Fabrication and characterization of electrospun poly(ε-caprolactone) / TiO2 nanocomposite membranes with synergistic antibacterial property with gentamicin against MRSA

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Abstract -Electrospinning is an efficient technique for the fabrication of polymer nanofiber composites. In this study, micro to nanoscale fiber composites of poly(ε-caprolactone) (PCL) / Titanium Dioxide (TiO_2) were prepared through electrospinning by varying the concentration of TiO_2 nanoparticles and electrospinning parameters such as applied voltage, feed-rate and distance between the tip and the collector. These electrospun fibers were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-Ray Diffractometry (XRD) and Differential Scanning Calorimeter (DSC). The diameters of the fiber and the interstitial pore spaces of the as prepared electrospun fibers were measured. The fiber diameter was in the range of 130 nm – 4 µm and the interstitial pore space was approximately between 200 nm – 20 µm. Furthermore, variation in the fiber diameters and the interstitial pore with the electrospinning parameters as well as concentration of TiO_2 nanoparticles were also discussed. Both FTIR and XRD have been used to analyze the filler/polymer interaction and crystallization behavior of PCL nanofibers in the presence and absence of nanoparticles. The FTIR spectra showed an increase in absorbance of PCL as the TiO_2 nanoparticle concentration is increased. From XRD spectra it was observed that the incorporation of TiO_2 nanoparticles does not affect the structure of the PCL, instead, the degree of crystallinity has been increased significantly. However, at higher concentrations of TiO_2 nanoparticles, crystallinity has decreased to a lower value. DSC thermograms have also confirmed this behavior. The present study was carried out to evaluate the efficacy of drug loaded PCL/TiO2 nancomposite electrospun fibers against a wound isolate of multiple drug resistant Methicillin resistant *Staphylococus aureus* (MRSA). On loading the PCL/TiO_2 nancomposite electrospun fibers with gentamicin, there was a significant synergistic inhibitory effect on MRSA. The study invariably proved that antibiotic loaded PCL/TiO_2 nanocomposites can be used in the designing of scaffolds for wound healing and the control of wound associated infections.

Key words- Electrospinning, Poly(ε-caprolactone), TiO_2 nanoparticles, Nanofibers

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1. Introduction:

The preparation of polymer nanocomposite fibers has got great attention due to their unique properties and applications. Electrospinning is a widely used method to fabricate nano-to-micro scale fibers for tissue engineering applications [1-5]. The parameters which influence the diameter of the electrospun fibers are (1) Polymer and nano-filler concentrations (2) Applied electric field (3) Feed-rate (4) Distance between the syringe-tip and the collector (5) Nature of solvent (6) Ambient conditions such as humidity and solution temperature inside the chamber [5, 6].

The basic requirements for a biomaterial to be scaffold are it should be biocompatible and the surface properties of the material should favor the cellular attachment, proliferation and differentiation. These biomaterials can either be natural or synthetic. There are a lot of polymers such as polylactic acid, poly(ε-caprolactone), polyglycolic acid and their co-polymers, natural polymers such as proteins and polysaccharides are used in the field of tissue engineering scaffolds [7]. They can be filled with nanoparticles such as carbon meshes, carbon nanotubes, chitosan whiskers, metal oxide nanoparticles etc. [8, 9, 12]

The primary objective of this study is to optimize the electrospinning parameters to form the nanocomposite fibers of PCL/TiO_2 with diameter in nanoscale which can be used as scaffold in bone tissue engineering. Poly (e-caprolactone) (PCL) is a well-known FDA approved biocompatible, biodegradable polymer which has shown to be capable of supporting a wide variety of cell types [10]. In order to enhance the mechanical properties of the PCL nanofibers, we have introduced TiO2 nanoparticles into the PCL matrix. Nanoscale metal oxides like ZnO nanoparticles have been incorporated in electrospun tissue engineering scaffolds to tune the morphology, improve the mechanical property, impart antibacterial property and improve cell adhesion property [11]. Our recent work revealed that electrospun PCL membranes incorporated with ZnO nanoparticles can enhance cell proliferation, cell migration and scar-free wound healing [12]. TiO_2 nanoparticles have been recently proposed as the attractive filler material for biodegradable polymer materials. TiO_2 nanoparticles have recognized as most effective photocatalytic material which is used for the photo-assisted degradation of bacteria and organic molecules. Addition of TiO_2 nanoparticles will enhance the mechanical strength of the PCL scaffold and will help to attack the invading bacteria. In this study, we have carried out morphological analysis of electrospun PCL with various concentrations of TiO_2 nanoparticle under varying electrospinning parameters.

