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Nanofibrous Structure of Chitosan for Biomedical Applications

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Abstract

Over the past decade, there has been a strong growing interest in using several forms of chitosan, more specifically nanofibers, for various biomedical applications. Chitosan has several impressive biological characteristics including but are not limited to its great biocompatibility and biodegradability, anti-bacterial properties, and cytocompatibility. In order to create nanofibers from this natural polymer, the electrospinning has been widely used as the most effective technique to produce a stable structure. Overtime, a number of challenges have been overcome through the development of mechanically and structurally intact, biocompatible and multi-functional nanofibers. The recent progress of the nanofibrous structure of chitosan and their biomedical applications in tissue engineering, drug delivery, wound dressing, and antimicrobial are discussed.

Keywords: Chitosan; Nanofiber, Electrospinning, Biomedical applications

Introduction

Chitosan is an N-deacetylated product of chitin, a helical polysaccharide macromolecule found in the exoskeleton of crustaceans such as crabs, shrimp, insects, and other arthropods and is the second most abundant natural biopolymer after cellulose. Both chitin and chitosan have shown to have remarkable biological properties such as bioresorbable degradation products, hydrophilicity, biocompatibility, cellular binding capability, and acceleration of wound healing which accounts for their wide variety of applications in food, cosmetic, biomedical, and pharmaceutical industries [1]. Commercially, chitosan is produced by exhaustive deacetylation of chitin with concentrated solution of sodium hydroxide. Alternatively, a controlled and mild method has been developed by using chitin deacetylase, an enzyme that catalyzes deacetylation of N-acetyl-D-glucosamine residues of chitin in to D-glucosamine residues of chitosan [2,3]. Once the degree of deacetylation (DD) for chitin reaches approximately 60 to 70%, it is referred as chitosan [1,4]. Figure 1 shows the multistep process involved to obtain chitin from crab and shrimp shells, and comparative chemical structures of chitin and chitosan.

The presence of primary aliphatic amines in the chemical structure of chitosan makes this polymer distinct from other commonly available polysaccharides such as cellulose, hyaluronic acid, alginate, dextran, etc. The primary aliphatic amines of chitosan can be protonated under acidic conditions (amine pKa is 6.3) which makes them cationic polyelectrolyte [5]. The cationic nature of the polymer allows it to become water-soluble after the formation of carboxylate salts, such as formate, acetate, lactate, malate, citrate, glyoxylate, pyruvate, glycolate, and ascorbate, etc. Important information of chitosan's physical and chemical properties can be found in the several review articles and American Standard Testing Materials (ASTM) standard guides and in the U.S. Pharmacopoeia (USP) [6,7].

Chitosan has many versatile properties which make it an excellent excipient in controlled release formulations including non-viral vectors for DNA-gene and drug delivery, and imaging applications [8-11]. Chitosan has been prepared with a variety of different shapes, geometries, and formulations that include liquid gels, powders, beads, films, tablets, capsules, microparticles, sponges, textile fabrics, and inorganic composites [12]. In each preparation chitosan is either physically associated or chemically cross-linked to form stable hydrogel networks. Hydrogels are high water content materials prepared from cross-linked polymers that are able to provide sustained, local delivery of a variety of therapeutic agents, encapsulation of cells and proteins in bio-friendly environment [13]. The advanced development of chitosan hydrogels has led to new drug delivery systems that release their payloads under varying environmental stimuli. In order to satisfy the requisite features of a hydrogel, the chitosan polymer network must satisfy two conditions: (1) inter-chain interactions must be strong enough to form semi-permanent junction points in the molecular network, and (2) the network should promote the access and residence of water molecules inside the polymer network. Figure 2 shows the schematics of four major physical interactions (ionic, polyelectrolyte, interpolymer complex, and hydrophobic associations) that lead to hydrogel network formation in chitosan [13-14]. There are many recent reviews surveying the hundreds of papers related to chitosan processing and geometries for various applications, but little information is available on nanofiber processing of chitosan and their applications.

Polymeric nanofibers are of great scientific and technological interest because of their wide-range of applications in biomedicine and biotechnology [15-17]. Once polymer fiber materials began to be created in the diameters of submicrons to few tens of nanometers, they were referred to as nanofibers. In comparison to conventional large diameter fibers, they have larger surface area to volume ratio, flexibility in surface functionalities, and superior mechanical performance compared with any other known form of the material used [18]. These outstanding properties make the polymer nanofibers optimal candidates for many important applications such as tissue engineering

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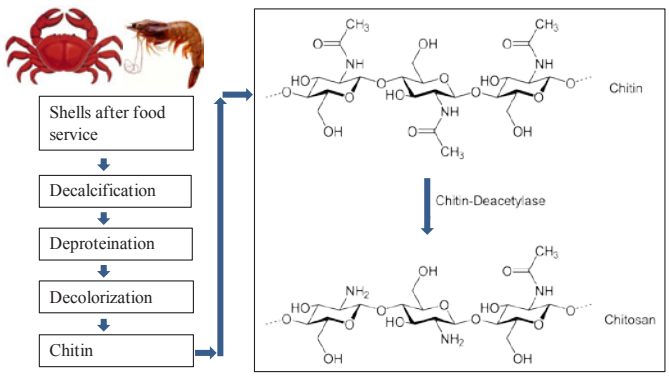

Figure 1: Illustration of multistep process involved to convert crustacean's shells such as crabs, shrimps, lobsters etc., into chitin. Chemical structures show enzymatic conversion of N-acetyl-D-glucosamine residues of chitin into D-glucosamine residues of chitosan during deacetylation process.

