Development of Ultrafine Chitosan Fibers Through Modified Wetspinning Technique

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ABSTRACT: Chitosan has been extensively exploited in biomaterials research because of easy tailorable properties. Chitosan fibers are produced through either wetspinning or electrospinning. However, it is difficult to produce few microns fibers using either of these techniques. Present study focuses on production of ultrafine chitosan fibers through modified wetspinning technique by injecting homogenous chitosan solution through a very fine hole of silicone tube into either sodium tripolyphosphate (STPP) or sodium hydroxide (NaOH) bath by applying positive pressure. The gelation behavior of the chitosan was evaluated with STPP and NaOH solution through rheological study for comparative spinnability of chitosan in STPP and NaOH bath. Although gel strength of chitosan–NaOH system (240 Pa) was four times higher than that of chitosan–STPP system, gel breakdown rate was higher in previous case. From Fourier infrared spectroscopy (FTIR) analysis, ionic cross-linking between TPP and chitosan molecules in chitosan–TPP fibers was confirmed. Scanning electron micrographs showed fine chitosan fibers with average diameter of $\sim$ 10 μm. These nonwoven fibers/scaffolds with interconnected porosity may find potential biomedical applications. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 1550–1557, 2011

Key words: chitosan fiber; wetspinning; ionic cross linking; gelation kinetics; fiber diameter

INTRODUCTION

Chitosan, an amino polysaccharide, is a biodegradable biopolymer. Chemically, chitosan is [α (1-4)-2-amino-2-deoxy β-D glucan] and is a copolymer of glucosamine and *N*-acetyl glucosamine units.[1] Chitosan is an attractive fibrous biopolymer endowed with excellent biocompatibility, biodegradability, antimicrobial activity, and accelerated wound-healing potential.[2–5] This biopolymer has been extensively studied and found wide application as a biomaterial for different clinical applications like drug delivery devices,[6,7] bioactive dressings,[8] scaffolds for tissue engineering.[9,10]

Chitosan fibers are usually fabricated through wetspinning and electrospinning techniques. Melt-spinning cannot be used as chitosan decomposes prior to melting,[11] making it impossible to draw into fibers. Wetspinning is an ageold process and widely used in textile industries.[12] Interest in this technique for biomedical and pharmaceutical applications is because of its reproducibility, inexpensiveness, and environment friendly process with easy scalability. In wetspinning, usually highly viscous polymer solution is used to enhance the fiber drawing and production in short period of time.[13] Chitosan fibers can be produced in different alkaline media through pH induced coagulation, as in aqueous solution of NaOH,[14] KOH,[15] NaOH-40% methanol,[16] or NaOH-Na_2SO_4/AcONa mixture.[17] Fiber forming ability of chitosan has been investigated by several researchers,[18] and considerable efforts have also been made to improve mechanical properties.[4,19–21] Electrospinning technique is being used because of its ability to produce nanofibres. Recently, technological innovations made this process scalable for sustained industrial production of nanofibers. In this process, relatively low viscosity and loading is required in comparison to wetspinning. Chitosan nanofibers were produced either using organic solvents, which are toxic and not suitable for biomedical use or in combination with other polymers.[22,23]

It is challenging to produce fibers of few microns ($\sim$ 10 μm) diameter either by wetspinning or electrospinning technique.[13] In case of wet spinning, high drawing ratio facilitates production of fine fibers. Whereas in electrospinning, highly viscous polymer dope is required to inject through a fine needle.[24] Wetspinning is typical for production of fiber with dimension of tens of microns. For production of fibers with fidelity at the cellular dimension ($\sim$ 10

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μm),[25] it is necessary to use spinneret with very fine diameter (<25 μm). It is really difficult to inject high viscous solution through such a narrow capillary for wetspinning. So, it is extremely difficult for mass scale production of fibers with few microns diameter either by electrospinning or wetspinning. Thus, a novel approach may be necessary to produce fibers of few microns based on wetspinning technique. Further, scaffolds prepared from these fibers have inter fiber voids or porosity well beyond the range of cellular dimensions, which is very effective for cell penetration.[26] Nano/micro fibers have high surface to volume ratio, which is especially beneficial for delivery of drugs and signaling molecules through surface. Moreover, these nano/micro fibers account for enhanced cell-material interaction[27] and improved matrix production.[28]

