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## Perspectives of Chitin and Chitosan Nanofibrous Scaffolds in Tissue Engineering

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### Abstract

Chitin and its deacetylated derivative, chitosan, are non-toxic, biodegradable biopolymers currently being developed for use in biomedical applications such as tissue engineering scaffolds, wound dressings, separation membranes, antibacterial coatings, stent coatings, and sensors. Recently, nano fibrous scaffolds based on chitin or chitosan have potential applications in tissue engineering. Tissue engineering is one of the most exciting interdisciplinary and multidisciplinary research areas today, and there has been exponential growth in the number of research publications in this area in recent years. It involves the use of living cells, manipulated through their extracellular environment or genetically to develop biological substitutes for implantation into the body and/or to foster remodeling of tissues in some active manners. Electrospun chitin and chitosan nano fibrous scaffolds would be used to produce tissue engineering scaffolds with improved cytocompatibility, which could mimic the native extracellular matrix (ECM). Electrospinning is truly a feasible means of producing nano fibrous scaffolds that resemble the ECM, however, moreover than this, it is imperative that the effects of an artificial matrix has on cell growth, proliferation, and differentiation. This review summarizes the recent progress in chitin and chitosan based nano fibrous scaffolds with an emphasis in tissue engineering applications.

### 1. Introduction

Chitin, the second most abundant natural polysaccharide, is synthesized by a number of living organisms. Chitin occurs in nature as ordered microfibrils, and is the major structural component in the exoskeleton of arthropods and cell walls of fungi and yeast. The main commercial sources of chitin are crab and shrimp shells, which are abundantly supplied as

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waste products of the seafood industry. Because chitin is not readily dissolved in common solvents, it is often converted its more deacetylated derivative, chitosan (Kurita, 2001; Rinaudo, 2006; Pillai et al., 2009). Chitosan is often identified by its degree of deacetylation (DD), a percentage measurement of free amine groups along the chitosan backbone. Generally, the material is considered chitosan when it becomes soluble in dilute acidic solutions, which occurs when  $DD \geq 60\%$  (Roberts, 1992). Because of its ease of solubility, chitosan is preferred over chitin for a wide range of applications.

Chitin and chitosan are biocompatible, biodegradable, and nontoxic, and are anti-microbial and hydrating agents. Chitin and chitosan are easily processed into gels (Nagahama et al., 2008a; 2008b), membranes (Jayakumar et al., 2005; Jayakumar et al., 2007; Jayakumar et al., 2009; Madhumathi et al., 2009a; Madhumathi et al., 2009b), nanofibers (Schiffman & Schauer, 2007a; Shalumon et al., 2009), beads (Jayakumar et al., 2006), microparticles (Prabaharan & Mano, 2005), nanoparticles (Anitha et al., 2009), scaffolds (Madhumathi et al., 2009c; Maeda et al., 2009) and sponges (Muramatsu et al., 2003; Portero et al., 2007) forms. There are a number of promising applications of nanoscale thin films and fibers of chitin/chitosan (Wang & Hon, 2003; Rinaudo, 2006; Pillai et al., 2009).

Recently, much attention has been paid to electrospinning process as a unique technique because it can produce polymer nanofibers with diameter in the range from several micrometers down to tens of nanometers, depending on the polymer and processing conditions. In electrospinning, a high voltage is applied to create electrically charged jets of a polymer solution. These jets dry to form nanofibers, which are collected on a target as a non-woven fabric. These nanofibers are of considerable interest for various kinds of applications, because they have several useful properties such as high specific surface area and high porosity. Nanofibrous non-woven fibers, containing chitin or chitosan, yield potential applications in areas such as filtrations, recovery of metal ions, drug release, dental, tissue engineering, catalyst and enzyme carriers, wound healing, protective clothing, cosmetics, biosensors, medical implants and energy storage (Zhang et al., 2005; Fang et al., 2008). In this review, we are reporting about the different preparation and tissue engineering applications of electrospun chitin and chitosan based nanofibers in detail.

## 2. Electrospinning of chitin and chitosan

### 2.1. Electrospinning of chitin

Chitin is insoluble in most of the organic solvents. Due to its insolubility, its applications are limited in many applications. Chitin dissolves only in specific solvents such as N, N-dimethylacetamide (DMAC)-LiCl (Cho et al., 2000), hexafluoroacetone, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Kurita, 2001) and saturated calcium solvent (Jayakumar & Tamura, 2008; Nagahama et al., 2008a; Nagahama et al., 2008b). Min et al., 2004 performed electrospinning of chitin using HFIP solvent. Before electrospinning the chitin was depolymerized by gamma radiation to improve the solubility. Although as-spun chitin nanofibers had the broad fiber diameter distribution, most of the fiber diameters are less than 100 nm. Junkasem et al. 2006 reported that nanocomposite fiber mats composed of electrospun poly(vinyl alcohol) (PVA) containing  $\alpha$ -chitin whiskers prepared from shells of *Penaeus merguensis* shrimps have been fabricated. This research was conducted since both PVA had previously been electrospun (Ding et al., 2002) in water and chitin/PVA nanocomposite films containing  $\alpha$ -chitin whiskers have also been fabricated (Sriupayo et al.,

2005a; Sriupayo et al., 2005b). A maximum tensile strength value of  $5.7 \pm 0.6$  MPa was obtained when the chitin whisker to PVA ratio was approximately 5.1%, after this point, increasing the chitin content decreased the strength of the mats.

