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Single step electrospinning of chitosan/poly(caprolactone) nanofibers using formic acid/acetone solvent mixture

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ABSTRACT

A fibrous scaffold comprising of chitosan (CS) and poly(ε-caprolactone) (PCL) was electrospun from a novel solvent mixture consisting of formic acid and acetone. CS concentration was varied from 0.5% to 2% by fixing PCL concentration as a constant (6%). Selected CS concentration (1%) was further blended with 4–10% PCL to obtain fine nanofibers. The composition of mixing was selected as 25:75 (1:3), 50:50 (1:1) and 75:25 (3:1) of CS and PCL. Lower concentrations of PCL resulted in beaded fibers where as 8% and 10% of PCL in lower compositions of chitosan resulted in fine nanofibers. Viscosity and conductivity measurements revealed the optimum values for the spinnability of the polymer solutions. Optimized combination of CS and PCL (1% CS and 8% PCL) in 1:3 compositions was further characterized using SEM, FTIR, AFM and TG–DTA. The developed electrospun CS/PCL scaffold would be an excellent matrix for biomedical applications.

1. Introduction

Electrospinning (Li & Xia, 2004; Reneker & Chun, 1996) has been used as an effective method to fabricate biomimetic non-woven scaffolds that are comprised of a large network of interconnected fibers and pores. A number of processing methods, such as drawing (Jayaraman, Kotaki, Zhang, Mo, & Ramakrishna, 2004), self assembly (Hartgerink, Beniash, & Stupp, 2002), template directed synthesis (Hulteen, Chen, Chambliss, & Martin, 1997), phase separation (Ma & Zhang, 1999), and electrospinning are already developed to fabricate micro or nanoscale fibrous scaffolds (Lu et al., 2008). Electrospinning, a suitable technique for the production of small-diameter fibers, was developed in the first half of the 20th century (Formhals, 1934), but recently Reneker and coworkers have investigated the process in more detail demonstrating its versatility (Reneker & Chun, 1996). To date, electro spinning has been used for the fabrication of scaffolds from numerous biodegradable polymers, such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycoside) (PLGA) and polyurethane (PU). In addition, natural proteins have also been used as well including collagen, elastin and gelatin (Bhattarai, Edmondson, Veiseh, Matsen, & Zhang, 2005; Khil, Kim, Kim, Park, & Lee, 2004). Recently there is seen a growing interest in the production of scaffolds using natural polymers like chitin, (Shalumon et al., 2009) chitosan, (Sajeev, Anoop, Deepthy, & Nair, 2008) alginate, etc. due to their non-toxicity, enhanced biocompatibility, cell adhesion and proliferation. Since natural polymers have certain limitations like low stability, release of toxic degradation products that can be harmful to cells, natural polymers are often blended with synthetic polymers (Yingshan et al., 2008; Ying Wun, Xiaoying, & Siqin, 2008) in order to enhance the mechanical properties, degradation stability and enhanced affinity to cellular components. For electrospun nanofibrous scaffolds in biomedical applications, like drug delivery, wound dressing, tissue engineering, (Binulal et al., 2009; Jayakumar, Prabaharan, Nair, & Tamura, 2010; Khil et al., 2004; Xu, Inai, Kotaki, & Ramakrishna, 2004) its physical and biological properties are largely determined by the materials' chemical compositions. Based on polymer physics, copolymerization and polymer blending are two effective means to combine different polymers to yield new materials properties. Thus, by selecting a combination of proper components and by adjusting the component ratio, properties of electrospun scaffolds can be tailored with desired new functions.

In this work, we fabricated nanofibrous scaffolds using the synthetic polymer PCL and a natural polymer CS. Since the PCL is hydrophobic and chitosan is hydrophilic, it is difficult to obtain a single solvent for both. The amino groups and hydroxyl groups in its backbone not only render chitosan itself quite hydrophilic, but also make chitosan weakly basic. CS is a linear biopolymer and is usually obtained by alkaline deacetylation of chitin. It has been widely used in different biomedical areas because of its various advantages like biocompatibility (Jayakumar, Nwe, Tokura, & Tamura, 2007;