For the rapid wound healing, the biomaterials used for the wound treatment should create an optimal environment for the healing by eliminating wound infection [13] and fluid loss [14]. Extensive use of antibiotics led to the recent trends of increased resistance of many pathogenic bacteria to antibiotics. Individuals may succumb to multiple drug resistant (MDR) infections, especially in the developing countries [15]. This tendency has serious negative effects on disease control and therapy against pathogenic bacteria. *Staphylococcus aureus* displays important virulence properties and causes a wide range of infectious diseases including pneumonia, septicemia and endocarditis [16]. This bacterium is a prominent nosocomial pathogen often carried asymptomatically on the human body. Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of infections in hospitals and, more recently, in the community [17]. MRSA includes those strains that have acquired a gene giving them resistance to methicillin and essentially all other beta-lactam antibiotics [18]. The prevalence of MRSA appears to be increasing at an alarming pace in India [19]. It is very necessary to promote the appropriate therapeutic choices for these surgical wound infections under the observation of resistance in pathogens which is causing the infections. Hence, these pathogens bear prime significance as antibiotic resistant agents. Scientists are in search of novel strategies that can tackle the problems related to MRSA and the wound infections caused by them. Hence the present work also explored the in vitro efficacy of gentamicin loaded PCL/TiO_2 nanocomposite fiber membranes to inhibit these bacteria.

2. Materials and Methods

2.1 Materials

Poly(e-caprolactone) (PCL, Mn= 50,000 – 80,000) and TiO_2 nanoparticles (10 – 20 nm) were purchased from Sigma – Aldrich USA, Acetone and Dimethyl suphoxide (DMSO) were obtained from Merck, India, Tryptone Agar, peptone water and gentamicin sulphate were purchased from HiMedia, India.

2.2 Preparation of PCL/TiO_2 electrospun nanocomposite membranes

In this work, TiO_2 nanoparticles were dispersed in acetone. Dispersed nanoparticles were thoroughly stirred with the 15 wt% of PCL (in acetone) solution for 24 hours. Then the mixed solution was taken into a syringe pump and subjected to electrospinning to obtain the fiber nanocomposites. Different parameters like concentration of PCL, concentration of TiO_2, tip to collector distance, applied voltage and feed rate were varied to find the effect of these parameters on the electrospinning process of PCL/TiO_2 nanocomposites.

2.3 Characterization

The resultant electrospun fibers were characterized using Scanning Electron Microscope (JSM – 6390) and the fiber diameter was quantified from the images using imageJ software. The fibers with various concentrations of TiO_2 were characterized using Fourier Transform Infrared Spectroscopy (Perkin Elmer- Spectrum 400) and X-Ray Diffraction analysis. XRD measurements were performed on the Bruker D8 Advance Tools Diffractometer operating in the reflection mode with Cu-Kα radiation (40 kV, 20 mA) and diffracted beam monochromator. The degree of crystallinity has also calculated using "EVA" software via equation as follows:

Percentage of Crystallinity = Crystalline Area/Total Area x 100%

The thermal properties of the samples (neat PCL fiber and different TiO_2 nanoparticles concentrations) were analyzed by Differential Scanning Calorimeter (Perkin Elmer Pyris Diamond). All the samples were sealed in aluminum pans and the weights of the samples taken were in between 5mg – 10mg. The heating and cooling cycles of the sample were investigated at a heating or cooling rate of 5 ºC/min from -75 ºC to 80ºC. The degree of crystallinity has been calculated from DSC using the following equation;

% Crystallinity = [ΔHm] / ΔHm ° X 100%

ΔHm and ΔHm° are the enthalpy of melting of the samples and enthalpy of melting of 100% crystalline PCL respectively (ΔHm° = 139.5 J/g).