Park et al. 2006a developed electrospun chitin/poly(glycolic acid) (PGA) blend nanofibers in HFIP was investigated to fabricate biodegradable and biomimetic nanostructured scaffolds for tissue engineering. PGA was chosen because it was a biocompatible and biodegradable polymer. The PGA/chitin blend fibers have average diameters of around 140 nm, and their diameters have a distribution in the range 50–350 nm. In vitro degradation studies, conducted in phosphate-buffered saline (PBS), pH 7.2 demonstrated that the blend fibers degraded faster than pure PGA fibers. Also, electrospun chitin/silk fibroin (SF) nanofibers in HFIP solvent was reported (Park et al., 2006b). The average diameters of chitin/SF blend fibers decreased from 920 to 340 nm, with the increase of chitin content in blend compositions. Shalumon et al., 2009 developed a novel electrospun water-soluble carboxymethyl chitin (CMC)/PVA blend was successfully prepared by electrospinning technique. The concentration of CMC (7%) with PVA (8%) was optimized, blended in different ratios (0–100%) and electrospun to get nanofibers. Fibers were made water insoluble by cross-linking with glutaraldehyde vapors followed by thermal treatment.

## 2. 2. Electrospinning of chitosan

The electrospinning process is determining the optimal and suitable solvent system. Since chitosan is soluble in most of the acids. However, the protonation of chitosan changes it into a polyelectrolyte in acidic solutions. Chitosan is the only pseudonatural cationic polymer (Kurita 2001; Rinaudo, 2006; Pillai et al., 2009) and in general there are only a few reports on ionic polymers or polyelectrolytes that have successfully been electrospun (Duan et al., 2004). It has been theorized by Min et al., 2004 that the repulsive force, between ionic groups within polymer backbone that arise due to the application of a high electric field during electrospinning, restrict the formation of continuous fibers and often produce particles. There has been some work (Mckee et al. 2004; Mckee et al. 2006) towards developing an empirical equation for fiber diameter and the effects that the intermolecular associations state of a polyelectrolyte have on electrospinning was reported.

An electrospun nonwoven fabric of chitosan, was successfully prepared (Ohkawa et al., 2004). This study focuses on the effect of the electrospinning solvent and the chitosan concentration on the morphology of the resulting nonwoven fabrics. The solvents tested were diluted hydrochloric acid, acetic acid, formic acid and trifluoroacetic acid (TFA). As the chitosan concentration was increased, the morphology of the deposition on the collector changed from spherical beads to interconnected fibrous networks. The addition of dichloromethane to the chitosan-TFA solution was improved the homogeneity of the electrospun chitosan fiber. Under optimized conditions, homogenous (not interconnected) chitosan fibers with a mean diameter of 330 nm were prepared. Ohkawa et al., 2004 proposed that TFA is the main constituent in the successful solvent system for chitosan because the amino groups of the chitosan can form salts (Hasegawa et al., 1992) with the TFA, which can effectively destroy the rigid interactions between the chitosan molecules thus facilitating electrospinning. Similarly, Haider & Park, 2009 developed electrospun chitosan nanofibers for absorbing metal ions. A follow-up study was conducted by Ohkawa et al. 2006 which focused on idealizing the viscosity of their solutions (Baumgarten et al., 1971; Fridrikh et al., 2003) in order to decrease the average fiber diameter. It was determined

that fiber diameter and polymer concentration have an inverse relationship, there was a linear increase of fiber diameter as the concentration of chitosan in solution decreased. In another study, Homayoni et al., 2009 developed electrospinning of chitosan. The problem of chitosan high viscosity, which limits its spinability, is resolved through the application of an alkali treatment which hydrolyzes chitosan chains and so decreases its their molecular weight. Solutions of the treated chitosan in aqueous 70–90% acetic acid produce nanofibers with appropriate quality and processing stability. Decreasing the acetic acid concentration in the solvent increases the mean diameter of the nanofibers. Optimum nanofibers are achieved with chitosan, which is hydrolyzed for 48 h. Such nanofibers result in a moisture regain, which is 74% greater than that of treated and untreated chitosan powder. The diameter of this nanofiber, 140 nm, is strongly affected by the electrospinning conditions as well as by the concentration of the solvent.