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Jayakumar, Prabaharan, Reis, & Mano, 2005; Rinaudo, 2006) bio degradability, hydrophilicity, non-antigenicity, non-toxicity, antimicrobial activity, bio adherence and cell affinity, which make chitosan the ideal candidate for uses in a wide range of applications (Berger et al., 2004; Chirkov, 2002; Dodane & Vilivalam, 1998; Kumar, 2000; Subbiah, Bhat, Tock, Parameswaran, & Ramkumar, 2005; Vartiainen et al., 2004). Many attempts have also been made to electrospin chitosan and its derivatives (Shalumon et al., 2009) into nanofibers from the traditional dilute aqueous acetic acid solvent (Bhattarai, Edmondson, Veiseh, Matsen, & Zhang, 2005; Duan, Dong, Yuan, & Yao, 2004; Jia et al., 2007; Li & Hsieh, 2006; Ohkawa, Cha, Kim, Nishida, & Yamamoto, 2004), which can reduce toxicity and enhance biocompatibility of the nanofibrous membranes for biomedical applications. For the latter, because of the inherent poor processability of the CS itself, fiber-forming facilitating additives such as poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA) were typically employed to improve the electrospinnability of chitosan (Jia et al., 2007; Jian-Wei, Yong, Zhao, Ping, & Jian, 2006).

The fabrication of nanofibrous scaffold of CS/PCL with solvent systems trifluoro acetic acid (TFA) (Prabhakaran et al., 2008) and hexafluoro-2-propanol (HFIP) (Yang, Chen, & Wang, 2009) has been reported. But these solvents are relatively costlier than conventional solvent systems and their drastic nature may leads to faster degradation of the polymers. Moreover, in the other hand, conventional solvent of CS in acetic acid needs a binder. Recently, formic acid/acetone mixture was found as a good solvent for mutual dissolution of PCL and CS (Vera, Sofia, Natalia, & Joao, 2009) and we were successful to obtain nanofibers through single-step electro spinning from the same solvent system. The delayed degradation rate and increased mechanical strength of PCL can be made use of to improve the strength of the CS scaffolds.

2. Materials and methods

2.1. Materials

Poly(caprolactone) (PCL) ($M_W \sim 43{,}000$–$50{,}000$) was purchased from Polysciences Inc., USA. Chitosan (degree of deacetylation 80–85%, M_W: 100–150 kDa) was purchased from Koyo chemicals Co. Ltd., Japan. Formic acid and acetone purchased from RFCL Ltd. and Qualigens Fine Chemicals Ltd., India, respectively. Syringes and needles were purchased from BD Sciences Ltd., Spain. All chemicals were used as received.

2.2. Preparation of CS/PCL solutions

CS solutions of 0.5%, 1% and 2% were prepared by dissolving 0.5, 1 and 2 g of chitosan powder in 100 ml of formic acid/acetone mixture (70:30). Since the solvent mixture is the common solvent for both the polymers, CS/PCL solutions with PCL concentration 6% were prepared by dissolving 6 g of PCL in 100 ml of different CS solutions at room temperature. All the polymers were mixed well for 2 h. The maximum possible percentage of CS for electrospinning was selected by blending different concentrations of CS and PCL in three different ratios (1:3, 1:1 and 3:1). Similarly, CS/PCL solutions with varying PCL concentrations like 4%, 6%, 8% and 10% were prepared by dissolving 4, 6, 8 and 10 g of PCL in 100 ml of formic acid/acetone mixture containing 1% CS in different mixing compositions (1:3, 1:1 and 3:1). The blending compositions for electrospinning solutions are shown in Tables 1 and 2.

2.3. Electrospinning of CS/PCL solutions

All the concentrations of CS/PCL were electrospun at room temperature. Electrospinning setup includes a high voltage DC power supply and a multi terminal distribution box (Model RR30P, 0–30 kV, Gamma High Voltage Inc., USA). The polymer solutions were delivered from a syringe pump KDS 220, (KD Scientific Inc., USA) fitted with 10 ml syringes having blunt ended metal needles of 21 gauges. A distance of 8 cm was maintained between the aluminium target and spinneret. All the sample solutions were spun at a rate of 0.5 ml/h to a grounded aluminium target. The aluminium targets were fixed on a wooden stand from the tip of the needle by applying a voltage of 10 kV.

2.4. Characterizations

The viscosity of the electrospinning solutions was measured using Brookfield viscometer (Model DV-II + Pro). All the electrospinning solutions were kept at 22 °C to obtain uniform temperature and from which 10 ml of each well-mixed solutions were taken in the built-in stainless steel container of the viscometer. Measurements were done at the same temperature using S32 spindle at an RPM of 10. For conductivity measurements, 10 ml of each solution was taken in a plastic container and conductivity was measured using EUTECH COND 610 conductivity meter at 22 °C.

Table 1
Variation in viscosity and conductivity with CS concentrations 0.5–2% and PCL (6%) at different compositions of CS and PCL.

