Recent Advances in Electrospinning of Some Selected Biopolymers

Masoumeh Valizadeh, Seyed Abdolkarim Hosseini Ravandi and Seeram Ramakrishna

Abstract—**The growing need for materials suitable for sustainable development and improved healthcare has motivated scientists and engineers worldwide to investigate biopolymers. Success in this direction hinges on our ability to tailor properties of biopolymers and find cost effective processing methods.**

Authors applied a versatile nanofiber production technique known as electrospinning to process a variety of nanoassemblies with high specific volume and porosity from biocompatible, biodegradable and nontoxic natural biopolymers. These nanofibers and nanowebs with tailored physical, chemical, biological and mechanical properties are attractive for designing ecologically friendly products in a range of applications from personal care to healthcare such as hospital apparel, drug delivery systems, and scaffolds for tissue engineering and tissue regeneration.

The present study reports on electrospinning of natural biopolymers chitosan and alginate and their blends with other biocompatible polymers. Several solvent systems and different processing conditions are investigated for effective electrospinning of biopolymers. Optimum processing conditions for producing desired electrospun nanowebs are discussed.

Key words: Biopolymers, electrospinning, nanofibers, characteristics, medical application.

I. Introduction

There are more than three manufacturing approaches to fabricate nanofibrous structures; i.e. electrospinning, phase separation, and self-assembly. Structures created by each of these approaches are quite different and thus have their own unique advantages. The phase separation technique allows the control of pore architectures, while structures produced by electrospinning provide more control on morphology and high aspect ratio, variable pore-size distribution, and high porosity [1].

The main mechanism for a generic self-assembly is the intermolecular forces that bring the smaller units together and the shape of the smaller units of molecules which determine the overall shape of the macromolecular nanofiber. Compared to the other approaches, this one is a good method for obtaining smaller nanofibers but it involves a complex process [2].

In more recent years, much attention has been paid to the use of high electrostatic potentials for the fabrication of ultra fine structured and nano fiber based networks from biopolymers by the electrospinning technique [3, 4, 5, 6] . Electrospinning, which is a relatively old idea in fiber spinning, is currently used as one of the most advanced methods in manufacturing high performance nanofibers, which have been introduced into various technological fields because of their distinct specifications, such as high aspect ratio, porosity, and special chemical and physical properties which have originated from their unique structure.

Specifically, there is a wide range of spinnable polymers; however, only a few of them have the necessary parameters required in such critical applications. Although there are a lot of polymers which are spinnable, only a few of them have the required parameters suitable in the complex procedure of electrospinning.

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II. Biopolymers

Natural and synthetic biopolymers have quickly found their way into a multiplicity of applications in materials science and biointerface engineering. Beyond the interest in biopolymers as genuine materials, their use as a matrix for composite material is one of the exciting challenges to build high performance and competitive functional materials because of their particular properties like biodegradability and biocompatibility. This interest in biopolymers is driven by two scientific developments. The first is our increasingly sophisticated understanding of polymer structure–function. The second is the maturation of applying high technologies such as biotechnology and nanotechnology, which allow these materials to be synthesized in large yields with precise control over the chemistry, morphology and structure [7, 8].

On the other hand, the use of biopolymers and bio-resources is considered as one of the many strategies to minimize the environmental impact of petroleum-based plastics.

There are different important parameters which have to be fulfilled according to the final intended application for a certain biomaterial. Figure 1 shows the main properties of the biopolymers. Other properties mentioned in Figure 1 may be revealed only by some special types of biomaterials.

A. *Biodegradability*

The biological base of biopolymers provides a unique opportunity to incorporate the highly demanded property of these materials, i.e. the biodegradability. It must be

noticed that among the plastic wastes, there are products with a high degree of contamination and recycling involves a high energy cost. However, the use of biodegradable polymers represents a real step in the right direction to protect us from environmental pollution. [9, 10]. A vast number of biodegradable polymers have been synthesized or formed in nature during the growth cycles of all organisms. Some microorganisms and enzymes, capable of degrading them, have been identified. Figure 2 shows a classification of biopolymers in 4 categories [11- 14].

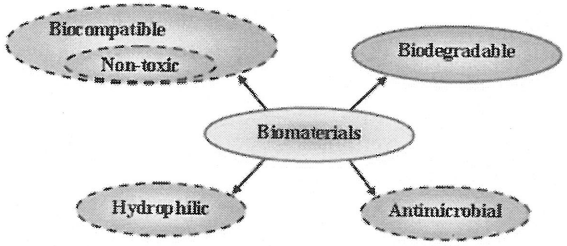


Fig. 1. Some main characteristics of biopolymers.

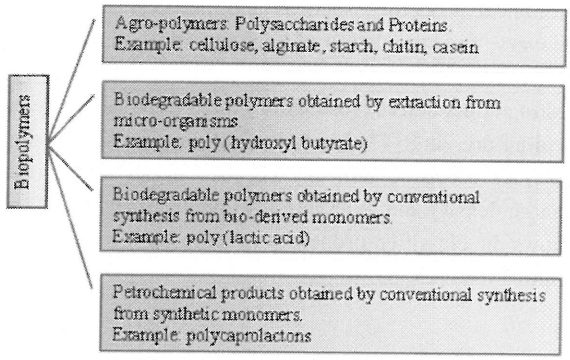


Fig. 1. Classifications of biopolymers.

B. Biocompatibility

Biocompatibility is actually the most important factor which distinguishes a biomaterial from any other material. Biocompatible polymers are able to exist in contact with tissues of the human body without causing an unacceptable degree of harm to the tissues [8, 15].