The electrospun various grades of chitosan and cross-linked them using glutaraldehyde vapor, utilizing an Schiff base imine functionality by Schiffman & Schauer, 2007a. Chemical, structural, and mechanical analyses have been conducted. Additionally, the solubilities of the as spun and cross-linked chitosan mats have also studied. The solubility was greatly improved after cross-linking. The as-spun medium molecular weight chitosan nanofibers have a Young's modulus of  $154.9 \pm 40.0$  MPa and display a pseudo-yield point that arose due to the transition from the pulling of a fibrous mat with high cohesive strength to the sliding and elongation of fibers. As-spun mats were highly soluble in acidic and aqueous solutions. After cross-linking, the medium molecular weight fibers increased in diameter by an average of 161 nm, have a decreased Young's modulus of  $150.8 \pm 43.6$  MPa, and were insoluble in basic, acidic, and aqueous solutions. Schiffman & Schauer, 2007b demonstrated that Schiff base cross-linked chitosan fibrous mats was produced by a one-step electrospinning process that is 25 times faster and, therefore, more economical than a previously reported two-step vapor-cross-linking method. These fibrous mats were insoluble in acidic, basic, and aqueous solutions for 72 h. This improved production method results in a decreased average fiber diameter, which measures  $128 \pm 40$  nm.

Sangsanoh & Supaphol, 2006 reported an additional method for cross-linking or neutralizing electrospun chitosan nanofiber mats. Further utilization of chitosan nanofibrous membranes that are electrospun from chitosan solutions in TFA with or without dichloromethane (DCM) as the modifying co solvent is limited by the loss of the fibrous structure as soon as the membranes are in contact with neutral or weak basic aqueous solutions due to complete dissolution of the membranes. Dissolution occurs as a result of the high solubility in these aqueous media of  $-\text{NH}_3^+\text{CF}_3\text{COO}^-$  salt residues that are formed when chitosan is dissolved in TFA. Traditional neutralization with a NaOH aqueous solution only maintained partial fibrous structure. Much improvement in the neutralization method was achieved with the saturated  $\text{Na}_2\text{CO}_3$  aqueous solution with an excess amount of  $\text{Na}_2\text{CO}_3(\text{s})$  in the solution. Sangsanoh & Supaphol, 2006 also reported that electrospun chitosan nanofibrous mats, after neutralization in the  $\text{Na}_2\text{CO}_3$  aqueous solution, could maintain its fibrous structure even after continuous submersion in phosphate buffer saline (pH = 7.4) or distilled water for 12 weeks.

Besides electrospinning chitosan in TFA, the second solvent system that has been demonstrated to effectively produce nanofibers is concentrated acetic acid. Geng et al., 2005 attempted to electrospin three kinds of demineralized and deproteinized chitosan powders. However, uniform fibers were only fabricated from 7% chitosan in 90% aqueous acetic acid

solutions. It was noted that the surface tension and charge density were the key factors in determining the spinnability of the system. In preliminary attempts to produce chitosan nanofibers for wound or alternate medical applications, or the removal of metals from solutions for environmental applications, Vrieze et al., 2007 conducted a feasibility study concerning the electrospinning of chitosan in formic, acetic, lactic and hydrochloric acids. It was determined that only the use of concentrated aq acetic acid solutions resulted in fibers. It is notable that the electrospun chitosan in acetic acid has been reported (Li & Hsieh, 2006; Sangsanoh & Supaphol, 2006). Torres-Giner et al., 2008 developed electrospun chitosan nanofibers using TFA and dichloromethane solvent. In addition, Torres-Giner et al., 2008a also developed porous electrospun chitosan nanofibers using pure trichloromethane solvent.

Recently, electrospun chitosan composite nanofibrous mats have been fabricated using synthetic biodegradable polymers such as PVA (Miya et al., 1994; Zheng et al., 2001), poly(ethylene oxide) (PEO) (Yilmaz et al., 2003), poly(vinyl pyrrolidone) (PVP) (Ignatova et al., 2007), poly(lactic acid) (PLA) (Peesan et al., 2006; Torres-Giner et al., 2008b) and poly(ethylene terephthalate) (PET) (Jung et al., 2007). These composite fiber mats have more advantageous over the electrospinning of pure chitosan. Because, the mechanical, biocompatible, antibacterial and other properties of the chitosan nanofibers was drastically enhanced by the addition of PVA, PEO, PLA, PVP and PET. Researchers have electrospun composite nanofibers to try to increase their understanding of the role that chitosan plays during electrospinning. Finally, in order for chitosan-containing non-wovens to be applicable for a variety of biomaterial applications, particular mechanical, chemical, biocompatible, and other properties are required. Each of the composite fibrous mats created contain their specific properties and the applications are widen.