Polymer (in formic acid/acetone)		Viscosity (cP) at 22 °C			Conductivity (mS) at 22 °C		
CS (wt.%)	PCL (wt.%)	Blending composition (CS:PCL)			Blending composition (CS:PCL)		
		1:3	1:1	3:1	1:3	1:1	3:1
0.5	6	64	129	218	0.922	1.147	1.473
1.0	6	147	553	1515	1.067	1.592	2.240
2.0	6	927	7063	52,009	1.410	2.441	3.614

Table 2
Variation in viscosity and conductivity with PCL concentrations 4–10% and CS (1%) at different compositions of CS and PCL.

Polymer (in formic acid/acetone)		Viscosity (cP) at 22 °C			Conductivity (mS) at 22 °C		
CS (wt.%)	PCL (wt.%)	Blending composition (CS:PCL)			Blending composition (CS:PCL)		
		1:3	1:1	3:1	1:3	1:1	3:1
1.0	4	83	479	1485	1.324	1.694	2.275
1.0	6	147	553	1515	1.067	1.592	2.240
1.0	8	187	615	1590	1.001	1.472	2.068
1.0	10	210	649	2178	0.818	1.381	1.977

The morphological evaluation of the as-spun fibers for all the combinations of PCL and CS was done using a JEOL SEM model JSM 6490 LA. Samples on the aluminium foils were cut into small sizes and sputter coated with platinum prior to SEM imaging using a JEOL auto fine coater (model JFC-1600) at 10 mA for 120 s.

Atomic force microscopic evaluation for the surface topography of the scaffolds was performed using JEOL, JSPM 5200 with Silicon Nitride SPM probes having a spring constant of 4.5 N/M. All the fibers were electrospun into a flat mica sheet of 1 cm^2 area and were analyzed for the surface topography.

For confirming the presence of CS and PCL in the PCL/CS electrospun scaffold, spectroscopic evaluation of PCL, CS and CS/PCL samples were carried out separately by Infrared Spectroscopy using a FTIR (Perkin-Elmer Spectrum RX1) in the range between 4000 and 400 cm^{-1}, with a resolution of 2 cm^{-1}.

Thermal stability of the electrospun scaffolds was examined by differential thermal analysis (DTA)–thermogravimetric (TGA) experiments using SII model EXTAR TG/DTA 6200. All the samples were pre weighed and allowed to undergo programmed heating in the temperature range of 23–400 °C at a rate of 10 °C /min.

3. Results and discussion

3.1. Viscosity and conductivity measurements

In viscosity measurements, when CS concentration increases from 0.5% to 2%, by fixing the PCL concentration as 6%, viscosity of the solution was found to be increasing significantly. For 0.5% of CS, lower composition of CS with PCL (1:3) give comparatively very low viscosity. But as the composition ratio of CS to PCL increases to the higher values like 3:1, viscosity also doubled to that of lower composition. Similar behavior was observed for 1% and 2% of CS. In this case, increase in viscosity for the higher CS composition (3:1) with respect to lower CS content (1:3) was nearly 10.3 times and 56 times higher, respectively, for 1% and 2% of CS. Consequently, in all cases, except for 2% CS, 1:3 composition of CS/PCL system was found to be more suitable for electrospinning. The viscosity and conductivity data of different CS/PCL compositions is shown in Table 1. Similar behavior was observed in conductivity of all the CS/PCL combinations. For 0.5% of CS, 1:3 composition gives lower conductivity but 3:1 combination it was higher (1.6 times). For the higher CS concentrations, like 1% and 2%, the conductivity for 3:1 combinations was 2.1 and 2.6 times higher than that of 1:3 combination. When the concentration of CS was fixed as 1% and PCL was changed from 4% to 10%, an increase in trend was observed for viscosity but opposite behavior was obtained for conductivity (Table 2). The increase in viscosity and reduction in conductivity of the solutions are attributed to the concentration of PCL. As the PCL composition increases, ionic concentration of the solution decreases, which in turn reduces the conductivity. Even higher conductivity is preferable for electrospinning, the possible surface tension of higher concentrations of CS polymer solutions might prevent the formation of fibers. From all the results, it is logical that lower concentrations and compositions of CS are more suitable for electrospinning than higher ones.