It is important to study the in vivo reaction of biomaterials, since the biological effects play a crucial role in therapy or biological applications. For example, the use of organic solvents may significantly reduce biocompatibility and induce cytotoxicity; therefore, for every small change in engineered biopolymer for medical uses, the in vivo biocompatibility should be tested independently. For biodegradable biomaterials, the biological, chemical, physical and mechanical properties vary with time. Therefore, the active biocompatibility of these materials must be demonstrated over time whereas the produced degradation products can have different levels of tissue compatibility when compared to the starting parent material. An example of this status is the scaffolds containing calcium in the form of calcium nitrate, which could cause long-term toxicity [8, 16- 20].

C. Hydrophilicity

All polymeric materials can be classified in to either hydrophobic or hydrophilic based on their water contact angle, which is related to the wettability of the polymer surface. This property is definitely important in applications like cell proliferation, drug delivery and wound healing. The surface properties of a biomaterial, especially hydrophilicity, influence cell adhesion and proliferation on the media. For example, the studies show that a very hydrophobic polymer such as poly tetra fluoro ethylene (PTFE) with a contact angle of 105°-116° inhibits the cellular adhesion [21]. Meanwhile, hydrophilic polymers are known to facilitate cellular adhesion. There are some hydrophilic polymers such as poly ethylene oxide which prohibit cellular adhesion. Here the assumption is that as the proteins have both hydrophobic and hydrophilic regions, an area with appropriate proportions of hydrophilicity to hydrophobicity would be the most favorable [21- 25]. Martins *et al.* have demonstrated that the best adhesion can be assigned to the polymers with contact angle of 65°-80° [26].

III. NATURAL SOURCES BIOPOLYMERS

In the field of biomedical applications, biodegradable polymers have been reasonably more considered in comparison to biostable polymers. Perhaps, serious problems of synthetic materials, troubles of pollution and shortage of petroleum resources as the main source of raw material for synthetic polymers have been the actual reason for the flourishing trend of biopolymers. However, the irrefutable superiorities of biopolymers to synthetic products in medical applications along with the particular properties noticed by different industries as well as all environmental considerations have made the natural polymers and their derivatives more remarkable, but their utilization is still limited because of their high cost and/or their low performance [8, 27- 29]. This part of the review refers to the most notable biopolymers, with a particular focus on their medical scopes.

A. Sodium Alginate

Alginate or algin is a linear polysaccharide copolymer of D-Malnnuronic acid (M) and L-Guluronic Acid (G) units (Figure 3). Alginate is found in the cell walls of brown seaweeds. The actual chemical structure of the alginate varies from one genus to the other. Therefore, the properties of the alginate depend on the source of seaweed and the proportion of M:G [30- 32].

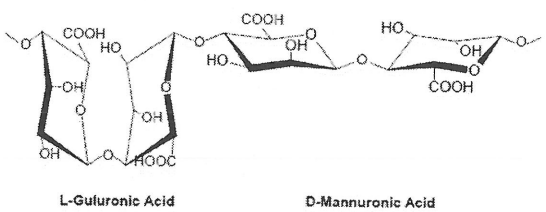


Fig. 2. Chemical structure of Alginic acid.

Alginate is a biodegradable, biocompatible anionic polymer due to its colloidal properties, which make it a film forming, stabilizing, suspending, gel producing, thickening and emulsion stabilizing agent. Alginate is a highly hydrophilic biopolymer which can react with polyvalent metal cations to produce strong gels or a low soluble polymer. Because of these properties, alginate is an active biopolymer which has received considerable attention in various industries [30, 33]. However, there is a widespread interest in alginate for Pharmaceutical and medical applications such as drug carrier, wound dressing and tissue engineering scaffolds [34- 38]. Offering many advantages over traditional cotton and viscose gauzes, alginate-based products are currently the most popular dressings used in wound management among the various fibrous and hydrogel products. As a wound dressing alginate which is usually used in the form of calcium alginate, it utilizes its ion exchange properties. When a dressing of calcium alginate is applied to a wound, it starts to absorb the exudates of the wound. In a reverse ion exchange mechanism, calcium ions are gradually exchanged against sodium ions present in the blood and wound secretions, starting to swell and in the presence of sodium ions, turning into a moist gel that fills and securely covers the wound. Calcium alginate produces a highly absorbent, non-adherent dressing that transmits oxygen and moisture vapor. A moist wound environment promotes curing and leads to a better cosmetic heal of the wound [39- 41].

Alginate has recognized as a prospective material for encapsulation and immunoprotection of transplanted cells. Stable cultures in alginate beads have been achieved with a number of cell types including II chondrocytes, Schwann cells, islets, myoblasts, fibroblasts, kidney cells, epithelial cells, bone-marrow stromal cells, and hepatocytes [36, 42, 43]. Alginate hydrogel systems have been used as bio-interactive scaffolds for various tissue engineering applications. The hydrophilic character of the alginate enabled the efficient proliferation of cells into the alginate scaffolds [44- 46].

B. Chitosan

Chitin is a co-polymer of N-acetyl-glucosamine and N-glucosamine units. The chemical structure of chitin is 2-acetamido-2-deoxy-b-d-glucose monomers attached via $\beta(1 \rightarrow 4)$ bonds. Chitosan is the deacetylated derivative of chitin (Figure 4) [47, 48].