PVA is used for a variety of biomedical applications such as bone implants (Allen et al., 2004; Zhang et al., 2005), and artificial organs (Chen et al., 1994). Several researchers have developed nanofibers of PVA with chitosan by electrospinning because PVA has good fiber-forming characteristics (Li & Hsieh, 2006; Zhou et al., 2006; Huang et al., 2007; Jia et al., 2007; Zhou et al., 2007). The electrospun composite chitosan/PVA nanofibers in different ratios for biomedical applications were reported (Ding et al., 2002; Koski et al., 2004; Ohkawa et al., 2004; Zhang et al., 2005; Lin et al., 2006; Zhang et al., 2007; Zhou et al., 2008). Chitosan can be used as a thickener to improve the rheological properties of an aq solution to be electrospun because chitosan is compatible with other biocompatible polymers such as PVA (Miya et al., 1994; Zheng et al., 2001), PEO (Yilmaz et al., 2003) etc. A common defect observed when electrospinning is the formation of beads, to counter this, additives such as salts (Fong et al., 1999) or surfactants (Lin et al., 2004) are added. Alternatively, the addition of cationic and anionic polyelectrolytes (Son et al., 2004) would also increase the conductivity of a solution and thus decrease fiber diameter. Since chitosan is a linear cationic polymer, it was determined that the polyelectrolyte chitosan can act like other ionic additives and does reduce fiber diameter thus producing thinner, uniform, bead free fibers was noted (Lin et al., 2006; Jia et al., 2007). It was also noted by Jia et al., 2007 that the formation of a crystalline microstructure is restricted during electrospinning because the stretched molecular chains solidify rapidly and at high elongation rates. This effect has additionally been noted elsewhere (Deitzel et al., 2001; Zong et al., 2002).

Zhou et al., 2007 developed electrospun fibers from chitosan/PVA in aqueous acrylic acid solutions and in a second article (Zhou et al., 2006) they thermally cross-linked the fibrous

mats using triethylene glycol dimethacrylate (TEGDMA) utilizing a higher concentration of an alternative acid than all of the previously mentioned researchers whom electrospun chitosan/PVA. Zhou et al., 2007 was also capable of spinning up to a 90/10 ratio of chitosan/PVA in as high as a 90% concentration of aqueous acrylic acid. To allow for utilization of the chitosan/PVA mats in a variety of applications, the amount of TEGDMA can be chosen to match the desired mechanical properties. It was found that by adding TEGDMA prior to spinning followed by heat-treating the as-spun mats for 2 hr at 80°C cross-linked fibrous mats could be fabricated.

The development of bioinspired or biomimetic materials is essential and has formed one of the most important paradigms in today's tissue engineering research. Zhang et al., 2008a reported a novel biomimetic nanocomposite nanofibers of hydroxyapatite (HAp)/chitosan was prepared by combining an in situ co-precipitation synthesis approach with an electrospinning process. A model HAp/chitosan with the HAp mass ratio of 30 wt% was also synthesized through the co-precipitation method so as to attain homogenous dispersion of the spindle-shaped HAp nanoparticles (ca. 100-30 nm) within the chitosan matrix. Similarly, biocomposite nanofibers were also prepared using of HAp with chitosan/PVA for biomedical applications (Yang et al., 2008).

PEO is also a biocompatible polymer (Griffith, 2000) that has been used as a wound dressing (Yoshii et al., 1999) and cartilage tissue repair (Sims et al., 1996; Subramanian et al., 2005). The PEO had previously been electrospun (Doshi & Renekar, 1995; Fong et al., 1999; Deitzel et al., 2001a; Deitzel et al., 2001b) with chitosan. Duan et al. 2004 electrospun chitosan/PEO based on previous research showing that PEO aided in the electrospinning of silk and collagen (Huang et al., 2001; Jin et al., 2002). The authors stated that the conductivity, surface tension, and moreover the solution viscosity is what allowed for electrospinning to occur when there was a mass ratio of PEO/chitosan of 2/1 or 1/1. Spasova et al. 2004; Duan et al. 2004; Desai et al., 2009, reported the electrospinning of chitosan/PEO system. It was observed that as the proportion of chitosan increased, the fiber diameter was decreased. Bhattarai et al., 2005 also developed electrospun chitosan with PEO. When PEO was added specifically to reduce the viscosity of solution so a higher polymer concentration within a solution would be spinable. It was found that a chitosan/PEO ratio of 9/1 was an appropriate candidate for bone tissue engineering because these mats retained good structural integrity in water and promoted good adhesion of chondrocyte and osteoblast cells. In addition, Vondran, 2007 has also studied the mechanical properties of chitosan/PEO nanofibrous mat as well as mats that were cross-linked by glutaraldehyde vapor. Chitosan/PEO nanofibers with surfactants for filtration applications was reported (Kriegel et al., 2009a; Kriegel et al., 2009b).