When diameter of fibers is in the order of few microns, it is impossible to draw and collect fibers by rotary collector used in conventional wetspinning process. During collection of such fine fibers, there is a chance of coalescence or tearing of the fibers. Rather, it is preferable to collect the fibers by auto deposition instead of mechanical drawing. As the fibers spun in coagulating solution without any cross linking were unstable in acidic condition and absorb *in vivo* quickly, cross linking of chitosan fibers is an integral step toward developing matrices for medical application, which require a biodegradable, biocompatible, porous fibrous matrix that would be stable in body for the intended period. The amine group of chitosan has been explored for intermolecular crosslinking using variety of ionic and covalent crosslinkers, including diisocyanates, phosphate and phthalate ions,[21] *N*,*N*-disuccinimidyl suberate,[29] epichlorohydrin,[4] genipin,[30] hexamethylene 1,6-di(aminocarboxysulfonate),[31] and glutaraldehyde (GA).[32] In the context of wetspinning, ionic crosslinking has more significance because of their instantaneous crosslinking owing to complex formation. Shu et al. studied the effectiveness of several anions, like citrate, sulphate, tripolyphosphate (TPP) for formation and stability of chitosan micro beads through ionic complexation. The chitosan–TPP complex was relatively stable in aqueous media of varied pH with retention of the spherical shape even after drying.[33] Recently, chitosan microfiber has been fabricated using microelectromechanical systems (MEMS) technique by a microfluidic chip using sodium tri polyphosphate (STPP) as coagulant with diameters of around 50–250 μm.[34] However, a modified cost effective technique can be explored for obtaining finer fibers based on wet spinning.

Present study describes a process for production of fibers with few microns (∼ 10 μm) diameter through modified wetspinning technique. Chitosan fibers were produced by injecting low viscous solution through a very fine flexible hole of a silicone tube fitted to a syringe. The free end of the tube was immersed into a STPP coagulation bath. The low viscous chitosan solution can easily be injected through fine hole into fibers because of the instantaneous coagulation cum cross linking effect of TPP. The gelation behavior of the chitosan–TPP system was evaluated through rheological study for the first time for comparative spinnability of chitosan in STPP and NaOH bath. The gelation kinetics and gel strength of chitosan gel either with NaOH or STPP was evaluated rheologically. Gelation kinetics was evaluated through complex viscosity (η^*) measurement of the chitosan solution before and after addition of NaOH or STPP. Further, complex modulus (G^*) was measured as function of strain for gel strength evaluation. The fine chitosan fibers were characterized by Fourier transform infrared (FTIR) spectroscopy to evaluate the interaction between chitosan and TPP. Microstructure and average diameter of fibers were examined by scanning electron microscopy (SEM). Swelling behavior of the fibers was also assessed in phosphate buffered saline (PBS) under physiological condition.

MATERIALS AND METHODS

Preparation of chitosan solution

A chitosan stock solution (4 wt %) was prepared by dissolving required quantity of chitosan powder (Brookfield viscosity 800 cps, >75% deacytylated, high molecular weight, Sigma-Aldrich, Germany) in 0.5 M acetic acid by overnight stirring. Further, chitosan solutions of 0.5 and 1 wt % concentrations were prepared by diluting the stock solution with double distilled water. The homogeneous solution was filtered through a filter cloth under positive pressure, deaired by centrifugation and used for spinning of fiber.

Spinning of fiber

A very fine hole was created on a silicone rubber tube (TYGON^R, Tarsons, India) in the slightly inflated state, which makes it possible to extrude fiber into few microns diameter by injecting polymer solutions into coagulation bath through a syringe placed on a syringe pump. Fibers were extruded at different flow rates by exerting 0.2 MPa and 0.3 MPa pressure, respectively, to investigate their variation in property with flow rate (Table I). Fibers were collected in the coagulation bath containing either 1% (w/v) STPP/ethanol (pH 8.6) or 1 M NaOH/ethanol (1 : 1 volume ratio) with continuous stirring using a magnetic stirrer (Fig. 1). Optimization of coagulation bath was carried out through prior experiment.

TABLE I
Chitosan Fibers Produced in Different Coagulation Bath Using Different Pressure

Sample name[a]	Chitosan solution (wt %)	Coagulation bath	Injection pressure (MPa)	Flow rate (mL/min)
C0.5S-P2	0.5	STPP	0.2	3.5
C0.5S-P3	0.5	STPP	0.3	6.7
C1S-P2	1	STPP	0.2	2.8
C1S-P3	1	STPP	0.3	5.3
C0.5N-P2	0.5	NaOH	0.2	3.5
C0.5N-P3	0.5	NaOH	0.3	6.7
C1N-P2	1	NaOH	0.2	2.8
C1N-P3	1	NaOH	0.3	5.3

[a] "C," "S," "N," and "*P*" represent chitosan, STPP, NaOH, and pressure, respectively.