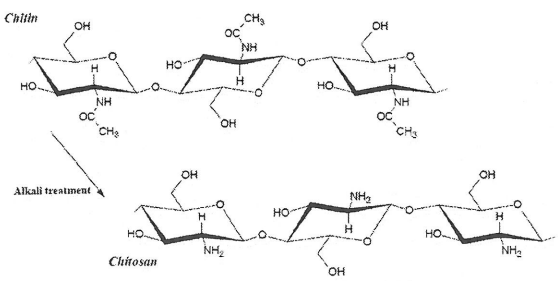


Fig. 3. Chitosan is the deacetylated derivative of chitin.

Chitosan and its derivatives have been recently explored intensively in different industries such as cosmetics, biotechnological, agricultural, food, and non-food industries like water treatment, paper, and textile [49].

Chitosan is an excellent biosorbent for metal cation removal because of its large number of NH_2 groups, and ion exchange activity. The reactive amino group selectively binds to transition metal ions but not to alkali and alkaline-earth metal ions. The excellent adsorption characteristics of chitosan for heavy metals can be attributed to high hydrophilicity, which is due to the large number of hydroxyl groups of glucose units, the presence of a large number of functional groups, high chemical reactivity of these groups and flexible structure of the polymer chain [50- 52].

Chitosan is a versatile biopolymer known for its biocompatibility and biodegradability. It is the only pseudo natural cationic polymer that possesses a broad range of interesting and unique biological activities. During the recent years, chitosan and its molecular derivatives have attracted attention because of their potentially beneficial biological properties such as antimicrobial activity, drug delivery, and stimulation of healing and tissue-engineering scaffolds. The broad spectrum antimicrobial activity of chitin and its derivatives enables them to be used in wound healing dressing [47, 53- 61]. Chitosan is also a potential polymer for gene delivery because of its high positive charge density and relatively low cytotoxicity [62]. Table I shows the classified properties of chitosan in some medical applications [23, 63- 67].

C. silk

Silk fiber has been of interest because of its apparel and clothing applications. Over the past decades, this biopolymer has also been used in pharmaceuticals, cosmetics creams, lotions and makeup powders. Silk fibroin (SF) is a precious candidate material for biomedical applications because of its unique biological properties including biodegradability, biocompatibility, minimal inflammatory reactions, non-toxicity, blood compatibility, good oxygen and water permeability, and high mechanical strength.

IV. Electrospinning of Biopolymers

Typical electrospinning units include a syringe containing polymer solution (or melt), that is charged through a high voltage supply. Figure 5 shows the schematic of a typical electrospinning unit. A charged metallic plate is placed at a fixed distance from the needle tip of the syringe. The high voltage electrical field overcomes the surface tension of the fluid droplet at the tip of the metal syringe needle and the Taylor cone forms, whereby a fluid jet is ejected and subjected to whipping instabilities due to electric Maxwell stresses.

TABLE I
CLASSIFIED CHARACTERISTICS OF CHITOSAN ACCORDING TO ITS MEDICAL APPLICATION

Tissue engineering	Wound healing	Drug delivery
• Non-toxic	• Active initiation of the healing process	• Non-toxic
• Rapidly biodegradable	• Promotion of granulation tissue and re-epithelization	• hydro-soluble
• Mucoadhesive	• Intrinsic antimicrobial activity	• positive charge enables chitosan to interact with negatively charged polymers, macromolecules, and many certain polyanions
• Suitable for controlled release of:	• Controlled release of exogenous antimicrobial agents to prevent infection	• special possibility of adhering to the mucosal surfaces within the body
– Cytokines	• Entrapment of growth factors to accelerate the healing	• capacity of opening tight junctions between epithelial cells though well organized epithelia
– Extracellular matrix components	• Limitation of scar formation and retraction	• improve targeting efficiency of the drug
– Antibiotics	• Stimulation of integrin-mediated cell motility and increased in vitro	
• Suitable for	• angiogenesis	
– Retention of the normal cell morphology	• Integrin-dependent regulation of the pro-angiogenic transcription	
– Promotion of the cell attachment	• factor Ets1	
– Proliferation and viability of tissue cells including stem cells	• Stimulation of cellular activities	
• Easy to develop in various forms		
• Chemically and enzymatically modifiable		

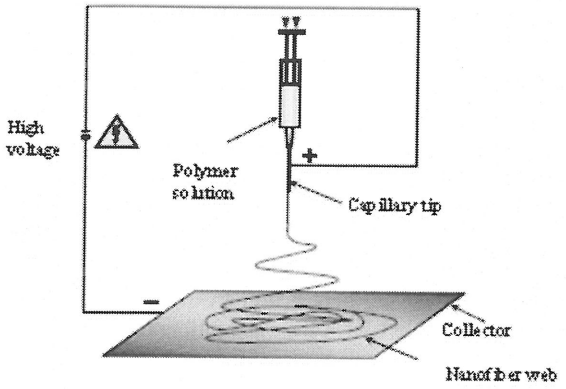


Fig. 4. Schematic of electrospinning setup.

Electrostatic interactions between charges on nearby segments of the same jet elongate it as the jet moves toward the collector surface. Meanwhile, the polymer jet precipitates; the solvent is evaporated and finally, the polymer jet is solidified into nanofibers. In the electrospinning method, the electrical and rheological properties of polymer and solvent play very important roles due to influence of the viscosity, surface tension, dielectric strength and conductive identities of the solution. An electro-spinnable solution must have sufficient concentration to entangle the polymer chains; It should also have a suitable viscosity at this concentration so that a droplet can be maintained and the solution can be pumped through the syringe [68, 69]. In electrospinning of biopolymers, these tasks are less under control and more complicated. Molecular weight, electrical charge of polymer chains, hydrophilicity and the tendency to form hydrogels are the affective parameters determining the complex behavior of many certain biopolymers such as chitin, chitosan, alginate and gelatin.