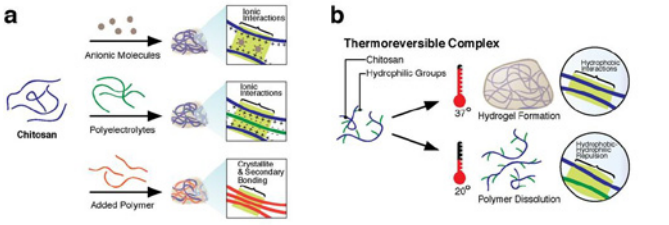

Figure 2: Schematic representation of chitosan based hydrogel networks derived from different physical associations: (a) networks of chitosan formed with ionic molecules, polyelectrolyte polymer and neutral polymers; (b) thermoreversible networks of chitosan graft copolymer resulting semi solid gel at body temperature and liquid below room temperature. (from ref [13] with permission).

scaffolds, wound dressing materials, therapeutic drug delivery devices, filtration devices, etc. A number of manufacturing processes have been explored to fabricate nanofibers which include drawing [19], self–assembly [20], template-directed synthesis [21], and phase separation [18,22]. Drawing is a process that is very similar to dry spinning in the fiber industry, having the ability to produce long single nanofibers one-by-one. Self-assembly is a process in which individual, pre-existing components organize themselves into desired patterns and functions. For template-directed synthesis, a nanoporous membrane is used as a template to make nanofibers of solid (fibril) or hollow (tubule) shape. Phase separation consists of dissolution, gelation, and extraction using an alternate solvent, freezing, and drying resulting in nanoscale porous foam. Unfortunately, all these techniques have substantial disadvantages such as high time consumption, low cost effectiveness and noncontiguous fiber formations etc.

Electrospinning: A fascinating technique for nanofiber fabrication

Electrospinning is a fastest growing trend in the production of fibers in laboratory to industrial scale [23-26]. Recently, these techniques have become popular due to their ability to produce various classes of ultrafine fibers such as polymer, ceramics, metals, composite etc., with diameters in the range several micrometers down to tens of nanometers. An electrostatic field is created between a syringe tip holding electrospinning fluids (e.g. polymer solution or melts) and a collector of good conductance so that spherical shaped droplet at the end of the syringe tip turned into a conical shape, i.e. Taylor cone. Once the electric field goes beyond a threshold value a charged fluid jet is ejected from the tip of the cone. If the molecular entanglements in the fluid are sufficiently high, stream breakup does not occur (if it does, an electrospray droplet is formed) and a charged liquid jet is formed. As the jet dries in flight, the charge migrates to the surface of the fiber. The jet is then elongated by a whipping process caused by electrostatic repulsion initiated at small bends in the fiber, until it is finally deposited on the grounded collector. The elongation and thinning of the fiber resulting from this bending instability lead to the formation of ultrafine fibers [27-29]. If polymer solution is used as electrospinning fluid, the twisting and bending of the jet generates highly stretched polymeric fiber with simultaneous rapid evaporation of the solvent. Polymer and solution properties such as molecular weight, viscosity, conductivity, and surface tension are very important parameters to control the fibers size and morphology. Some other parameters which can change the electrospinning process are applied voltage, tip-to-collector distance, feeding rate, etc [23-26].

Two of the most fascinating characteristics of polymeric nanofibers are the very large surface area-to-volume ratio and high porosity with very small pore size. For these reasons, electrospun nanofibers have shown to be promising candidates for biomedical applications such as tissue templates, medical prostheses, artificial organ, wound dressing, drug delivery, and pharmaceutical composition [30-35]. Electrospun nanofibers of various biopolymers, both synthetic and natural origin have been widely used for these applications. Among the degradable synthetic polymers, poly (lactic acid), polycaprolactone (PCL), and poly (D,L-lactide-co-glycolic acid) (PLGA) have been investigated as fibrous scaffolds due to their bioresorption properties required for tissue engineering applications and good mechanical properties. However, synthetic polymers are typically hydrophobic and lack cell-recognition sites for support of cell adhesion [35,36]. Natural polymers exhibit particular advantages over synthetic polymers because of their proven biocompatibility and their resorbable degradation products that can be used in a wide variety of ways in biomedical technology. Collagen, gelatin, hyaluronic acid, chitosan, and alginate are the most commonly used natural polymers [37,38]. Chitosan, a biodegradable, non-antigenic biopolymer, bears a proxy structure of glycosaminoglycan (GAG), a major component of the native extracellular matrix in human and animal tissues provides mechanical support and regulates cellular activities [39]. The ability to generate nanofibers from chitosan, a natural polysaccharide derived from leftover shells after food processing, may provide virtually unlimited resources for the development of biocompatible scaffolds to restore damaged or dysfunctional tissues.