2.4 Evaluation of the antibacterial activity of nanocomposite fibers on S. aureus

Antibacterial property of the PCL/TiO_2 membranes was examined by our previously reported protocol [12]. 5mm diameter PCL membranes discs were made. 8 µL of gentamicin sulphate (2mg/ml) was loaded separately in PCL and PCL/TiO_2 membranes, and kept for drying at 40°C for 30 minutes. 18 hrs broth culture of S. aureus equalized to 0.5 Mc Farland standard was prepared. This culture was swabbed on separate Mueller-Hinton agar plates and the surface was allowed to dry for 5 minutes. Plain electrospun membrane discs without TiO_2 and with TiO_2, gentamicin loaded PCL membrane discs and gentamicin loaded PCL/TiO2 membrane discs were placed on the agar with the help of a sterile forceps and pressed gently. The plates were incubated at 37 °C overnight. After incubation, zone of diameter was checked and the results were recorded.

3. Results

3.1 FTIR analysis

The FT-IR spectra of the PCL/TiO2 nanocomposite membranes are shown in Figure 1. From the IR spectra, strong bands of carbonyl stretching mode around 1727 cm-1, asymmetric CH2 stretching at 2949 cm-1, symmetric CH2 stretching at 2865 cm-1, C – O and C – C stretching in the crystalline phase at 1293 cm-1, Asymmetric COC stretching, OC – C stretching at 1190 cm-1, symmetric COC stretching at 1170 cm-1, and finally C – O and C – C stretching in the amorphous phase at 1157 cm-1 were observed. As TiO_2 concentration increases the IR absorbance of the material increases [20]. It indicates the successful incorporation of TiO_2 nanoparticles in the PCL matrix.

However, fundamentally, the IR spectrum of PCL was not affected by the presence of nanoparticles. This indicates that there was no chemical interaction between the nanoparticles and the PCL.

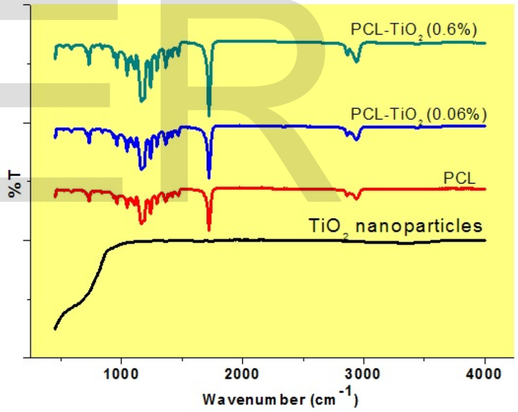


Figure 1: FTIR spectra of electrospun PCL / TiO2 nanocomposites (PCL=15 wt% electrospun at 20 kV applied voltage, 15 cm tip to collector distance and a flow rate of 1 ml/hr)

3.2 Effect of the concentration of PCL on fiber diameter

The most important quantity related with electrospinning is the individual fiber diameter of the electrospun membrane. As the polymer concentration increases the fiber diameter were also increased with less bead formation which is evident from the Fig. 2(c). At lower concentrations, there were a lot of beads in the membranes as shown in

Fig. 2(a) & 2(b). The viscosity of the polymer solution increases as the concentration of the polymer increases and then the bead formation will be lower and fiber diameter will be getting increased [21]. On the other hand, at lower polymer concentrations, the solution splits up and form thinner diameter fibers with lots of beads in the fiber. Such a behavior has been confirmed by Baumgarten in 1971, by preparing acrylic microfibers [22]. Doshi et al. [23] and Fong et al [24] have also investigated and reported such behavior of viscosity on fiber diameter.