Different methods to collect the electrospun nanofibers lead to different qualities and characteristics for the product. Recently, a novel, innovative, high-performance, and simple method called Electro-centrifuge spinning has been developed to produce polymeric nanofibers based on using electrical and centrifugal forces [70- 76]. Badrossamay *et al.* produced nanofiber assembly by rotary jet-spinning technique which proved to be advantageous for building uniaxial aligned nanofiber structures in polymers not amenable to fabrication by electrospinning [77].

Given the high aspect ratio of nano fibers and the high porosity, which is as a result of random deposition of the fibers, electrospun nanowebs are able to mimic the structure of natural extracellular matrix [78].

A. *Electrospinning of chitosan*

Electrospinning of most biopolymers is difficult due to their ionic character in dissolved state, limited solubility in most organic solvents and three-dimensional networks of strong hydrogen bonds which make the behavior of the polymer solution more complex. These parameters influence the rheological characteristics of polymer solution directly.

The functional properties of chitosan, like metal binding, viscosity, surface tension, solubility, spinnability, film-formation, and antimicrobial activity, change depending on its molecular weight and degree of acetylation [79- 81]. The molecular weight of chitosan as a polysaccharide and the distribution of molecular weight affect physical and chemical properties, such as the rheological properties, mechanical properties and pore size of membranes and microcapsules of chitosan. The electric charge density on the molecule can be changed by ionic strength, variable pH, concentration, time, or the nature of the solution. Rheological studies have found that chitosan behaves as a compact sphere in acetic acid (AcOH) (1%)-NaCl (2.8%) solution or as a random coil in urea. In dilute aqueous AcOH, chitosan behaves as a cationic polyelectrolyte [52, 82]. The linear charge density of chitosan molecules in acidic solution varies with the number of amine group along the polymer chain, probably affecting the coil sizes in solution [83]. The intrinsic

viscosity of chitosan decreases with increasing ionic strength. The intrinsic viscosity was changed slightly as the solution pH was increased from 3 to 4. But it was decreased rapidly as the pH was increased from 4 to 5 [82]. On the other hand, the intrinsic viscosity of chitosan and the radius of gyration increased when the degree of deacetylation (DDA) was increased at the same molecular weight, because the charge density was increased along the polymer chain with increasing DDA [84].

Geng *et al.* (2005) have enumerated the surface tension of chitosan solution as the most important factor in the electrospinning process as it can affect the formation of droplets [85]. Viscoelacticity forces, due to lower surface tension of the spinning solution, help electrospinning to occur at lower electric field. As such, because surface tension and viscosity are the two most crucial parameters in the electrospinning of polymer solutions, there is a need for an efficient method which either decreases solution viscosity, or optimizes both surface tension and viscosity. In order to achieve these improvements, decreasing the molecular weight of chitosan was investigated [80].

Depending on the purification and deacetylation method and the origin of chitin used, the molecular weight of chitosan may vary from few kDa to over 1500 kDa [86]. Polycationic nature of chitosan, the rigid structure of repeating units, its high crystallinity and the ability to form strong hydrogen inter-molecular bond lead to the poor solubility of chitosan, increase in viscosity as well as surface tension and some limit in its processing. Decrease in molecular weight and crystallinity improves the solubility of chitosan in dilute acids significantly and enables chitosan processing into membrane, bead and fiber forms [87].

There are different methods to modify the molecular weight of chitosan for certain purposes such as ultrasonic, enzymatic and chemical techniques [79, 80, 87]. Many attempts have been made to electrospin chitosan, but this has been proved to be very difficult. There are several reports on electrospinning of chitosan blends, but the electrospinning of pure chitosan is still a challenging task [87, 88]. Few successful works on electrospinning of pure chitosan have applied different methods:

i: Electrospinning of chitosan in acetic solutions, mainly AcOH [85, 89, 90].

ii: Electrospinning of chitosan using triflouroacetic acid (TFA) and dichloromethane (DCM) [91, 92].

iii: Electrospinning of chitin from 1,1,1,3,3,3-hexaflouro-2-propanol (HFIP) solution and deacetylation of electrospun chitin nano fibers in order to have chitosan nanofibers [93, 94].

iv: Chitosan nanoweb can be directly prepared from the chitin whisker. Chitin whiskers are produced via acid hydrolysis of chitin flakes and deacetylated via alkali treatment to lead to chitosan nanoweb [95].

v: A combination of electrospraying and subsequent freeze drying can produce chitosan fibrous 3D network structures from low concentration chitosan solutions. There are applications including tissue engineering, cell or drug delivery, and membranes [96].

However, the limitation of the last methods is that TFA, DCM and HFIP are volatile, toxic, and expensive solvents and need drastic attention in further extraction of the solvent from the final product to be used in medical applications. Electrospinning of chitosan in concentrated AcOH is limited to the rather low molecular weight chitosan. There are successful reports on electrospinning of chitosan with molecular weights of $\overline{M}_V = 106$ kDa, 54%DDA [85], $\overline{M}_V = 210$ kDa, 78%DDA [92] and $\overline{M}_V = 294$ kDa, 75-85%DDA [90] (Figure 6).