Fibers were washed thoroughly with distilled water until the rinsed water exhibited a neutral pH and incubated in 100% ethanol overnight followed by vacuum drying at room temperature. The chitosan fibers produced in 1*M* NaOH/ethanol (1 : 1 volume ratio) bath were designated as C0.5N-P2, C0.5N-P3, C1N-P2, and C1N-P3, and chitosan–TPP fibers produced in 1% (w/v) STPP/ethanol (pH 8.6) were designated as C0.5S-P2, C0.5S-P3, C1S-P2, and C1S-P3 (C, N, S, and *P* represented chitosan, NaOH, STPP, and pressure, respectively, numerical followed by "*C*" and "*P*" denote wt % of chitosan solution used for fiber production and applied pressure, respectively).

Rheological study

Viscosity of chitosan solutions was measured to evaluate flow behavior at different shear rates. Rheological study of chitosan solution was carried out to evaluate gelling behavior with 1*M* NaOH/ethanol (1 : 1) or 1% (w/v) STPP/ethanol (1 : 1) solution as coagulant at 25°C temperature using Bohlin CVO rheometer (Malvern Instrument, Malvern, UK) with a cone and plate geometry (CP 4°/40 mm diameter) maintaining a gap of 150 μm. Gelation kinetics of the chitosan solution with different coagulants as mentioned were studied with time (t) sweep complex viscosity (η^*) measurement in oscillation mode. Gel strength was evaluated with amplitude sweep measurement after formation of gel by incubating chitosan solution with coagulant for 5 min.

Further, gel strength of both the systems was evaluated at 25°C and 70–75% relative humidity using universal testing machine (Model H25KS, Hounsfield, UK) with 25-N load cell. In this experiment, 1 wt % chitosan solution was allowed to gel in petridish with either NaOH or STPP bath for 15 min. Subsequently, gel strength was measured using a 25-mm circular disk mounted on a cylindrical rod. The gels were compressed at a rate of 1 mm/min up to 70% compression and correlated with amplitude sweep measurements. A set of three samples were tested for each group.

FTIR analysis

FTIR spectra of vacuum dried chitosan, STPP powder, chitosan fiber (C1N-P2), and chitosan–TPP fiber (C1S-P2) were obtained with FTIR spectrophotometer (Model NEXUS-870, Thermo Nicolet Corporation, Madison, WI), and spectra were analyzed for relative comparison. Samples were individually mixed with approximately five times of vacuum dried KBr and pressed into pellets by hydraulic press for obtaining FTIR spectra.

Swelling study of fibers

Swelling study was carried out in PBS (pH 7.4) to assess the extent of swelling under physiological condition. Fibers were allowed to swell until reached to a saturated condition. At different time intervals (30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, and 48 h), the fibers were weighed after wiping out the surface water with a tissue paper. The percentage swelling was calculated using the following formula:

$$\% \text{ Swelling} = [(\text{Wet weight} - \text{dry weight})/\text{dry weight}] \times 100$$

Mechanical testing

The collected fibers were directly placed into 96-well tissue culture plate to shape as cylindrical fibrous scaffold for evaluation of mechanical properties. The samples were tested at 25°C and 70–75% relative humidity under compression mode in both fully dried and wet conditions using a universal testing machine (Model H25KS, Hounsfield, UK) with 100-N load cell. For wet samples, the fully dried scaffolds were allowed to swell in PBS solution overnight and mechanical test was carried out afterwards. All scaffolds were compressed up to 70% of

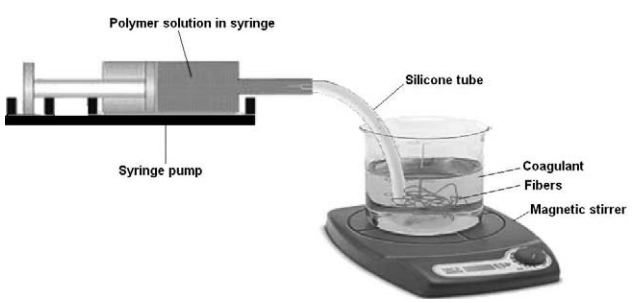

Figure 1 Schematic of modified wetspinning set up.

their initial height at a rate of 1 mm/min. Elastic modulus and compressive strength of the scaffolds were measured for each sample. A set of five samples were tested for each group.

SEM analysis

The fully dried samples were placed on sample holder using double-sided adhesive carbon tape and coated with gold using plasma coater for 30 s. The microstructures of the fibers were examined using scanning electron microscope (JSM-5800, Jeol, Japan) under vacuum. Images were taken at different magnification for evaluation of fiber diameter and analysis of scaffold architectures.