Progress in eletrospinning of chitosan

Recently, a library of various polymer-solvent combinations which has a crucial role in transforming chitosan into nanofibers has been developed for many ground breaking applications in different disciplines of biomedical technology such as such as in tissue engineering, wound healing, drug delivery, and anti-bacterial applications [33,34,40-47]. These studies as well as steady state growth of number of publications of chitosan based nanofibers in each year (Figure 3) demonstrate enormous potential of chitosan nanofibers in the biomedical field.

Challenges and successes in electrospinning of chitosan

Physical and chemical parameters of polymer solution such as viscosity, electric conductivity, and polymer concentration can

determinedly affect the formation and morphology of electrospun fibers. These parameters are well defined for electrospinning several synthetic polymers solutions in organic solvents. A majority of natural polymers, including chitosan, do not dissolve in organic solvents with an exception of highly corrosive halogenated organic acids. Although chitosan dissolves in water at acidic pH, a major complication arises in electrospinning due to its high viscosity in aqueous solution. At low polymer concentrations, solutions do not contain sufficient material to produce stable solid fibers. With increasing polymer concentration, the number of direct inter- and intra-chain associations of chitosan molecules in the solution increases rapidly and reaches a critical value of forming a 3-D network structure—a highly viscous gel, rendering the solution difficult to electrospin [48]. The associative properties of chitosan chains arises due to the presence of strong hydrogen bonding between -NH_2 and -OH groups of chitosan molecules causing it to be unspinnable. Nevertheless, several research groups have succeeded in the preparation of chitosan based fibers by either blending it with other surfactants like synthetic polymers such as polyethylene oxide (PEO) and polyvinyl alcohol (PVA) or by mixing it with strong organic acids [33,49,50]. The decrease in viscosity with addition of synthetic polymers can be attributed to the change in inter and intramolecular interactions of chitosan chains. The additive molecules bound onto chitosan backbone disrupt the self-association of chitosan chains by forming new hydrogen bonds between its OH groups and water molecules [48,51]. Physically, this modulation in associative forces by surfactants is manifested as an increase in chitosan solubility and a decrease in its solution viscosity which a suitable condition for electrospinning.

Electrospinning of chitosan-trifluroacetic acid

The most common way to produce chitosan nanofibers is by preparing chitosan solution in trifluoroacetic acid (TFA) and/or acetic acid [50,51] . These solvents are best known to improve the fluidity in chitosan solution by disrupting the 3-D networks of chitosan so that ultrafine fibers can be produced. Ohkawa et al. [50] was able to electrospin a chitosan-TFA solution to develop nanofiber mess sheets. Jang et al. [52] was able to produce nanofibers by using 7% chitosan concentration solution (chitosan Mw = 106,000 g/mol) using 90% acetic acid. The most important solution parameter in the electrospinning of chitosan is increasing the acetic acid concentration. More concentrated acetic acid in the solution progressively decreases the surface tension of chitosan and concomitantly increases charged density of the jet without significant effect on solution viscosity. The higher the charge density carried by the jet the smoother the fiber, due to a stronger whipping instability of the jet [52]. Unfortunately, these solvents can cleave the backbone structure the chitosan molecules so that the resulting nanofibers are very weak in mechanical strength and also causing them to degrade very quickly in an aqueous environment. Chemical crosslinking is a way to make the fibers insoluble and to retain their structural integrity but it requires the use of toxic chemicals. At the end, any trace amount of leftover crosslinking chemicals in the fiber can alter its cytocompatibility. Therefore, alternative ways of making chitosan nanofiber are warranted.

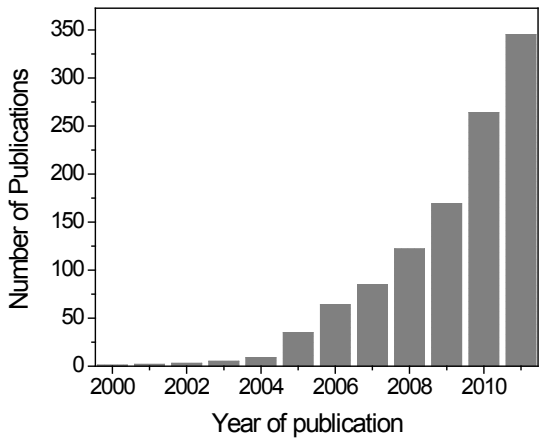

Figure 3: Scientific publications on chitosan nanofibrous structures found in the Science Direct search system with key words “chitosan” and “electrospinning”.