3.2. Characterization of CS/PCL

3.2.1. SEM studies

The SEM images of all different compositions of CS/PCL are shown in Fig. 1. In the optimization, PCL concentration was fixed as 6% and CS concentration was varied from 0.5% to 2%

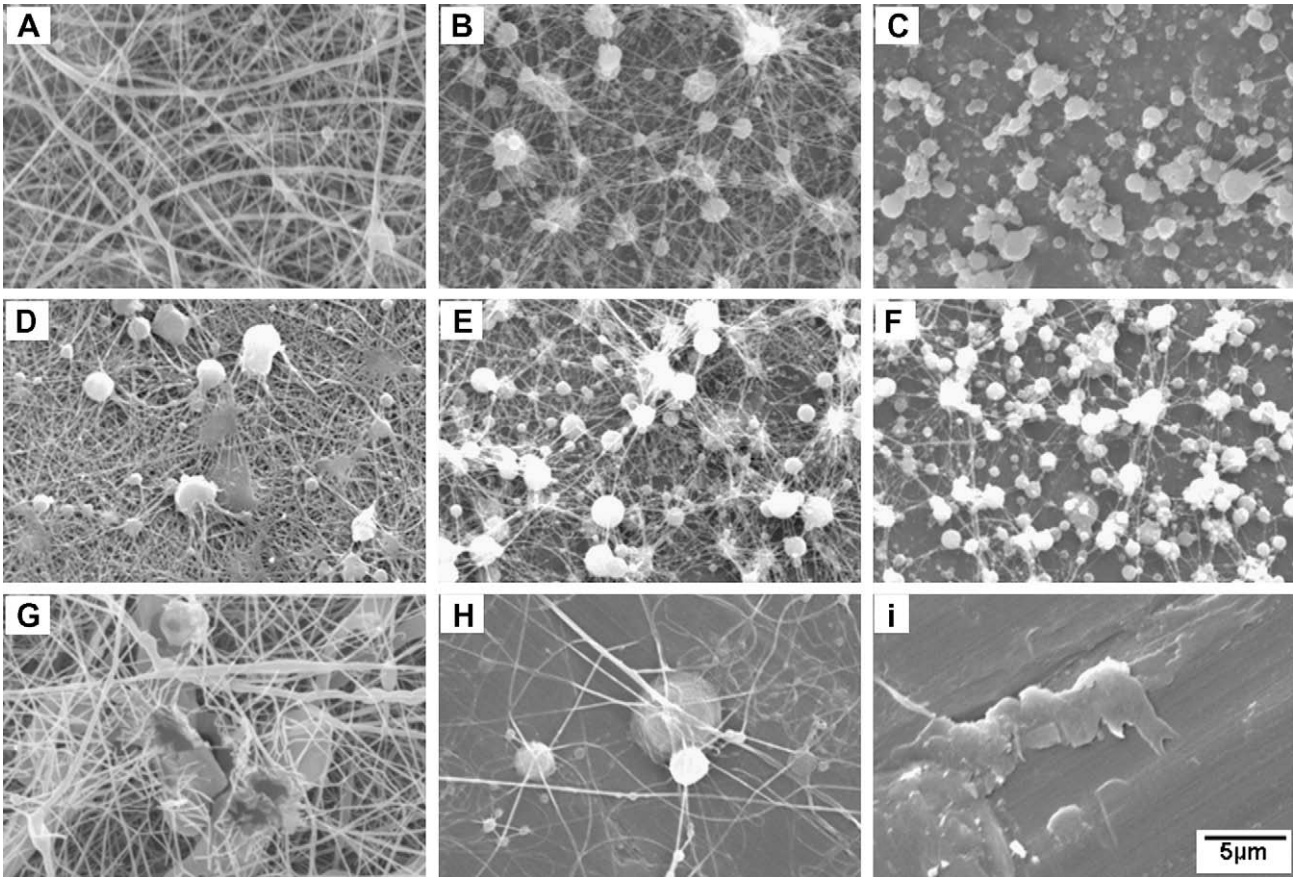

Fig. 1. SEM images of electrospun fibers of 0.5–2% of CS with 6% PCL in different compositions: (A–C) CS (0.5%)/PCL = 1:3, 1:1 and 3:1 (D–F) CS (1%)/PCL = 1:3, 1:1 and 3:1 (G–I) CS (2%)/PCL = 1:3, 1:1 and 3:1. (Scale 5 μm.)

(Fig. 1A–I). In 0.5% concentration of CS, three different compositions of CS/PCL (1:3, 1:1 and 3:1) were electrospun to get nanofibers. In 1:3 compositions, fine nanofibers with non-uniform morphology were obtained (Fig. 1A). But as the CS composition increased to 1:1 and 3:1, (Fig. 1B and C) fibers with higher density of beads were obtained. When CS concentration was increased to 1% and 2%, morphology of the fibers changed to beaded structure. The SEM images of 1% and 2% CS compositions are shown in (Fig. 1D–F) and (Fig. 1G–I), respectively. It is noticeable that, at higher concentration and compositions of CS, electrospinning was difficult and the fiber formation ability of the polymer solution was very poor.