RESULTS AND DISCUSSION

The silicone rubber tube was used as a carrier and a pressure sensitive injector. In normal condition, the hole was closed because of its elastic nature and opens up to a different extent at different pressure above a threshold. During operation, two opposing forces always act against each other. As pressure increases, the solution pressure at one point overcomes the pressure associated with elasticity of silicone rubber and solution comes out through the hole. Below 0.15 MPa pressure, no fiber formation was observed as the applied pressure was not sufficient to open up the hole with a clear-cut dimension. At 0.2 MPa pressure, fiber was formed as the solution was injected through the hole to coagulation bath, and fiber can be formed easily with enhanced rate at elevated pressure up to 0.3 MPa. Beyond 0.3 MPa pressure, polymer jet was disrupted because of sudden pressure release prior to gelling of chitosan with NaOH or STPP. Thus, a pressure window does exist for production of fiber using this pressure sensitive silicone rubber tube with fine hole. This further helps in clearance of blockage from lump of coagulated polymer by upward pressure fluctuation. Chitosan fibers produced under different conditions are shown in Table I. Although very fine fibers can be produced using 0.5% chitosan solution in NaOH bath, the fibers disintegrated with mild agitation may be because of lack of crosslinking and lower polymer content. While fibers produced in STPP bath with 0.5% chitosan solution were quite stable, maintain their integrity and was used for scaffold fabrication.

Prior experiments revealed that a high concentration of crosslinker in coagulation bath would cause higher cross linked density resulting in formation of brittle fiber. The concentration of coagulant was reduced to 1% STPP for production of chitosan–TPP fiber with adequate flexibility. Chitosan solution was gelled instantaneously in contact with NaOH or STPP solution through pH sensitive gelation or ionic cross-linking, respectively. As chitosan–TPP fiber is formed by ionic complexation, the fibers may be unstable in presence of different Lewis base/anionic ligands. For evaluation of stability, chitosan–TPP complex was immersed in aqueous media (pH 7) at 25°C for 7 days and found reasonably stable because of strong ionic complexation between cationic chitosan and anionic phosphate in comparison to H_2O as ligand. Similar study by Shu et al. (2002) also revealed effectiveness of TPP in comparison to citrate and sulphate ions for stability of micro beads in aqueous media.[33] The produced chitosan–TPP fiber may find suitable biomedical application because of the mild spinning condition, non-toxic solvent, and coagulation system used.

Rheological study

Viscosities of 1 and 0.5 wt % chitosan solutions were 0.31 and 0.07 Pas, respectively, at shear rate of 50 s^{-1} showing Newtonian flow behavior at shear rates ranging from 40 s^{-1} to 100 s^{-1}. The complex viscosity (η^*) and gel strength of 1 wt % chitosan solution before and after addition of coagulants are shown in Figure 2. From amplitude and frequency sweep measurements, the strain value of 0.05 and frequency value of 0.5 Hz were obtained from the linear viscoelastic region and further used for gelation kinetics study. As gelation progresses, polymer–coagulant systems gradually deviate from linear viscoelastic region and as a result complex viscosity increases significantly with time because of increase in stiffness of the gel. Further, gel strength of the polymer–coagulant system was evaluated through amplitude sweep test by keeping the constant frequency from linear viscoelastic region.

From Figure 2(a), it is evident that the complex viscosity in both conditions started increasing instantaneously after addition of coagulant and increased significantly in 10 min time. Thus, chitosan solutions transformed into gel instantaneously in contact with 1*M* NaOH/ethanol (1 : 1) or 1% (w/v) STPP/ethanol (1 : 1), which is an important requirement for successful wetspinning. Interestingly, magnitude of complex viscosity of chitosan gel with NaOH (65 Pas) was two times higher than that of chitosan–TPP gel. This may be due to the fact that chitosan–TPP gel was formed through ionic cross linking with interpenetrating hydrated gel network structure with relatively lower strength in comparison to pH assisted phase separation/solidification in NaOH solution. Further, Figure 2(b) shows the final gel strength of both the systems through evaluation of complex modulus (G^*) against amplitude sweep measurement. The gel strength of chitosan–NaOH system (240 Pa) was four times higher than that of