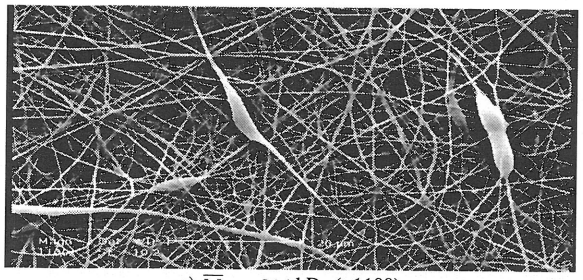
Table II reveals the required condition for electrospinning of chitosan, with different molecular weights, DDA, and the solvents which have resulted in quality nanofibers. Table II includes the electrospinning parameters, i.e., applied voltage, flow rate and tip collector distance, and the consequential nanofibers diameter. Each of the presented parameters of this table has a significant effect on the diameter and the quality of the electrospun fibers [85, 92- 94, 97].

The electrospinning process is initiated at a point at which the electrostatic force in a solution overcomes the surface tension of the solution. This requires high voltage. Specifically, increasing the voltage could help higher stretching obtain finer fibers as well as higher extrusion of polymer from the needle that could cause thicker fiber formation. However, there is no agreement on the effect of applied voltage on the nanofibers diameter in electrospinning.

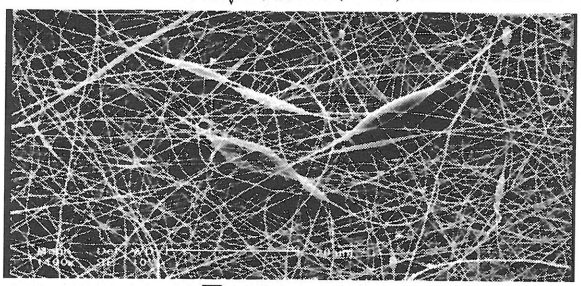
Flow rate is another important parameter determining the amount of solution available. An increase in flow rate, in turn, increases fiber diameter and bead size. Additionally, the internal diameter of the tip has a certain effect on electrospinning: a smaller internal diameter reduces clogging, as well as the number of beads, leading to less exposure of the solution to the atmosphere during the process [98].

Tip-collector distance has a direct influence on jet flight time and electric field strength. A decrease in this distance shortens flight times and solvent evaporation time, and increases the electric field strength, which results in more bead formation. The effect of decreasing tip-collector distance is almost the same as that of increasing voltage [2, 99]. On the other side, solvent and its dielectric specifications affect the electrospinnability of the polymer in the electrical field. This factor along with the concentration of polymer in solutions determines the resistance of polymer solution against stretching and fiber forming in electrospinning technique. For example, as Table II shows, TFA and TFA:MC generally give thicker nanofibers when compared to AcOH as solvent. If we consider AcOH as the solvent, 90% concentrated AcOH leads to fine nanofibers ranging 90-140 nm, but decreasing the concentration of AcOH to 70% increases the diameter of electrospun fibers. Dissolving chitosan in strongly concentrated AcOH in water results in lowering surface tension and increasing charge density of aqueous chitosan

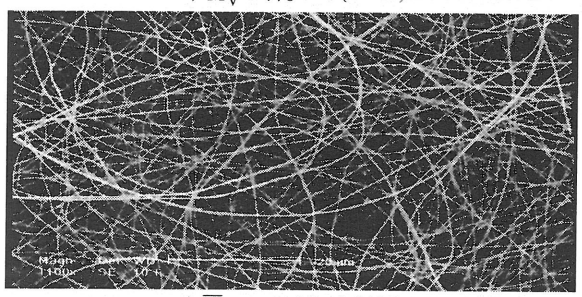
solution [85, 90, 97]. Figure 7 illustrates the effect of AcOH concentration on quality and diameter of chitosan nanofibers, and Figure 8 shows the effect of the concentration of chitosan solution in 90% AcOH.



a) $\overline{M}_V$ = 914 kDa (×1100)



b) $\overline{M}_V$ = 770 kDa (×1100)



c) $\overline{M}_V$ = 294 kDa (×1100)

Fig. 5. 5wt% chitosan in AcOH 90%.

B. Electrospinning of silk

Silk electrospun scaffolds can be fabricated to mimic topographic structure of the extracellular matrix (ECM), [100- 102].

Among silkworms, the wild silkworms such as Samia cynthia ricini, or A. pernyi can also produce silk fiber, but their primary structure is different considerably from domesticated Bombyx mori (B. mori) silk fiber which is well known. Silk consists of two types of proteins, fibroin and sericin. Fibroin is the protein that forms the filaments of silkworm silk. Silk proteins contain highly repetitive crystalline domains periodically interrupted by less crystalline or amorphous regions. The crystalline region from B. mori fibroin is a 59 amino acid repeat [GAGAGSGAAG[SGAGAG]8Y], with a 3:2:1 ratio of glycine, alanine, and serine [7].

There have been several attempts to address electrospinning of **SF**. The first researches on silk electrospinning encountered problems of selecting the proper solvents and controlling the conformational transitions of fibroin during electrospinning. The physicochemical properties of **SF** fibers strongly depend on crystallinity and molecular conformation. Physical or chemical treatments (mechanical, thermal or solvent treatment) can induce different levels of crystallinity in **SF**. The most common method used to convert the random coil conformation of **SF** into β-sheet one is to treat it in an organic solvent, particularly methanol. The main concerns in choosing the **SF** solvent or the treating solvents are that the solvent should not interfere with the biocompatibility of the processed material when exposed to cells in vitro or in vivo; it should also maintain the secondary structure of silk (β-sheets) in the electrospun fibers, which are necessary to attain optimal mechanical properties [103]. The preparation of electrospinning dope of **SF** requires a pretreatment on silk. This pretreatment has two stages [101, 103- 109]:

i- Degumming: Raw silk is degummed at boiling temperature in aqueous solution of 0.02_M Na_2CO_3 to remove the sericin gum for 30 minutes.