To obtain fine nanofiber structure from beaded to non-beaded morphology, CS concentration was fixed as 1% and the PCL concentration was varied from 4% to 10%. To elucidate the effect of PCL concentration on the fiber formation ability of the electrospun scaffolds, CS was mixed with PCL in three different compositions. All the CS/PCL systems in 3:1 combinations resulted in beaded or irregular fiber geometries. When PCL concentration was 4%, both 1:3 and 1:1 compositions of CS/PCL gives beaded fibrous structure (Fig. 2A1 and B1). When PCL concentration was increased to 6%, similar result was obtained for all the combinations of CS and PCL (Fig. 2C1 and D1). This also implies that the composition of PCL is not much enough for the formation fibers. In contrast, when the PCL concentration was raised to 8%, a drastic difference in fiber morphology was observed. For 1:3, and 1:1 combinations, fiber morphology was uniform with average fiber diameters 102 ± 24 and 116 ± 27 nm, respectively (Fig. 2E1 and F1). Similar kind of result was obtained for 10% of PCL as well (Fig. 2G1 and H1). But a negligible difference in fiber morphology with very little bead density was observed for the 1:1 combination of both 8% and 10% PCL. In 10% PCL, average fiber diameter was 136 ± 32 and 147 ± 30 nm, respectively, for 1:3 and 1:1 combinations. It clearly reveals the effect of PCL concentration on the fiber diameter of the electrospun scaffold. From these results, 8% PCL and 1% CS was selected as the better combination for the fabrication of suitable scaffold. Fig. 2A2, C2, E2 and G2 are the corresponding higher magnification images of CS (1%): PCL system with PCL concentration 4–10% and mixing composition 1:3. Similarly, Fig. 2B2, D2, F2 and H2 are the higher magnification images of the same system with mixing compositions 1:1. From all the results, it is clear that, both 1:1 and 1:3 combinations of CS and PCL for 1% CS and 8% PCL are very suitable for making fine nanofibers. Fiber diameter distributions of both 8% and 10% PCL with 1% of CS is shown in Fig. 3.

3.2.2. AFM studies

Surface topography of the electrospun scaffolds was analyzed by AFM and is shown in Fig. 4. It shows the morphology of the electrospun scaffold of 1% CS and 8% PCL in 1:3 combination and Fig. 4B is the representative single fiber image. When the composition of chitosan was increased to 1:1, scaffold geometry was varied a little, in which small concentration of beads were present (Fig. 4C and D). The presence of this negligible amount of beads in the 1:1 composition of the optimized concentration is due to the increased concentration of CS, which may retard the fiber formation ability of the polymer solution by preventing the initiation of taylor cone.