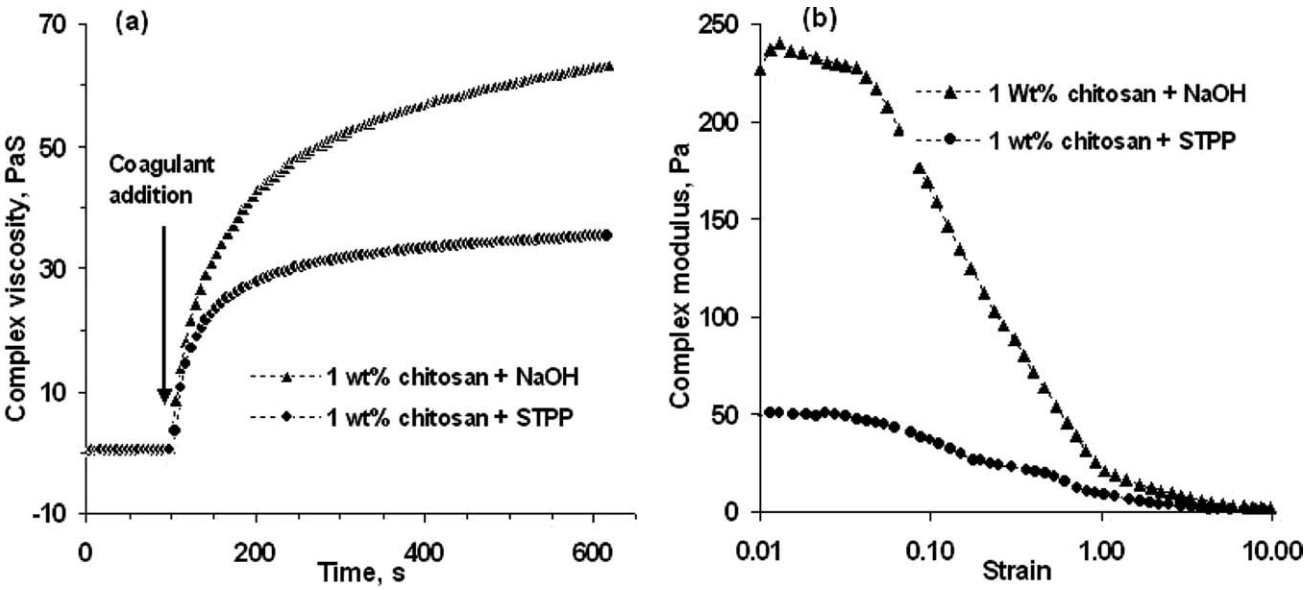

Figure 2 (a) Gelation kinetics of 1 wt % chitosan solution through complex viscosity measurement as a result of addition of 1*M* NaOH/alcohol or 1% (w/v) STPP/alcohol solution and (b) comparative gel strength of chitosan gel with 1*M* NaOH/alcohol and chitosan–TPP gel with 1% (w/v) STPP/alcohol.

the chitosan–TPP system as evident from complex modulus in Figure 2(b). During the amplitude sweep measurement, magnitude of complex modulus was almost constant up to strain of 0.03 and reduced gradually as the strain value increases because of breaking of the gel network. It is clear from the graph that the breakdown rate was much higher with chitosan–NaOH gel than that of chitosan–TPP gel. Both the gels completely broke down above strain value of 1 as magnitude of complex modulus dropped down to zero [Fig. 2(b)]. Further, gel strength of chitosan–NaOH and chitosan–TPP systems was found to be 14.7 kPa and 13.4 kPa, respectively, from mechanical testing under compression mode, which had resemblance to some extent with amplitude sweep gel strength measurement.

Ionic cross linking of chitosan–TPP system occurs from outward to inward of the polymer solution jet with formation of an immobilized skin through higher cross linked density at the surface. Diffusion of TPP slows down further through the immobilized skin, and as a result, cross linking continues for extended period of time. When fibers are removed instantaneously, some amount of chitosan at the central area may still remain uncross linked. This can be assured by formation of brittle chitosan–TPP fiber when fibers are left for extended period of time in the STPP bath. But pH assisted phase transformation is achieved through faster H^+ ion exchange by proton jump mechanism with protonated chitosan molecules in NaOH bath. As a result, all neutral polymer chains self assembled into fiber through intra- and inter-molecular H-bonding. Thus, the gel strength of both the systems is justified. As far as fiber integrity is concerned, chitosan–TPP fibers were superior to that of chitosan fiber from NaOH bath because of relatively stronger cross linked skin formation with STPP.

FTIR analysis

FTIR spectra of chitosan powder, STPP powder, chitosan fiber (C1N-P2), and chitosan–TPP fiber (C1S-P2) are shown in Figure 3. FTIR analysis of chitosan powder and fiber revealed two characteristic absorption bands at 1660 cm^{-1} and 1573 cm^{-1} attributed to amide I (C═O) and amide II (N—H), respectively; 1377 cm^{-1} attributed to the distorting vibration of C—C bond. Absorption band at 2921 cm^{-1} in

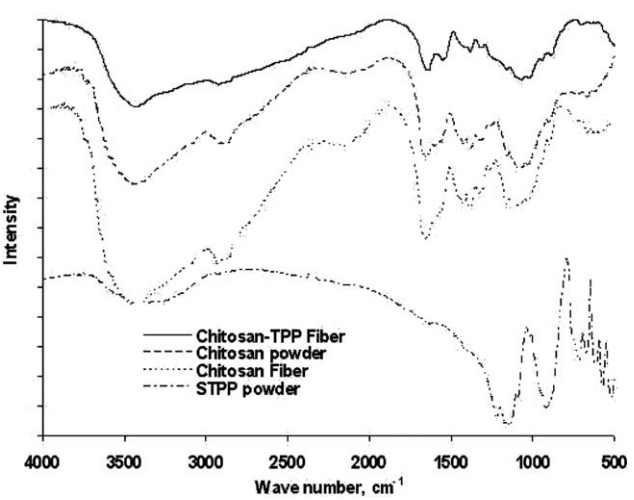

Figure 3 FTIR spectra of chitosan powder, STPP powder, chitosan fiber, and chitosan–TPP fiber.