ii- Preparation of regenerated **SF**: **SF** is dissolved in a mineral solvent such as:

a: A ternary solvent system of $CaCl_2/EtOH/H_2O$ (1:2:8 in molar ratio) at 70°C

b: 50% $CaCl_2$ at 100°C

c: 9M $LiBr_2$ at 55°C

Table III summarizes the best conditions of electrospinning of **SF** [100- 102, 110- 116] .The studies have shown that the nanofiber diameter increases exponentially with increasing the concentration of SF in solution [101]. Zhu *et al.* have revealed that the pH of **SF** solution has a remarkable influence on the rheological behavior and the crystallinity of regenerated **SF** aqueous solutions [116]. With the decrease in pH and the increase in concentration, regenerated **SF** aqueous solutions exhibit a transition from Newtonian fluid to non-Newtonian fluid, decreasing the average diameter of the electrospun silk fibers and facilitating the conformational transition of the electrospun silk fibers from random coil silk I conformation to silk II conformation.

C. Electrospinning of the blended biopolymer

Blending polymers are known to be a very effective way to produce new multipurpose advanced materials. Polymer blends have provided an efficient way to fulfill new requirements for material properties. Over the past few years, there has been a lot of interest in the study of polymer blends, especially biodegradable and sustainable materials. In electrospinning of biopolymers, application of a secondary polymer is noteworthy in electrospinning of biopolymers which have drastic difficulties in spinning due to their high molecular weight or surface tension, strong secondary bonds, high crystallinity special rheological behavior of their solution, etc [6].

1) Electrospinning of alginate blends

Physical properties of alginate (**Alg**) depend on the uronic acid composition and the relative amount of the three **M**, **G** and **MG** blocks. Alginate is a polymer commercially used in food and textile industries to

TABLE II
OPTIMUM ELECTROSPINNING CONDITION LEADING TO DESIRED CHITOSAN NANOFIBERS

Methods	Chitosan characteristics	Solvent; solution concentration	Applied voltage (kV/cm)	Flow rate of the solution (mm^3/hour)	Tip-collector distance (cm)	Nanofiber diameter (nm)
Electrospinning of chitosan	$\overline{M}_V$ =106 kDa; 54%DDA	AcOH 90%; 7-7.5wt%	3.5-4	1.2	N.R.	130
	$\overline{M}_V$ =112 kDa; 86.7%DDA	TFA; 3wt%	1.3	0.5	15	235
	$\overline{M}_V$ =210 kDa; 78%DDA	TFA; 7-8wt%	1	N.R.	15	490
	$\overline{M}_V$ =210 kDa; 78%DDA	TFA:MC= 70:30; 8wt%	1	N.R.	15	330
	$\overline{M}_V$ =296 kDa; 75-85%DDA	AcOH 70%-90%; 5-7.5wt%	1.06	1.6	16	140-284
	Chitin characteristics	Solvent; solution concentration	applied voltage (kV/cm)	Flow rate of the solution (cm^3/hour)	Tip-collector distance(cm)	nanofiber diameter (nm)
Electrospinning of chitin and deacetylation to chitosan	$\overline{M}_w$ = 920 kDa; 9%DDA	HFIP; 6wt%	216.7	N.R.	7	110-163

TABLE III
OPTIMUM ELECTROSPINNING CONDITIONS LEADING TO DESIRED SF NANOFIBERS

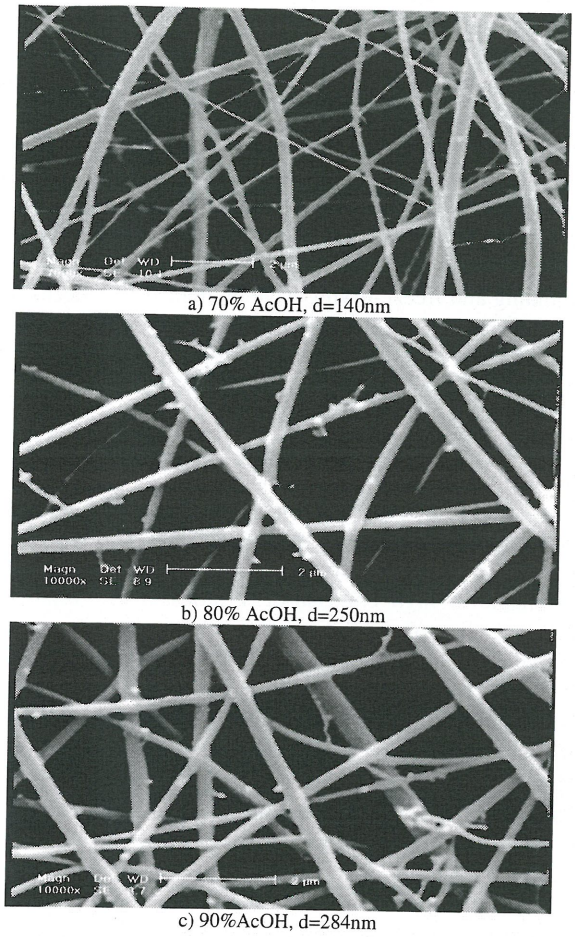
Applied solvent system	Regenerated SF concentration	Applied voltage (kV/cm)	Tip-collector distance (cm)	Nanofiber diameter (nm)
Hexafluoro-2-propanol 99%	0.74 wt%- 5 month	1.6–2	15	25
formic acid 98% for 3 hr	12-15 wt%	2.14	7	80
Dialyzed solution of SF in $CaCl_2$/EtOH/H_2O (1:2:8 in molar ratio)	34 wt%	1.1	18	500-1000nm
regenerated SF aqueous solution	25 wt% pH =4.8	4	N.R.	265
	30 wt% pH =5.6	4	N.R.	617
	33 wt% pH =6.9	4	N.R.	893
hexafluoroacetone. trihydrate (HFA.3H_2O)	3 wt%	1-1.6	10–15	~250nm

N.R.: Not reported

increase the viscosity of the system and to generate a solid matrix resistant to mechanically-induced strain. This is the result of gel formation in the presence of cross-linking polyvalent metal cations, specifically calcium ions. Gel forming properties are associated with the proportion and lengths of the **G** blocks.