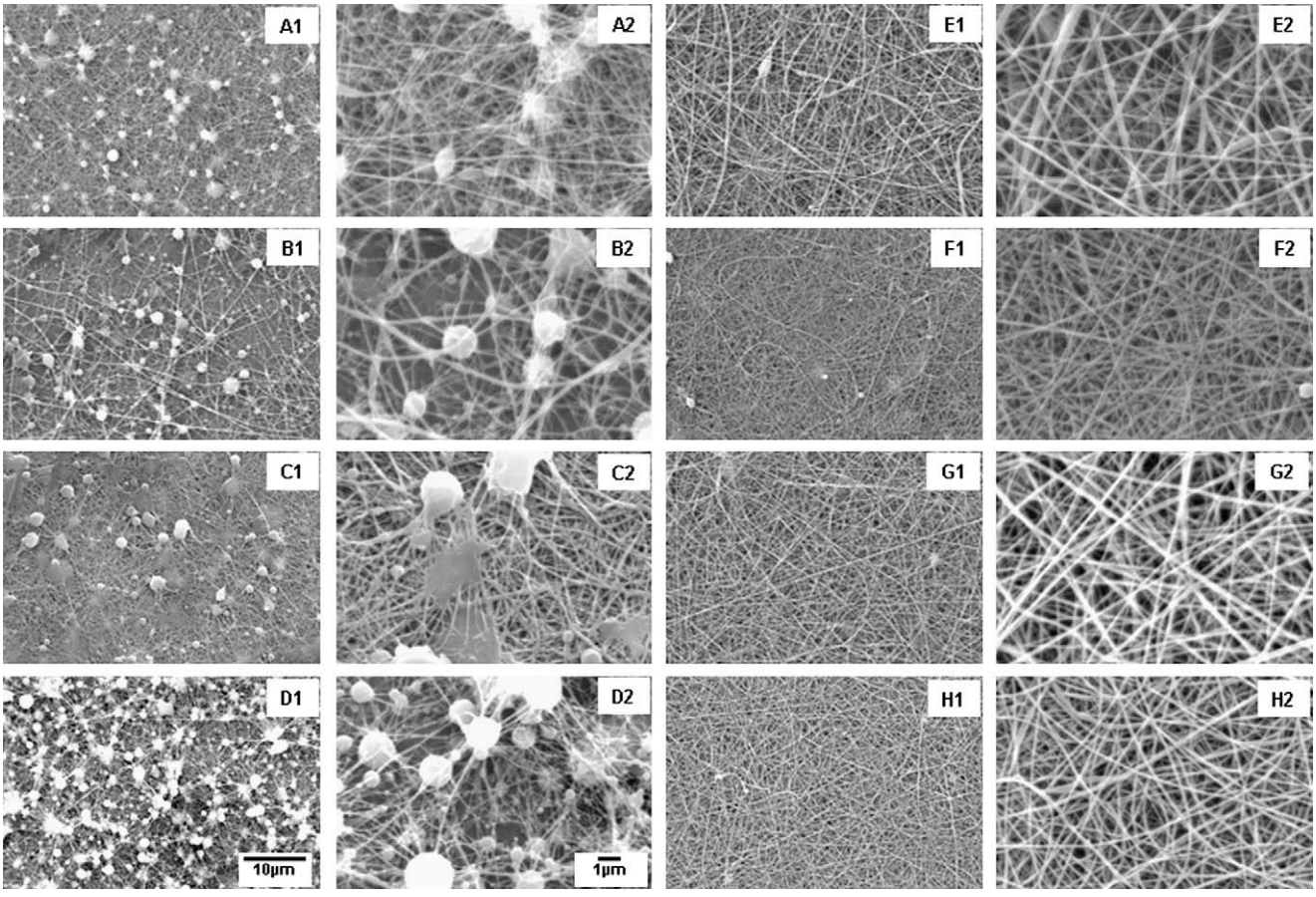

Fig. 2. SEM images of electrospun fibers of 1% CS with 4–10% PCL in different compositions: (A1 and B1) CS/PCL (4%) = 1:3 and 1:1, (C1 and D1) CS/PCL (6%) = 1:3 and 1:1, (E1 and F1) CS/PCL (8%) = 1:3 and 1:1 (G1 and H1) CS/PCL (10%) = 1:3 and 1:1. A2–H2 is the corresponding higher magnification images of A1–H1. (Scale A1–H1 10 μm and A2–H2 5 μm.)

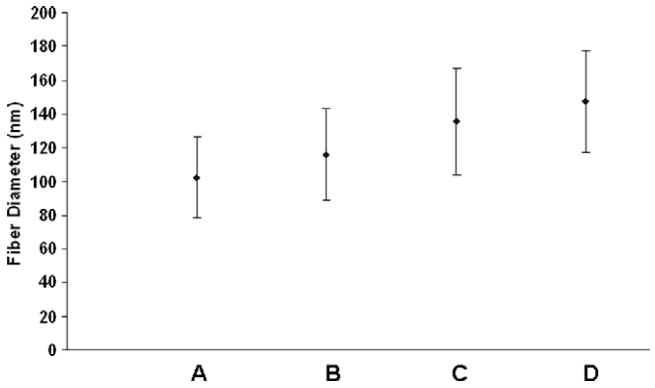

Fig. 3. Fiber diameter distributions of CS/PCL nanofibers. (A and B) CS (1%)/PCL (8%) = 1:3 and 1:1, (C and D) CS (1%)/PCL (10%) = 1:3 and 1:1.

3.2.3. FTIR

In FTIR, all the stretching and bending vibrations are found to be well matching with the theoretical values. The representative spectrum is shown in Fig. 5. In CS, the broad peak at 3421 cm^{-1} was due to N–H and hydrogen bonded O–H stretching. The peak at 2922 cm^{-1} was due to the asymmetric bending of C–H group. The N–H and –C–O–C peaks were observed at 1648 and 1100 cm^{-1}, respectively. In PCL, the peaks at 2929 and 1729 cm^{-1} represent the characteristic peaks for C–H and ester carbonyl groups, respectively. In PCL/CS scaffold, the O–H stretching has shifted to the lower frequency side and a sharp peak at 3437 $cm^{-1}$was observed. A slight bending at 1727 cm^{-1} is due to the presence of carbonyl group in PCL and another peak at 1166 cm^{-1} is due to C–O–C group. From all these results, it is clear that the combined electrospun scaffold contains both CS and PCL (Sarmila, Abhishek, Rajashree, Phani, & Nayak, 2009).