Chitin nanofibers to chitosan nanofibers

Being an ionic polymer, chitosan shows polyelectrolyte behavior in solution which can develop enormous repulsive forces between ionic groups within the polymer chain while it is charged under an electric filed. This may result in the lack of continuous fiber formation during the electrospinning process, especially during the jet stretching and bending. Electrospinning of chitosan using different solvent systems will have some limitation in obtaining continuous fibers. An alternative method has been developed by using neutral nonionic form of chitin solutions to make chitin fibers which later can be converted to chitosan fibers through deactylation process [4,26]. Chitin powder was first depolymerized by gamma irradiation to make it soluble. Next, it was dispersed in 1, 1, 1, 3, 3, and 3-hexafluoro-2- propanol (HFIP) to dissolve for three days with a concentration range of three to six percent by weight. Electrospun fibers were deposited on a target drum. Then the collected fibers were placed in concentrated solution of sodium hydroxide and then washed with distilled water. Finally, the fibers were dried under vacuum. Deacetylation reaction took place when chitin fibers were treated with concentrated base [26]. Nam et al. [4] was also able to generate chitosan nanofibers from chitin nanofibers by using a heterogeneous alkaline treatment to complete the transformation of chitosan nanofibers with different degrees of deacetylation.

Chitosan fibers form polyblends and surfactants

Arguably, the greatest challenge of producing chitosan nanofibers is the lack of appropriate solvents to produce a solution which is sufficient to make uniform nanofibers and at the same time, a solution formulation that is biologically friendly. Solvents such as acetic acid, trifluoroacetic acid (TFA) and HFIP are the most used solvents to prepare chitosan solutions. But, due to their highly corrosive nature, they are not compatible with biomolecules especially with cells, proteins, peptide drugs, etc. Avoiding the corrosive/toxic organic solvents and extra chemical processes during the nanofiber production is always challenging. Several studies have been explored using polyblend systems comprising of an aqueous solution of chitosan with hydrophilic- synthetic polymers such as PVA, PEO and surfactants such as Triton X-100 as one of the biologically friendly methods to make chitosan nanofibers [48,50].

Bhattarai et al. [48] has developed chitosan based nanofibers from a polyblend system of chitosan/PEO with desirable structure and material properties, and demonstrated that the nanofibrous matrix, when properly constructed, exhibited good structural integrity, promoted cell attachment, and thus can potentially serve as scaffolding material for tissue engineering. Preparation of a suitable solution for electrospinning was crucial for their success. The PEO and chitosan solutions were prepared separately and then mixed to create a particular range of chitosan/PEO blend solution (Figure 4). Blend solution was

further mixed with trace amount of dimethylsulphoxide (DMSO) and Triton X-100 to achieve better structural uniformity in naonfibers (Figure 5). PEO is a biocompatible polymer, one of the few synthetic polymers approved for internal use in food, cosmetics, personal care products, and pharmaceuticals. Therefore, this preparation method was well suited for several other biomedical applications and cited in several peer reviewed journal articles.

Another great advantage of chitosan is that it has good miscibility properties with a number of synthetic polymers in solutions. Good miscibility of polymer solution can create polyblend nanofibers with increased physico-chemical properties such as mechanical strength and biodegradation. Mechanical strength of chitosan nanofibers are relatively weaker compared to nanofibers of synthetic polymers. Chitosan also degrades much faster than synthetic polymers in body fluid environments. In polyblend nanofibers, chitosan and synthetic polymers such as poly (caprolactone) (PCL), poly (lactic acid) (PLA), and poly (D, L-lactide-co-glycolic acid) (PLGA) have the potential to complement each other very well. This is primarily because nanofibers can be engineered to retain the mechanical strength and durability of a synthetic component and the biological functionality of chitosan. In addition to combining properties, polyblends have been used to facilitate the electrospinning of chitosan in less rigorous processing that does not involve corrosive acids which can cause unwanted polymer degradation or damage [53-55]. An exhaustive list of chitosan and chitosan–synthetic polymer blend nanofibers is provided in Table 1.

Recently, Bhattarai, et al. [35] was able to develop chitosan-PCL polyblend nanofibers from electrospinning. Solution preparation for their set up was very critical to obtaining stable nanofibers. The electrospinning process was carried out immediately after mixing the chitosan/TFA solution with the PCL/TFE solution. The PCL is subject to the acidic hydrolysis by TFA overtime giving the resulting chitosan-PCL solution a certain time constraint. From their detailed research, it was found that the solution supply for electrospinning needed to be replaced every half hour with freshly prepared chitosan-PCL solution. For polyblend nanofibers containing a combination of natural and synthetic polymers, blend components are usually phase separated into the individual components and requires chemical crosslinking to retain their structural integrity and improve mechanical strength. This procedure requires the use of crosslinking agents which can cause problems in biomedical applications because any trace amount

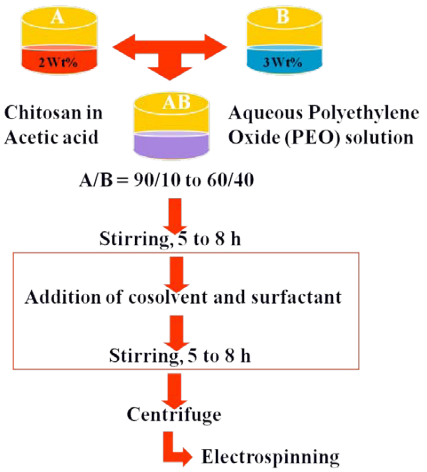

Figure 4: Preparation of chitosan/PEO solution for electrospinning [49].