Zhang et al., 2008b demonstrated that the preparation of nanofibers by introducing an ultra high-molecular-weight PEO (UHMWPEO) into aqueous chitosan solution. This system leads for certain the chitosan nanofibers good structural stability and handling property during practical applications compared to previous higher PEO loadings. Because of the excellent electrospinnability of the current solution system, it was able to electrospin both the extremely thin nanofibers (<100 nm in diameters) and large microfibers (few tens of micrometers in diameters), which have significant implications in developing biomimetic and bioactive 3-D cell-scaffold complex for engineering tissues. The results suggest that current eco-friendly and easily electrospinnable chitosan formulation could provide great potential for robust and scale-up production of the chitosan nanofibers for efficient practical

applications in wound dressings, tissue engineering, drug delivery, and other industrial uses.

PET is used in the textile and plastic industry, its antibacterial properties have been studied (Huh et al., 2001; Yang et al., 2002). Jung et al., 2007 reported that the electrospinning of chitosan with PET was useful for medical applications of the fibrous mats. Chitosan/PET and chitin/PET were electrospun in a TFA/HFIP solution. Antibacterial activity experiments indicated that chitosan/PET nanofibers inhibited the growth much more effectively than both the pure PET and the chitin/PET fibrous mats. Torres-Giner et al., 2008 developed chitosan/PLA blend nanofibers by electrospinning in TFA and trichloromethane mixture.

Collagen has also been used previously for electrospinning using HFIP solvent (Matthews et al., 2002; Matthews et al., 2003; Rho et al., 2006). To develop a better biomimetic extracellular matrix for the tissue engineering of functional biomaterials, a matrix fibrous mat of chitosan/collagen was electrospun and reported (Chen et al., 2007; Chen et al., 2008). Mo et al., 2007 reported about the electrospun chitosan/collagen in HFIP/TFA. Composite nanofibrous membranes of type I collagen, chitosan, and polyethylene oxide was fabricated by electrospinning, which could be further cross-linked by glutaraldehyde vapor (Chen et al., 2008). Nanofiber diameter was found to be  $134\pm 42$  nm, which increased to  $398\pm 76$  nm after cross-linking. The Young's modulus was increased after cross-linking, however, the ultimate tensile strength, tensile strain, and water sorption capability decreased after cross-linking.

Zein is a relatively straightforward biopolymer to electrospin (Miyoshi, Toyohara, & Minematsu, 2005; Torres-Giner et al., 2008b). Additionally, zein has low toxicity and has also been studied in a broad range of areas, such as the food, pharmaceutical, and biodegradable plastics industry (Corradini et al., 2006). Torres-Giner et al., 2009 developed novel antimicrobial ultrathin structures of zein/chitosan blend by electrospinning for biomedical applications.

SF is the fibrous protein that forms the filaments of silkworm silk. Due to its biocompatibility, biodegradability, low inflammatory responses, and good oxygen and water vapor permeability, it has been used for several biomedical applications (Santin et al., 1999; Park et al., 2001). The novel biomimetic nanofibrous scaffolds were prepared using chitosan/SF in formic acid (Park et al., 2004). While a pure chitosan system could not be electrospun, up to a 30/70 chitosan/SF in formic acid could be spun. The influence of a methanol treatment on the secondary structure of as-spun SF versus chitosan/SF was also investigated.

Recently, the chitosan derivatives based nanofibers has also been prepared for biomedical applications. Carboxymethyl (Vondran, 2007), carboxyethyl (Mincheva et al., 2007) and hexanoyl chitosan (Peesan et al., 2006; Neamnark et al., 2006) derivatives were used for the preparation of nanofibers. Hexanoyl chitosan was used for medical applications since it has been proven to be resistant to hydrolysis by lysosome (Lee et al., 1995) and is anti-thrombogenic (Hirano & Noishiki, 1985). Neamnark et al., 2006, prepared hexanoyl chitosan nanofibers in chloroform solvent. The prepared nanofibers displayed ribbon-like morphology with diameters ranging from 0.64 to 3.93  $\mu\text{m}$ . O-Carboxymethyl chitosan (O-CMCS) is a water-soluble derivative of chitosan (Muzzarelli et al., 1994; Chen & Park, 2003). O-CMCS has good moisture retention, gel-forming capability, is antibacterial, and non-cytotoxic, thus making it a good biomaterial (Chen et al., 2002; Chen et al., 2006). Vondran et



al., 2007 has prepared electrospun composite fibrous mats using CMCS/PEO using water. The fibers appear to be continuous and cylindrical, while some beading was observed. The average fiber diameter was calculated to be  $118.19 \pm 40.48$  nm. Similarly, Du & Hsieh, 2008 prepared O-CMCS blended PVA, PEO and poly(acrylic acid) (PAA) nanofibers by electrospinning. The optimal fiber formation was observed at equal mass composition of O-CMCS (89 kDa at 0.36 DS) and PVA, producing nanofibers with an average diameter of 130 nm. Heat-induced esterification (at 140 °C for 30 min) produced inter-molecular covalent cross-links within and among fibers, rendering the fibrous membrane water-insoluble. Membranes containing higher O-CMCS carboxyl to PVA hydroxyl ratio retained better fiber morphology upon extended water exposure, indicating more favorable inter-molecular cross-links. The fibrous membranes generated with less substituted O-CMCS were more hydrophilic and retained a greater extent of the desirable amine functionality.