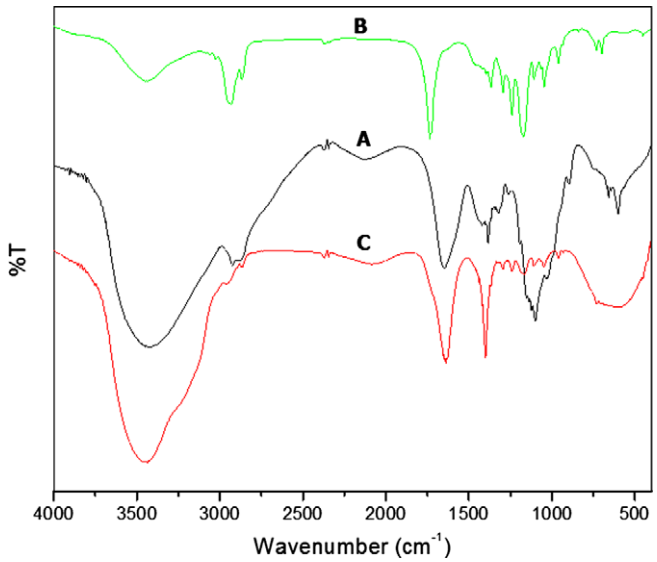

Fig. 5. FTIR spectra of (A) CS (B) PCL and (C) CS/PCL.

3.2.4. Thermal studies

Both 1:3 and 1:1 composition of the CS/PCL scaffold was permitted to programmed heating under nitrogen atmosphere. Fig. 6A represents the DTA diagram and Fig. 6B represents the TG diagram of PCL, CS and CS/PCL system as represented by a, b and c, respectively.

In TG, PCL has a decomposition temperature between 288 and 368 °C. CS has comparatively lower decomposition temperature of 226–314 °C. For CS/PCL system, the decomposition temperature starts from 249 °C and ends at 329 °C. In the CS/PCL system, the

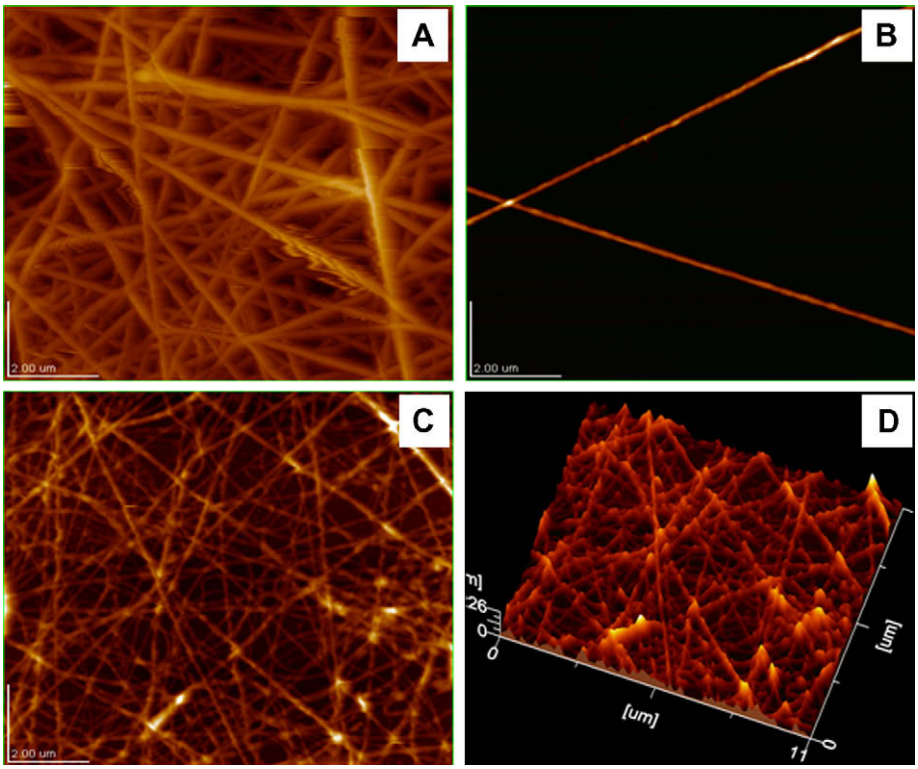

Fig. 4. AFM images of CS (1%) with PCL (8%): (A) topography of non-woven fibers of 1:3 composition, (B) topography of single fiber of 1:3 composition and (C and D) topography of non-woven fibers of 1:1 composition.