chitosan powder, chitosan fiber, and chitosan–TPP fiber attributed to asymmetrical stretching of —CH_2—. The wide absorption band around 3435 cm^{-1} was due to the stretching vibration of O—H and N—H, present in chitosan powder, chitosan fiber (C1N-P2), and chitosan–TPP fiber (C1S-P2). But, the peak at 3435 cm^{-1} becomes wider in chitosan–TPP fibers (C1S-P2), indicating enhanced hydrogen bonding. From comparison of FTIR spectra between chitosan powder and chitosan–TPP fiber (C1S-P2), it can be inferred that the characteristic absorption bands at 1660 cm^{-1} and 1573 cm^{-1} of chitosan are shifted to lower wave number at 1641 cm^{-1} and 1552 cm^{-1}, respectively, indicating interaction between NH_2 groups of chitosan molecule and TPP in the fibers. Absorption band at 1067 cm^{-1} of chitosan–TPP fibers is attributed to the presence of phosphate group in the fibers. The peak at 1218 cm^{-1} indicates P═O stretching appeared for STPP powder, which disappeared for the chitosan–TPP fiber may be because of hydrogen bonding between chitosan and phosphate group. The FTIR spectrum is consistent with the result of chitosan fiber modified by phosphate and is attributed to the linkage between phosphoric and ammonium ion.[29] So, it is confirmed that the tripolyphosphates are linked with ammonium groups of chitosan in chitosan–TPP fibers.

Swelling study

Swelling behavior of chitosan fibers (C1N-P2 and C1N-P3) and chitosan–TPP fibers (C0.5S-P2, C0.5S-P3, C1S-P2, and C1S-P3) is shown in Figure 4. The fibers showed a steady state of swelling in 48 h of soaking period without any disintegration or dissolution in the medium and preserved their physical integrity. From the graph, it is evident that the fibers showed a rapid swelling in first 30 min because of rapid diffusion of water molecules into the glassy region of fiber in a more or less well-defined front, then they gradually attained equilibrium in about 12 h and maintained equilibrium swelling state up to 48 h study period. Chitosan fibers (C1N-P2 and C1N-P3) showed higher swelling than that of chitosan–TPP fiber (C0.5S-P2, C0.5S-P3, C1S-P2, and C1S-P3) because of relatively strong ionic cross linking of chitosan with TPP in chitosan–TPP fibers. Fiber produced using 1 wt % chitosan (C1S-P2 and C1S-P3) showed more swelling than the fiber spun using 0.5 wt % chitosan (C0.5S-P2 and C0.5S-P3), which might be due to higher polymer/cross linking ratio. During swelling, water molecules permeate into fibers with expansion in volume through three dimensional stretching of molecular chain network. On the other hand, cross linked networks of fibers produce moderate stress to make the whole network shrink.

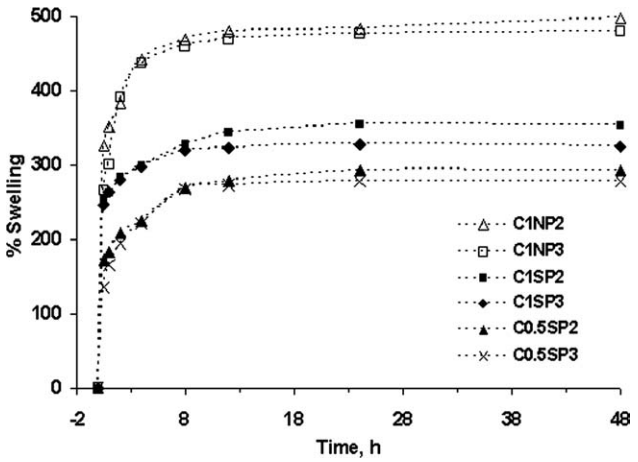

Figure 4 Percentage swelling of chitosan fibers and chitosan–TPP fibers in PBS.

When the two opposite forces balance each other, the swelling equilibrium is achieved.