Bicomponent nanofibers of N-carboxyethyl chitosan (N-CECS) and PVA were obtained by electrospinning was studied (Mincheva et al., 2007). The electrospinning of N-CECS containing nanofibers was enabled by the ability of PVA to form an elastically deformable entanglement network based on hydrogen bonds. The average diameters of the bicomponent fibers were in the range 100–420 nm. The average fiber diameter was tuned by varying the applied field strength and the N-CECS content in the spinning solution. The N-CECS/PVA nanofibrous mats were easily cross-linked by thermal treatment resulting in water-resistant materials, which retain their fibrous structure upon one-week contact with water. In addition, a novel biocomposite nanofibers were prepared using of HAp with N-CECS/PVA for biomedical applications (Yang et al., 2008).

Novel quaternized chitosan (QCS)/PVA blend nanofibers were developed for wound-healing applications (Ignatova et al., 2006). The average fiber diameter is in the range of 60–200 nm. UV irradiation of the composite electrospun nano-fibrous mats containing triethylene glycol diacrylate as cross-linking agent had resulted in stabilising of the nanofibres against disintegration in water or water vapors. Ignatova et al., 2007 also prepared QCS-containing nanofibers by electrospinning with PVP blending. A significant decrease in the fiber diameter of electrospun QCS/PVP mixed fibers and a narrowing of the fiber diameter distribution with increasing the QCS content was observed and explained by the increase in solution conductivity. An increase of the applied field strength led to greater fiber diameters and to broader diameter distribution. The photo-cross-linked QCS/PVP fibers irradiated for 10 h proved to be water insoluble.

The galactosylated chitosan (GC) nanofibrous scaffold was fabricated by electrospinning for tissue engineering applications (Feng et al., 2009) with an average diameter of ~160 nm using formic acid solvent. Jiang et al., 2004 prepared electrospun membranes composed of ibuprofen-loaded poly(lactide-co-glycolide)/poly(ethylene glycol)-g-chitosan (PLGA)/(PEG-g-chitosan) that would be suitable for a trial fibrillation due to the electrospun membranes composed of ultrafine fibers, have high porosity, and can conform to movements (Reneker & Chun, 1996). In this work, ibuprofen was incorporated into the fibrous mats in two different methods: electrostatically conjugated during the electrospinning process and covalently conjugated to the PEG-g-chitosan prior to spinning. This tri-component system had unique properties of being soluble in organic solvents, while being insoluble in neutral pH water (Ouchi et al., 1998). It was also demonstrated that the hydrophilicity, membrane shrinkage, and rate of drug release could be controlled within the system. The fibrous mats in vitro and in vivo biocompatibility and efficacy need to be

evaluated. Duan et al., 2006; Duan et al., 2007 simultaneously electrospun from two different syringes have PLGA and chitosan/PVA onto a rotating drum. The PLGA component is expected to enhance the mechanical properties, while the chitosan provides bioactivity and biocompatibility. The fibrous mats were cross-linked with 25% aqueous glutaraldehyde vapor for 4 hr at 37 °C (Chen et al., 2003). It was determined that tri-component systems might have potential in biomedical applications.

Wan et al., 2008 prepared novel poly(chitosan-g-DL-lactic acid) (PCLA) copolymeric nanofibers using an electro-wet-spinning technique. The diameter of fibers in different scaffolds could vary from about 100 nm to around 3  $\mu$ m. The prepared nanofibrous scaffolds exhibited various pore sizes ranging from about 1  $\mu$ m to less than 30  $\mu$ m and different porosities up to 80%. Two main processing parameters, that is, the concentration of PCLA solutions and the composition proportions of coagulation solutions, were optimized for obtaining desired scaffolds with well-controlled structures. The tensile properties of the scaffolds in both dry and hydrated states were examined. Significantly improved tensile strength and modulus for these fibrous scaffolds in their hydrated state were observed.

### 3. Applications of chitin and chitosan nano-fibers in tissue-engineering

Polymeric nanofibers that mimic the structure and function of the natural extracellular matrix (ECM) are of great interest in tissue engineering as scaffolding materials to restore, maintain or improve the function of human tissues. The natural ECMs in the body are mainly composed of two classes of extracellular macromolecules: proteoglycans and fibrous proteins with fiber diameters ranging from 50 to 150 nm, depending on tissue type (Elsdale & Bard, 1972). Studies showed that the material size feature can substantially influence the morphology and function of cells grown on the ECM, and that cells attach and proliferate well in micro and nanostructured materials (Laurencin et al., 1999; Teixeira et al., 2003).