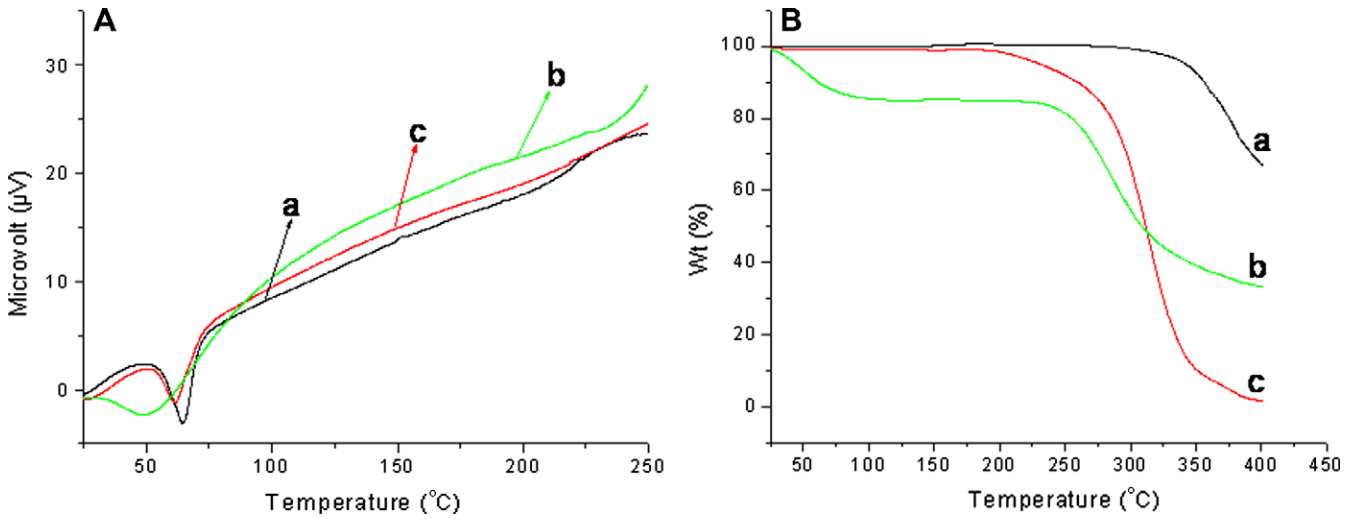

Fig. 6. (A) DTA diagram of (a) PCL (b) CS and (c) CS/PCL and (B) TGA diagram of (a) PCL (b) CS and (c) CS/PCL.

decomposition temperature was slightly shifted to higher values compared to CS, which imply the enhanced stiffness of the CS/PCL fibers.

In DTA, PCL shows an endothermic transition at 61 °C and CS shows a broad peak from 31 to 88 °C. For CS and CS/PCL, a slight shift in melting point towards lower temperature (59 °C) was observed. This transition is due to the decreased crystalline nature of the CS/PCL scaffold compared to PCL. A broad peak was observed for CS at 40–60 °C, which was due to the evaporation of moisture and hydroxyl entity from the CS structure (Jayakumar, Nagahama, Furuike, & Tamura, 2008).

4. Conclusions

CS/PCL nanofibers were prepared using formic acid/acetone solvent mixture. CS and PCL were blended in different concentrations and compositions and were evaluated for their viscosity and conductivity to examine the optimum values to get nanofibers. In the first, 0.5–2% of CS was blended with 6% PCL in different compositions to get the nanofibers. All the fibers show beaded or irregular morphologies. From the results, 1% of CS was again mixed with 4–10% of PCL to fine tune the fibrous structure. Fibers obtained from 1% CS and 8% PCL in 1:1 combination resulted in slightly beaded morphology with diameter 116 ± 27 nm, where as 1:3 compositions resulted in fine nano fibers of diameter 102 ± 24 nm. Representative evidence for the presence of CS and PCL in the optimized system was confirmed by FTIR. Thermal behaviors of PCL, CS and CS/PCL systems were also evaluated by TG–DTA and the combined effect of CS/PCL scaffolds was confirmed. The prepared electrospun scaffolds would be a better system for biomedical applications.

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