SEM) image of electrospun poly (vinyl alcohol) produced on laboratory electrospinning setup.

studies have found that the physical properties of nanofibers tend to be somewhat inferior to that of their film and resin counterparts of a similar thickness. [65, 71] This is believed to be a result of lower crystallinity due to rapid evaporation of the solvent followed by rapid cooling, which occurs in the final stages of the electrospinning process [58].

### 7.3. Nanofiber applications

Characteristics such as large surface areas and the ability to be engineered in various forms have allowed nanofibers to be used in applications including: filtration [51], composite reinforcement [96], multifunctional membranes [51], tissue engineering scaffolds [3, 45, 51, 97, 98], wound dressings [50, 65, 99, 100], drug delivery [100-102], artificial organs [65, 103], and vascular grafts [65, 102-104]. Although all of these areas of interest are, still studied, biomedical applications for polymeric nanofibers have made up a majority of the new growth in the field of nanofiber research (Figure 7). [65] This growth is in part due to increased understanding of the human body, cellular structure, and the body's reaction to foreign materials.



**Figure 7.** An estimation of the targeted nanofiber research fields based on the number of patent applications for electrospun nanofibers [65].

Researchers in the past have made attempts to electrospin chitosan in order to further utilize this material [33, 48, 49, 78, 80, 81, 84, 89, 93, 105]. Chitosan produces many challenges in being electrospun largely due to its high solution viscosity. Chitosan's rigid D-glucosamine structures, high crystallinity and ability to hydrogen bond lead to poor solubility in common organic solvents [105]. The smallest diameter fibers were reported using a poly(vinyl alcohol)/chitosan blend which resulted in nanofibers with average diameters between 20 and 100 nm [105]. Other studies have reported nearly defect free nanofibers, with slightly larger fiber diameters using a poly (ethylene oxide) (PEO)/chitosan blend [49, 89, 93, 105]. The successful electrospinning of pure chitosan has only been reported using a solvent system of 90 % acetic acid and a 7 wt. % concentration of chitosan [80].

The first successful reports of PEO/chitosan electrospun blends reported the electrospinning of nanofibers with diameters ranging from 40 to 290 nm, but that the most consistent and defect free fibers had an average diameter ranging from 200 to 250 nm (Spasova et al, 2004) Another study using a PEO/chitosan blend reported defect free nanofibers with diameters that ranged from 80 to 180 nm, but found that that the samples did not have consistent diameters. Using Fourier transform infrared spectroscopy and differential scanning calorimeter it was discovered that the two polymers had separated and the larger fibers largely consisted of PEO and the smaller fibers were predominately chitosan [93]. To further reduce the diameter of the electrospun PEO/chitosan blend fibers another research group introduced Triton X-100™ as a nonionic surfactant as well as dimethylsulphoxide as an additional solvent. These additions greatly improved the ability to electrospin PEO/chitosan blends with a high polymer concentration and produced fibers with diameters that ranged from 40 to 110 nm [49]. The same group also tested this nanomesh for cellular attachment and viability and found that cells more readily attached and were able to be sustained more efficiently than on a cast film of the same materials [49]. Another study was able to successfully electrospin PEO/chitosan blends with no additional additives, which resulted in fibers with an average diameter of 300 nm [48]. This group's main objective was to test the cellular viability of a chitosan blend in the electrospun nanomesh form. They concluded that chondrocyte cells showed good cell adhesion, proliferation and viability on the chitosan-based electrospun material. It also concluded that the electrospun material had a higher modulus compared to the control film made by solvent casting. [48]

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