Noh et al., 2006 studied the cytocompatibility assessment of chitin nanofibers. Chitin nanofibers was found to promote cell attachment and spreading of normal human keratinocytes and fibroblasts compared to chitin microfibers. This may be a consequence of the high surface area available for cell attachment due to their three-dimensional features and high surface area-to volume ratios, which are favorable parameters for cell attachment, growth, and proliferation. Cell studies conducted on chitin/PGA (Park et al., 2006a) and chitin/SF (Park et al., 2006b) fibrous mats proved that a matrix consisting of 25% PGA or SF and 75% chitin had the best results. The chitin/PGA fibers had a bovine serum albumin coating and were considered a good candidate for use as a tissue-engineering scaffold because normal human epidermal fibroblasts (NHEF) attached and spread. The chitin/SF fibrous mats had the highest spreading of NHEF and normal human epidermal keratinocytes (NHEK). Therefore, this scaffold was suggested for wound tissue engineering applications. Shalumon et al., 2009 developed CMC/PVA blend nanofibrous scaffold for tissue engineering applications. The prepared nanofibers were bioactive and biocompatible. Cytotoxicity and cell attachment studies of the nanofibrous scaffold were evaluated using human mesenchymal stem cells (hMSCs) by the MTT assays. The cell attachment studies revealed that cells were able to attach and spread in the nanofibrous scaffolds (**Fig. 1**). These results indicate that the nanofibrous CMC/PVA scaffold supports cell adhesion/attachment and proliferation and hence these scaffolds are useful for tissue engineering applications.

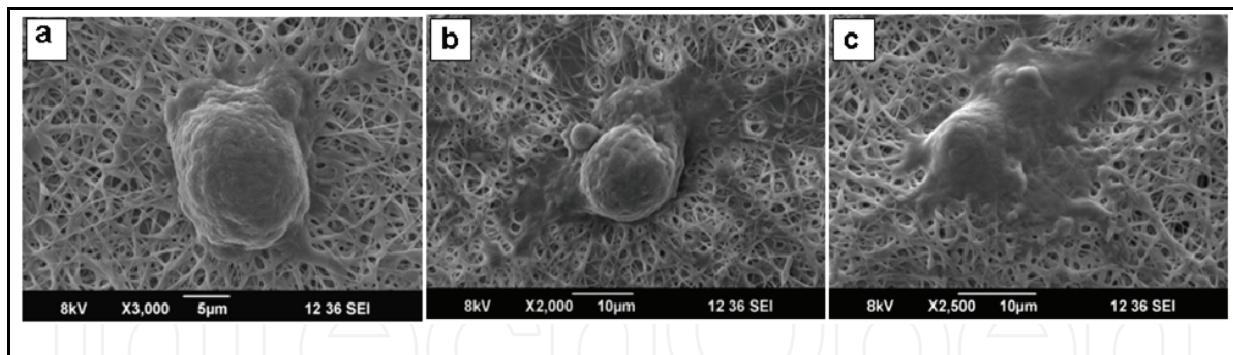


Fig. 1. SEM images of hMSCs attached on the surfaces of CMC/PVA scaffolds after (a) 12 (b) 24 and (c) 48 h of incubation.

Bhattacharai et al., 2005 reported that the chitosan/PEO nanofibrous scaffolds were promoted the attachment of human osteoblasts and chondrocytes and maintained characteristic cell morphology and viability throughout the period of study. This nanofibrous matrix is of particular interest in tissue engineering for controlled drug release and tissue remodeling. Similarly, Subramaniyan et al., 2005 prepared chitosan/PEO nanofibers for cartilage tissue engineering. In this study, chitosan/PEO nanofibers was evaluated the biocompatibility with chondrocytes. Cells were attached to the chitosan/PEO nanofiber mats slowly in the first week. After 10 days, the more cells were attached on the surface of the nanofibers. These results indicated that the electrospun chitosan/PEO mats have been used for cartilage tissue repair. Mo et al., 2007 also reported about that the smooth muscle cells attaching to the electrospun chitosan/collagen nanofibers after 30 days of culture.