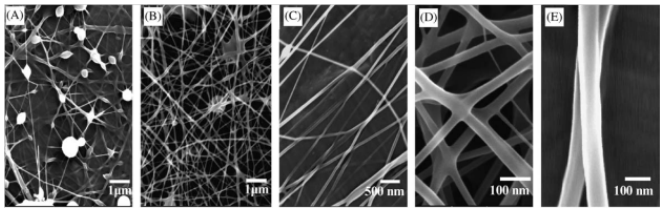

Figure 5: SEM images of electrospun structures (chitosan/PEO ratio 90/10) prepared from aqueous solution containing 0.5M acetic acid: (A) 0.3% Triton X-100™, (B) and (C) 0.3% Triton X-100™ and 10% DMSO. Fibers in (B) were collected on a stationary collector whereas fibers in (C) were collected on a cylindrical collector with a rotating speed of 2000 rpm. Images (D) and (E) are the high-magnification images of (B) and (C), respectively. (from ref [49] with permission).

of crosslinking agents found in the product can be toxic. However, chitosan/PCL polyblend system did not require any crosslinking agents and resulted in a successful nanofibrous product without undergoing any phase segregation.

Cooper A et al. [75] was also able to develop an aligned chitosan-PCL nanofibrous scaffold to investigate nerve cell organization and function using Schwann cells and PC-12 cells. These blended nanofibers with balanced hydrophilic nature, high structural integrity, and free amino groups are expected to have the potential application for nerve regeneration. Another study by the same group reported the fabrication of a lactic acid modified chitosan nanofiber [62]. Chitosan powder was freeze dried in a dilute lactic acid solution to prepare a chitosan lactate salt. The chitosan salt product was dissolved in a TFA/MC solvent for electrospinning. Once nanofibers were spun, they were further stabilized by thermal treatment causing amidation between chitosan and lactate salt [62].

Another form of composite polyblend nanofibers of chitosan is a version that contains inorganic nanoparticles for specific functional applications [67,76-78]. Over the past few years, several different electrospun nanocomposite fibers such as PCL/$CaCO_3$, Hap/gelatin, silk/Hap, PLA/Hap, and triphasic Hap/collagen/PCL have been devised and explored for potential bone regeneration applications. One particular study done by Zhang et al. [67] was able to electrospin composite nanofibers containing hydroxyapatite/chitosan for bone tissue engineering to design and fabricate bioactive scaffolds that resemble the native extracellular matrix. Preparation of these fibers involved a two-step method, which involves firstly preparing Hap/CTS nanocomposites by a co-precipitation synthesis approach and then fabricating the resultant composite nanofibers with ultrahigh molecular weight poly (ethylene oxide) (UHMWPEO) as a fiber-forming additive. Cell culture experiments using the human fetal osteoblasts compared the electrospun Hap/CTS to electrospun pure chitosan. The study was able to highlight very important features of HAp/CTS composite nanofibers in display of its great potential for bone tissue engineering applications [68].

Biomedical applications for chitosan nanofibers

Tissue engineering

Electrospun nanofibers are widely and successfully used is in the area of tissue engineering. Tissue engineering relies on the ability of a scaffold to foster cellular in growth and rapid repopulation of new tissue. This requires a 3-D synthetic matrix to emulate the native extracellular matrix (ECM) found in tissues such as bone, ligament cartilage, skin, nerve, vascular tissue, and others. Broadly, the ECM,