The rod-like shape of **G** blocks results in an alignment of two chain sections, yielding an array of coordination sites, with cavities suitable for polyvalent cations and in interconnected by this mechanism, promoting gel network suitable size. Several different chains may become formation. The higher, the degree of linkage, the greater the resulting viscosity [30, 117- 120].

High viscosity of this polymer is the source of instability in nanofiber formation through electrospinning method. However, it is not the only limiting factor. The repulsive force among the polyanions could be the key factor hindering electrospinning of sodium alginate.

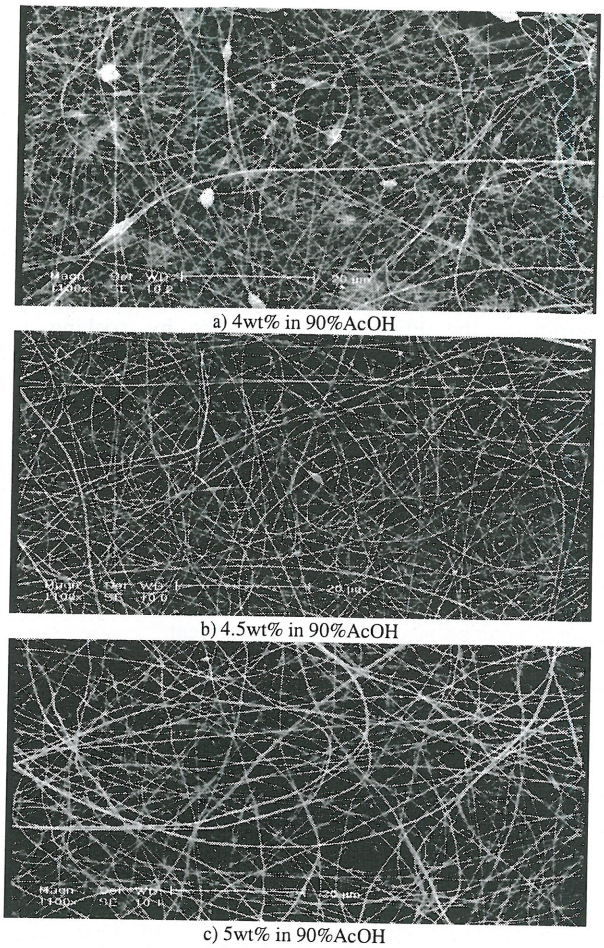


a) 70% AcOH, d=140nm

b) 80% AcOH, d=250nm

c) 90%AcOH, d=284nm

Fig. 6. The effect of AcOH concentration on the diameter of electrospun chitosan nanofibers.



a) 4wt% in 90%AcOH

b) 4.5wt% in 90%AcOH

c) 5wt% in 90%AcOH

Fig. 7. The effect of chitosan concentration on the quality of electrospun chitosan nanofibers.

There are a few studies on electrospinning of alginate. None of them succeeded to electrospin pure alginate, but its blend with other polymers such as PVA and PEO, which are known as secondary polymers (S.P.), leads to an electrospinnable polymer blends. A secondary polymer, mostly with a flexible molecular structure and low molecular weight, can function as a softening agent to reduce the average molecular weight, attenuate the powerful hydrogen bonds between molecular chains of primary polymer and finally, make it possible to electrospin the polymer blend solution. PEO is a biocompatible synthetic polymer which decreases the conductivity and viscosity of electrospinning solution and improves the processability of alginate. PVA is a non-toxic, water-soluble, biocompatible, and biodegradable synthetic polymer. It has been commercialized since 1950s. It is widely used in biomedical fields such as sutures and wound dressings. PVA is one of the best candidates to produce nano-bio fiber blends because of its ease of fiber formation in electrospinning. After blending with PEO or PVA, the interaction between PEO or PVA chains and sodium alginate polymer chains reduces the repulsive force between sodium alginate molecules, PVA or PEO as the secondary polymer eventually plays the role of plasticizer, thus allowing successful electrospinning of sodium alginate blends [37, 78].

Shalumon *et al.* prepared Sodium alginate (SA)/poly (vinyl alcohol) (PVA) fibrous mats by electrospinning technique [121]. SA/PVA electrospinning was further carried out by ZnO with different concentrations to get SA/PVA/ZnO composite nanofibers. SA/PVA/ZnO mats showed antibacterial activity due to the presence of ZnO as it could be an ideal biomaterial for wound dressing applications once the optimal concentration of ZnO gave the least toxicity.

Ma *et al.* reported that Core–shell structure nanofibers of sodium alginate/poly(ethylene oxide) were prepared via electrospinning their dispersions in water solution [122]. Furthermore, one-step cross-linking method, immersed in CaCl2 solution, was investigated to improve the anti-water property of the electrospun nanofibers mats and to facilitate their practical applications as tissue engineering scaffolds. It was assumed that the nanofibers membrane of electrospun SA/PEO could be used for tissue engineering scaffolds.