The biological evaluations of chitosan/HAp nanofibrous composite scaffolds have been reported (Zhang et al., 2008a). The chitosan/HAp nanofibrous scaffolds have significantly stimulated the bone forming ability as shown by the cell proliferation, mineral deposition, and morphology observation, due to the excellent osteoconductivity of HAp compared to the control chitosan. The results obtained from this study highlight the great potential of using the chitosan/HAp nanocomposite nanofibers for bone tissue engineering applications. A biocomposite of HAp with electrospun nanofibrous scaffolds was prepared by using chitosan/PVA and *N*-CECS/PVA (Fig. 2) for tissue engineering applications (Yang et al., 2008). The cell attachment of the prepared biocomposite nanofibers was studied using mouse fibroblast (L929) cell. The cell studies showed that the L929 cell culture revealed the attachment and growth of mouse fibroblast on the surface of biocomposite scaffolds. Similarly, the potential use of the *N*-CECS/PVA electrospun fiber mats as scaffolding materials for skin regeneration was evaluated *in vitro* was using L929 (Zhou et al., 2008). Indirect cytotoxicity assessment of the fiber mats indicated that the *N*-CECS/PVA electrospun mat was nontoxic to the L929 cell. Cell culture results showed that fibrous mats were good in promoting the cell attachment and proliferation. This novel electrospun matrix would be used as potential wound dressing for skin regeneration.

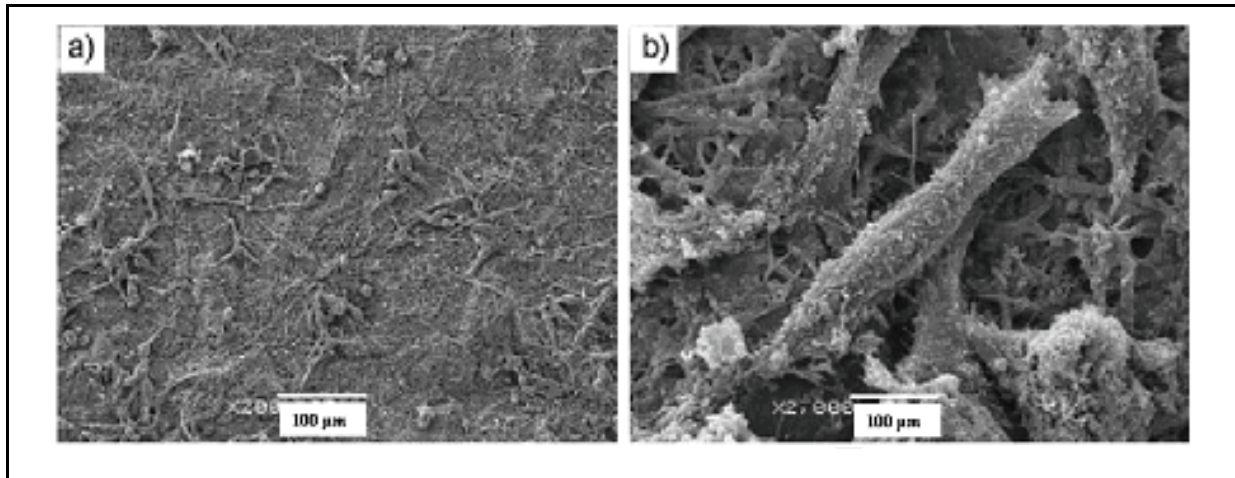


Fig. 2. SEM images of (a) and (b) L929 cells seeded on fibrous membranes of HAp-N-CECS/PVA after 48 h culture.

Liver tissue engineering requires a perfect extracellular matrix (ECM) for primary hepatocytes culture to maintain high level of liver-specific functions and desirable mechanical stability. Feng et al., 2009 developed a novel GC nanofibers with surface-galactose ligands to enhance the bioactivity and mechanical stability of primary hepatocytes in culture. The GC nanofibrous scaffolds displayed slow degradation and suitable mechanical properties as an ECM for hepatocytes according to the evaluation of disintegration and Young's modulus testing. The hepatocytes cultured on GC nanofibrous scaffold formed stably immobilized 3D flat aggregates and exhibited superior cell bioactivity with higher levels of liver-specific function maintenance in terms of albumin secretion, urea synthesis and cytochrome P-450 enzyme than 3D spheroid aggregates formed on GC films. These results suggested that the GC-based nanofibrous scaffolds could be useful for various applications such as bioartificial liver-assist devices and tissue engineering for liver regeneration as primary hepatocytes culture substrates.

#### 4. Conclusions

This review summarized the preparation and tissue engineering applications of chitin and chitosan based nanofibers. Additional studies are necessary before clinical applications and for commercialization of the chitin and chitosan based nanofibers. We hope that this review article will bring new innovative types of chitin and chitosan nanofibers for tissue engineering applications in the future.

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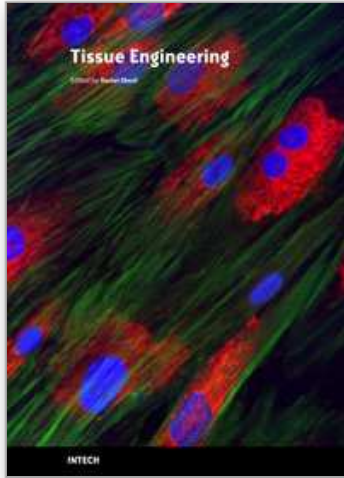
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The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient's needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

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