Polymer	Molecular Weight of Chitosan	Composition Ratio	Solvents	Targeted Applications	Reference
Single					
Chitin	910 kD		HFIP	-	[27]
Chitin	920 kD	-	HFIP		[4]
Chitosan	-	-	TFA, MC (70/30)		[57]
Chitosan		-	aq AA	-	[53]
Chitosan (alkali treatment)	Medium Weight	-	aq AA	-	[58]
Hexanoyl chitosan	576 kD	-	Chloroform		[59]
Blend					
Chitosan/PEO (w/ or w/o surfactant)	148 kDa and 68 kDa		aq AA/water, Tween 20	-	[60]
Chitosan/PEO (w/ or w/o metal ions)	50 kDa	30/70	aq AA	Protein resistance	[61]
Chitosan/PEO	190 kD /900 kD	90/10	aq AA, DMSO, Triton X-100™	Bone tissue engineering	[49]
Chitosan/PCL		80/20	TFA/TFE	Nerve therapy	[62]
Chitosan/UHMWPEO				Bone tissue engineering	
Chitosan/LA		-	TFA, MC (80/20)	Tissue engineering scaffold	[63]
Chitosan/PVA	120-1600 kD	10/90, 20/80, 25/75	aq AA	-	[64]
Chitosan/PVA	-	20/80	aq AA	-	[65]
Chitosan/PLA	-	-	TFA-TCM	Antimicrobial application	[66]
Chitosan/PET			TFA	Wound dressing	[67]
Chitosan/silk fibroin	220 kDa		FA		
Chitosan/HAp/ UHMWPEO		70/30--	HAc/DMSO (10:1)	Bone tissue engineering	[68]
Chitosan/collagen	1000 kDa	80/20, 50/50, 20/80	HFIP/TFA	Wound dressing	[69]
Chitosan-g-PEG/PLGA	-	-	DMF/THF	Wound dressing	[70]
Q-Chitosan/PVA	400 kDa		aq AA		
Q-Chitosan/PVP	380 kDa		Water		
CECS/PVA	120 kDa	100/0, 80/20 60/40, 50/50 30/70, 20/80 10/90, 0/100	Water	Skin regeneration	[71]
CECS/PVA	390 kDa	75/25	aq AcrA	Tissue scaffold	[72]
CMCS/PVA	405 kDa	50/50	Water	Antimicrobial application	[73]
Chitosan/gelatin w/ silver nanoparticles	51 kD	-	aq AA	Wound dressing	[74]
Chitosan-HOBt/PVA	110 kD	10/90 to 90/10	Distilled water	Drug delivery	[75]

Abbreviation: UHMWPEO: UltraHigh-Molecular-Weight Poly(Ethylene Oxide); PET: Poly(Ethylene Terephthalate); HAP: Hydroxyapatite; P(LLA-CL): Poly(L-Lactic Acid-co-ε-CaproLactone); CMCS: CarboxyMethyl Chitosan; CECS: CarboxyEthyl Chitosan; PVA: Poly(Vinyl Alcohol); Q-Chitosan: Quaternized Chitosan; PVP: Poly(Vinyl Pyrrolidone); DMF: DiMethylFormamide; THF: TetraHydroFuran; GA: GlutarAldehyde ; TFA: TriFluoroAcetic acid; aq AA: aqueous Acetic Acid solution; MC: Methylene Chloride; aq AcrA: Aqueous acrylic Acid solution; TCM: TriChloroMethane; DMSO: DiMethyl SulfOxide; HFIP: 1,1,1,3,3,3,-HexaFluoro-2-Propanol

Table 1: Electrospinning of chitosan based nanofibers.

a nanofibrous protein–polysaccharide hierarchical network provides mechanical support for cells to create functional tissues. Physico-chemical and biological properties of ECM ultimately control cell shape, define tissue architecture and help regulate its physiological function. Electrospun nanofibers of various natural and synthetic polymers mimic such hierarchical structures found in the natural ECM. In successful tissue engineering, scaffold should induce the formation of neo-tissue necessitating the breakdown and clearance of the originally implanted scaffolds. Chitosan based nanofibers, being a biodegradable polysaccharide under cell-induced proteolytic conditions, are believed to be an excellent proxy structure of ECM and well suited candidate for tissue engineering applications.

There have been several chitosan based nanofibrous structures developed in different geometries such as nonwoven sheets, fiber bundles and tubular constructs. By electrospinning polyblends of chitosan/PCL onto rotating spindles, nanofibers can be fabricated into tubular constructs [35]. These scaffolds are well suited as nerve guides for peripheral nerve regeneration, where severed nerve endings cannot be repaired with sutures. Synthetic nerve guides can take the place of autografts, to redirect nerve growth across the critical gap. Nerve conduit materials prepared with chitosan/PCL polyblend nanofibers have demonstrated strong mechanical properties, capable of being sutured to the nerve ends and maintaining structural stability *in vivo*. In addition, aligned chitosan-PCL nanofibers have shown to provide a favorable environment for nerve cell proliferation. In one study, a fiber mat with aligned nanofibers was found to enhance the neuritis extension and directionality of attached nerve cells [62,75]. Aligned electrospun fibers are also of major interest for development and remodeling of native and engineered heart tissues [49].

To mimic the biochemical and structural environment, certain tissue systems have additional demands. In the study of skeletal muscle tissue attachment and proliferation, Cooper et al. [79] developed a

chitosan/PCL based nanofibrous scaffold with unidirectional fiber orientation. Ideally, the matrix scaffold for muscle tissue engineering should provide a microenvironment with appropriate topographical and chemical cues to regulate muscle cell-material interaction. Visual confirmation of the muscle cell differentiation and corresponding gene expression was established by confocal fluorescence and PCR analysis. Highly aligned chitosan-PCL nanofibrous scaffolds exhibited superior tensile strength compared to randomly oriented nanofibers, promoting muscle cell proliferation and inducing the formation of elongated and anisotropically oriented myotubes (Figure 6). Muscle cells on the aligned nanofibers demonstrated up-regulation of differentiation-specific gene expression including troponin T and MHC. The study concluded that the aligned chitosan-PCL nanofibrous scaffolds could potentially serve as a cost-effective tissue-engineered construct for enhanced muscle tissue reconstruction.