Mechanical testing

Mechanical properties, in terms of elastic modulus and compressive strength, of the chitosan fibrous scaffolds are shown in Figure 5. The fibers produced in NaOH bath were inseparable visually after drying, as evidenced from SEM microscopy, and were excluded from mechanical characterization. Both elastic modulus and compressive strength of the scaffolds have been reduced in wet condition (100–300 kPa) extensively from dried condition (1000–2500 kPa) because of swelling of fibers more than three times as evidenced from swelling study. The mechanical properties were higher in 1 wt % chitosan scaffold (C1S-P2 and C1S-P3) than that of 0.5 wt % chitosan scaffold (C0.5S-P2 and C0.5S-P3), which may be due to higher polymer content. Interestingly, scaffolds were not disintegrated/crushed even after 70% compression during mechanical testing and were highly flexible in nature.

SEM analysis

SEM micrographs of the chitosan fibers are shown in Figure 6. All the fibers produced using this technique have average diameters in the range of 10–20 μm. Fibers produced in STPP bath under 0.2 MPa pressure (C0.5S-P2 and C1S-P2) were smooth and uniform in appearance [Fig. 6(a,c,e)], while that of 0.3 MPa (C0.5S-P3 and C1S-P3) were finer but quite non-uniform along the length [Fig. 6(b,d,f,g)]. At 0.3 MPa pressure, the flow rate was non-uniform may be because of excessive stretching of the elastic silicone tube causing variable hole diameters with time. Beyond 0.3 MPa, the fibers were disrupted because of sudden release of high pressure. Fiber produced

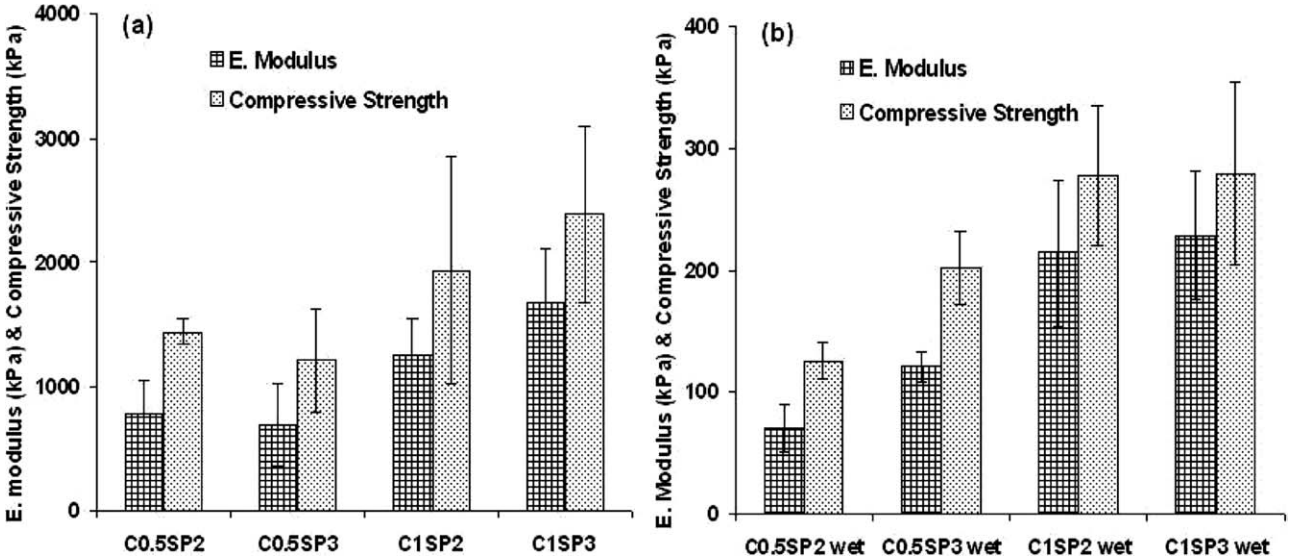

Figure 5 Elastic modulus and compressive strength of the chitosan scaffolds in (a) dry and (b) wet condition.

using 0.5 wt % chitosan solution (C0.5S-P2 and C0.5S-P3,) had average diameter of $\sim$ 10 μm while that of 1 wt % solution (C1S-P2 and C1S-P3) produced $\sim$ 20 μm. From the SEM micrograph, chitosan–TPP fibers seem to have joined with each other at the contact area, but they maintained their integrity during swelling. Although, chitosan fibers produced in NaOH bath (C1N-P2) were separable initially, but they coalesced together during drying and formed crumbled sheet [Fig. 6(h)]. It is to be noted that much finer fibers could be produced from 0.5 wt % polymer solution using NaOH bath, but after a while they couldn't retain shape and disintegrated easily even with small displacement because of lack of strength and less polymer loading as well. So, STPP may be considered as better coagulant/cross linker, as also evidenced from rheological study, for production of fine chitosan fiber using this technique.