Bonino *et al.* reported preparation of nanofibers containing alginate using two different molecular weights of (MWs)/ 37 kDa and 196 kDa. LowMWalginates are

attractive for in vivo tissue scaffolds, where degradation and clearance from the body are desirable, whereas higher MW alginates are amenable for topical use as wound coverage because of their better mechanical properties [123]. They used polyethylene oxide (PEO) as a carrier material to aid in electrospinning, and related the solution properties, including entanglement concentration, relaxation time, conductivity, and surface tension, to their ability to be electrospun. Finally, alginate-only nanofibers that are also water-insoluble were obtained by crosslinking the electrospun fibers with calcium and subsequently, removing the PEO and surfactants by soaking the nanofibers in water.

Fang *et al.* improved the electrospinnability of SA solutions by introducing Ca^{2+} cations to them [124]. Rheological behavior of the electrospinning solutions was investigated. The results showed that Ca^{2+} cations enhanced the intermolecular interactions of SA solutions, and improved the electrospinnability of SA solutions.

Alginate, PEO and PVA are soluble in water. This is an advantage as it ensures us that the product is neither toxic nor allergic because of the applied solvent.

Table IV provides the optimum electrospinning condition of sodium alginate blends with PVA and PEO. This table includes the polymer ratios, electrospinning settings and morphological information of the product according to different studies [37, 78].

Figures 9 and 10 show the effect of blend ratio for PEO and PVA as secondary polymers. As these figures reveal, the increase of alginate content results in nanofibers and bead formation [37]. PEO, in comparison with PVA, has more stability in electrospinning process. PEO has oxygen groups in its main polymer chains, which, along with methylene groups, make it a very flexible polymer with a better spinnability as compared to PVA.

2) Electrospinning of Chitosan blends

Low molecular weight chitosan has been shown to exhibit lower biological activities. That is why the researchers would like to employ higher molecular weight chitosan. However, the restrictions of electrospinning of high molecular weight chitosan (as is referred in section 4.2) constrain the electrospinning of pure high molecular weight chitosan.

The combination of chitosan with other biopolymers appears to be a common solution in various reports. Chitosan blends with other synthetic or natural polymers can imbibe the wide range of physical and chemical properties and processing techniques of synthetic polymers as well as the biological interactions and biocompatibility of natural polymers [86].

There are several studies on electrospinning of chitosan blends. Desai & Kit have reported the electrospinning of chitosan/poly (acryl amide) (PAAM) blend [125]. PAAM is a hydrophilic, synthetic polymer with NH_2 groups on side chains. This polymer is widely used for water treatment and antimicrobial applications. Their investigations have shown that temperature has a significant influence on the quality of Chitosan/PAAM nanofibers. Increasing temperature from 25°C to 75°C enhances the diameter, but at the same time, decreases the bead density of the nanofibers. Chen, Mo, & Qing have studied the electrospinning of chitosan–collagen complex to develop a biomimetic extracellular matrix for tissue engineering. Owing to numerous merits such as biological origin, nonimmunogenicity, excellent biocompatibility and biodegradability, collagen has been widely used for tissue engineering [126]. According to the reports, the hybrids of collagen–chitosan manufactured by crosslinking, wet/dry spinning and freeze-drying have biological and mechanical advantages as scaffold. chitosan can function as a bridge to increase the efficiency of collagen-based scaffolds owing to the large number of amino groups in its molecular chain [86].

Electrospinning of PEO/Chitosan and PVA/Chitosan blends has been reported in many studies [69, 80, 87, 88, 127- 133] . Bhattarai *et al.* used Triton X-100™ as a surfactant and dimethylsulphoxide (DMSO) as a co-solvent into chitosan solution to allow the solution to be spinnable at high chitosan/PEO ratios, and substantially improve the spinnability of the solution and the fibrous structure of as-spun nanofibers [127]. Ojha *et al.* have reported the production of core-sheath bi-component PEO/Chitosan fibers with PEO as sheath and chitosan as the core part [69]. As PEO is soluble in water, pure chitosan fibers can be obtained after removing the PEO sheath by washing it with deionized water .

Xie *et al.* have developed a novel way of chitosan-g-PLGA preparation and evaluated the copolymers in terms of their chemical characterization, their performance on electrospinning and their ability to support the culture [134]. In his work, Chitosan was initially modified with trimethylsilyl chloride, and catalyzed by dimethylamino pyridine. PLGA-grafted chitosan copolymers were prepared by reaction with end-carboxyl PLGA (PLGA–COOH).

Meng *et al.* have reported the electrospinning of both aligned and randomly oriented poly(D,L-lactide-co-glycolide) (PLGA)/chitosan nanofibrous scaffolds [135]. Morphological characterization using scanning electron microscopy showed that the aligned nanofiber diameter distribution obtained by electrospinning of polymer blend was widened with the increase of chitosan content. The drug release rate was increased with increasing the chitosan content because chitosan enhanced the hydrophilicity of the PLGA/chitosan composite scaffold. Moreover, they found that for the aligned PLGA/chitosan nanofibrous scaffold, the release rate was lower than that of randomly oriented PLGA/chitosan nanofibrous scaffold.

Jiang *et al.* have developed a novel 3-D chitosan/poly(lactic acid-glycolic acid) (PLAGA) composite porous scaffold by sintering together chitosan/PLAGA microspheres for bone tissue engineering applications [136]. Pore sizes, pore volume, and mechanical properties of the scaffolds could be manipulated by controlling fabrication parameters, including sintering temperature and sintering time. The