Chitosan based polyblend nanofibers with synthetic biopolymer polymers such as PEO, PLGA, PLLA, PVA and Hydroxyapatite (HAP) have been used as potential biomimetic scaffolds to engineer several others tissues such as bone, cartilage, tendon, etc., [48,67,76,80-84]. The ability of chitosan to support cell attachment and proliferation is attributed to its chemical properties. The polysaccharide backbone of chitosan is structurally similar to glycosaminoglycans, the major component of the extracellular matrix of bone and cartilage. Other advantages of chitosan based nanofibrous scaffolds for the tissue engineering include the formation of highly porous scaffolds with interconnected pores, osteoconductivity and ability to enhance bone formation both in vitro and *in vivo*. By modulating the fiber alignment and orientation, three-dimensional scaffolds can be formed to match the mechanical properties and microenvironments of varying tissue types (e.g. aligned ligament fibers versus spiral smooth muscle fibers). Fiber orientation of polyblends will play an increasing role in producing topographical cues that can direct cellular activity to regenerate functional tissues.

Wound dressing

Nanofibrous membranes are highly soft materials with high surface-to-volume ratios, and therefore can serve as excellent carriers for therapeutic agents that are antibacterial or accelerate wound healing. For quick healing of traumatic or postsurgical wounds, several factors should be considered while selecting a wound dressing, which includes eliminating infection, limiting inflammation, wound cleansing, maintaining moist environment of wound, controlling wound exudates, promoting tissue growth, and oxygenating the wound [85-88]. Chitosan based nanofibers along with other natural polymer based nanofibers have recently attracted a great deal of attention to be used as wound dressing. Chitosan is most suitable for this application because it meets several requirements such as histocompatibility, biodegradability, lack of antigenicity and also promotes wound healing [89-91].

In the area of wound healing, care, and management, antibacterial resistance of microorganisms is a major concern. Very recently, there has been a lot of focus on the development of new antibacterial to treat wounds infected with antibacterial resistant microorganisms. Chitosan derivatives with quaternary ammonium groups have been known to possess high efficacy against bacteria and fungi [93]. Several studies have documented the use of chitosan scaffolds to treat patients with deep burn, wounds, etc. Ignatova et al. [54] was able to use photo-cross-linked electrospun mats with quaternized chitosan to efficiently inhibit the growth of Gram-positive bacteria and Gram-negative bacteria. These results demonstrate that quaternized chitosan/PVP electrospun mats are favorable materials for wound dressing applications. A polyblend nanofibrous membrane of chitosan/collagen was found to induce cell migration and proliferation while assisting in wound healing. Chen et al. [93] reports that nanofibrous membrane have beneficial effects better than gauze and commercial collagen sponge when conducting animal studies. Nanofibers have greater water-retention capacity because of very high-specific surface area and are very soft, so that the dressing will not chafe the wound. A wound dressing made up of a chitosan based nanofiber would be a promising dressing material.

Drug release

Nanofiber scaffolds have been identified as local drug delivery vehicles owing to their large surface area and controlled degradation. Drugs can be loaded into the nanofiber by premixing the polymer solution with the therapeutic before electrospinning. In addition to blending the drug into the polymer mixture, a composite drug–polymer can be prepared by encapsulation of drugs or biomolecules by coaxial electrospinning, forming core-shell structures [95,96]. The drug or any biomolecule can be simply prepared in the core solution, protected from denaturing. The controlled release of the material can be regulated by the degradation rate or porosity of the polymer shell. Recently, chitosan nanofibrous structures have found their place as a unique drug release process [1]. Composite membranes composed of PLGA and PEG-g-chitosan prepared by electrospinning have shown the ability to be loaded with the anti-inflammatory drug known as ibuprofen [69]. The PEG-g-chitosan gave evidence of significantly reducing the initial burst of ibuprofen from electrospun composite membranes. Furthermore, ibuprofen can be joined to the side chains of PEG-g-chitosan to sustain its release for more than two weeks (Figure 7).