Thus using this modified technique, nonaligned fibrous scaffold with high interconnected porosity and micro architecture can be effectively produced with fibers as fine as 10 μm, which is closer to the cellular dimension. Because of the finer dimensions, they have high surface to volume ratio, which helps in enhanced cell-material interactions and delivery of substances (drugs, signaling molecules, etc.) through the surfaces for tissue engineering and absorption of large amount of exudates for application in wound dressing. Further, this technique holds promise of easy scalability because of its flexibility of making multiple holes in a tube, which acts like a pressure sensitive spinneret.

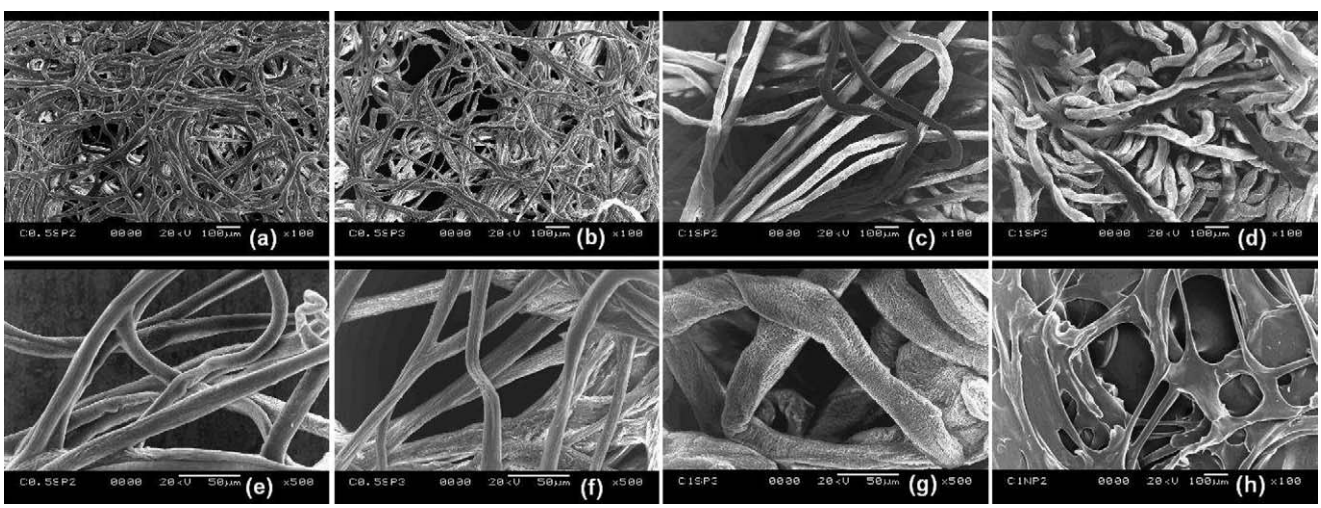

Figure 6 SEM micrographs of chitosan and chitosan–TPP fiber (a) C0.5S-P2, (b) C0.5S-P3, (c) C1S-P2, (d) C1S-P3 (×100 magnification), (e) C0.5 S-P2, (f) C0.5 S-P3, (g) C1S-P3 (×500 magnification), and (h) C1N-P2 (×100 magnification).

Thus, the production time for scaffolds can be effectively reduced.

CONCLUSIONS

Chitosan fibers were successfully produced using 1% (w/v) STPP/ethanol as coagulant cum cross linking agent through a pressure sensitive fine hole of silicone rubber tube by wetspinning route. However, chitosan fibers produced in 1 M NaOH/ethanol bath were distinctly separated initially, but disrupted with little agitation may be because of lack of crosslinking. Chitosan solution transformed into gel instantaneously in contact with either STPP or NaOH as evidenced in the rheological study, which was prerequisite for wetspinning. Though gel-strength of chitosan–NaOH system (240 Pa) was four times higher than that of chitosan–STPP system, the latter was superior in retaining its shape after drying. From FTIR analysis, cross linking between ammonium ions in chitosan and TPP in chitosan–TPP fibers was confirmed. Chitosan–TPP fibers (three times of dry weight) have shown lower swelling than that of chitosan fibers (five times of dry weight) from NaOH bath because of presence of strong ionic cross linking. Chitosan fibers as fine as 10 μm was produced through this technique as observed in SEM micrographs. The scaffolds made of fibers have compressive strength in the order of 280 kPa and 2400 kPa for wet and dry conditions, respectively, with 1 wt % chitosan solution. This novel and simple technique may be suitable for cost effective mass scale production of fine fibers of few microns diameter through a tube containing multiple holes, which acts like a pressure sensitive spinneret. Further, these fibers/scaffolds may be explored for their suitability in tissue engineering and wound dressing through biocompatibility study in an appropriate *in vitro/in vivo* model.

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