Recently, electrospun fibers as antitumor drug carriers have attracted a great deal of attention because it is a promising approach for the targeting delivery of the antitumor drugs at tumor tissue, especially in postoperative local chemotherapy. The drug release profile from these systems can be controlled by modulation of the nanofiber morphology, porosity, and composition. Chitosan and its derivatives have drawn a great attention as antitumor drug carriers such as doxorubicin hydrochloride (DOX) [96-98]. This is due to the set of

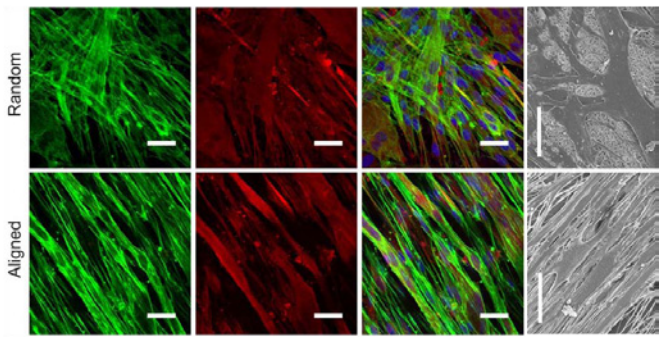

Figure 6: Confocal microscopy images showing immunocytochemistry analysis of actin (left column, green) and myosin heavy chain (MHC) (middle column, red) expressed by muscle cells grown on chitosan-PCL randomly oriented and aligned nanofibrous scaffolds after culture in fusion media for five days. The merged images with nuclei stained with DAPI (blue) are shown on the right column. SEM images showing morphology of muscle cells grown on chitosan-PCL nanofibers after 5 days of culture. Scale bars represent 40 μm. (from ref [80] with permission).

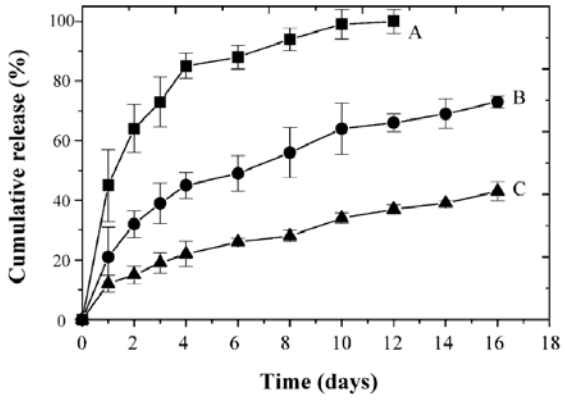

Figure 7: Release profiles of ibuprofen from an electrospun (A) PLGA membrane (5% ibuprofen), (B) a PLGA/PEG-g-chitosan membrane (5% ibuprofen), and (C) a PLGA/PEG-g-chitosan membrane conjugated with ibuprofen (4.4% ibuprofen). Electrospun membranes were incubated in 0.1 M PBS (pH 7.4) at 37°C. (from ref. [70] with permission)

advantageous properties of these polymers, for example, nontoxicity, biodegradability, biocompatibility, intrinsic antibacterial properties, and immuno-stimulating effect. Chitosan has shown good antitumor activity, which is mainly due to its polycationic nature. Ignatova et al. [99] developed a one-step preparation of DOX-containing nanofibrous materials by electrospinning of DOX/poly (l-lactide-*co*-d, l-lactide) (coPLA) and DOX/quaternized chitosan (QCh)/coPLA solutions. These nanofibers showed high antitumor activity which renders these types of nanofibrous materials promising candidates for the treatment of cervical tumor, which remains a critical public health problem.

Antimicrobial

Chitosan is known to have antimicrobial activity due to the fact that it is a cationic polyelectrolyte polymer. A polymer solution of chitosan/PET with TFA/HFIP as a solvent was electrospun to produce nanofibrous matrices to inhibit the growth of *S. aureus* and *K. pneumoniae*. Results showed that they were more effective than pure PET matrices[67]. Spasova et al. [100] examined the effect of potassium 5-nitro-8-quinolinolate (K5N8Q) intergrated into chitosan/PEO nanofiber matrices on antimicrobial and antimycotic activity against gram negative and positive bacteria (*E. coli and S. aureus*) and fungi (*C. albicans*). In comparison, only nanofibrous matrices containing K5N8Q comprised of sterile zones. Thin chitosan films can also be used to modify by nanofibrous matrices to further incorporate anti-bacterial properties. A simple chitosan film can be deposited on the electrospun nanofibers to increase the amount of chitosan along with the hemostatic activity of the matrices. PLA and PLA/PEG polyblend fibrous matrices were prepared by electrospinning and then coated with chitosan [100]. Both chitosan coated matrices demonstrated to have anti-bacterial activity against *S. aureus*.

Conclusion

This review gives an overall summary on how chitosan based nanofibrous structure research has evolved over the years and the biomedical applications in which they can possibly make a huge impact. Nanofibers prepared from electrospinning of chitosan represent a simple, efficient and scalable method that is well suited to prepare clinically relevant materials. The future challenges of chitosan nanofibrous structures include optimizing the fabrication process with the most effective solvents, the various combinations of electrospinning parameters and the hybrid of natural and/or synthetic polymers, chitosan derivatives, composites, etc. for each specific targeted application. Whether it's the regeneration of nerve tissue, delivery of anti-inflammatory drugs, or keeping a wound sterilized from infection, each form of biomedical application requires extensive research. The amount of research being conducted is growing rapidly every year to meet these demands. Although, thorough toxicity studies needs to be conducted before this new class of materials can be identified as suitable for human application.

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