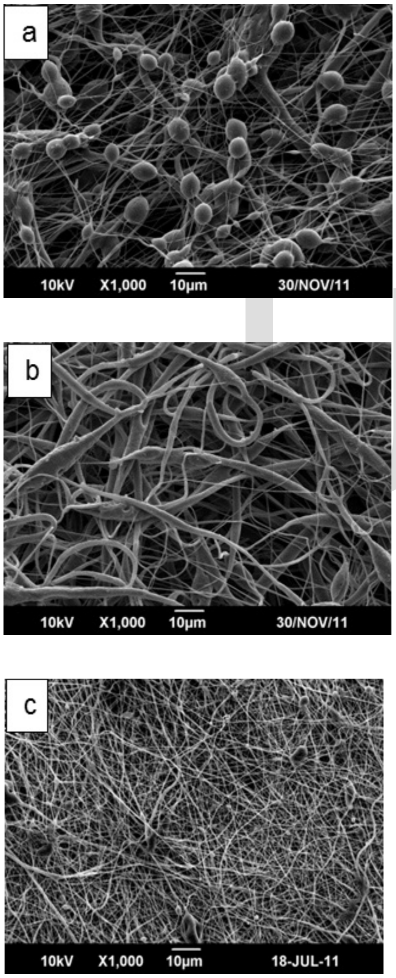
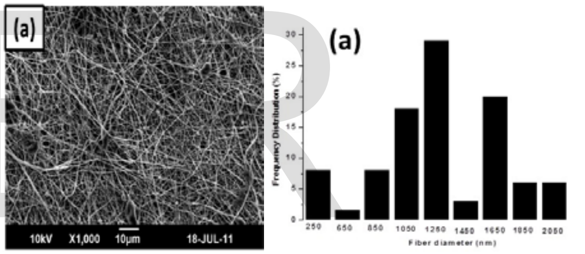
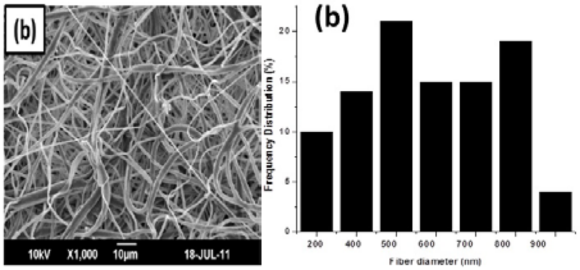


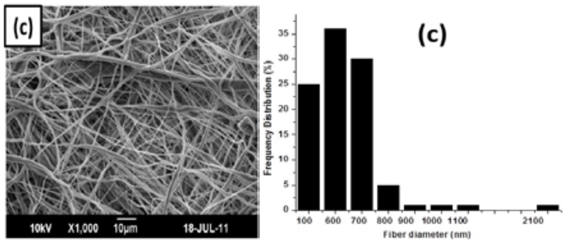
Figure 2: SEM micrographs of electrospun PCL nanofibers at different concetrations of PCL a) 8 wt%, b) 10 wt% c) 15 wt% electrospun at a constant applied DC voltage of 15 kV, tip to collector distance of 14 cm and a flow rate of 1 mL/hr.

3.3 Effect of feed-rate on fiber diameter

The SEM micrographs and their corresponding fibre diameter distribution curves with different feed rates, 1 mL/hr, 3 mL/hr and 5 mL/hr are given in Fig. 3, Fig. 4 and Fig. 5 respectively. The feed-rate is an important parameter which affects the fiber diameter. The fiber diameter increased as the feed-rate increases. During the travel of the jet from the syringe to the metal collector, the polymer solution jet gets elongated to form fibers. At this stage the fiber can be thin or thick which can also be controlled by changing the feed-rate of the polymer solution. At lower feed-rate, there is enough time for the elongation of jet to form thinner fibers with less ejection of solution. As the feed-rate increases more solution ejects from the syringe which causes less time to elongate and the diameter of the fiber increases. A feed-rate of 1 mL/hr or less than 1 mL/hr was seemed to be always better for getting good quality thin fibers.







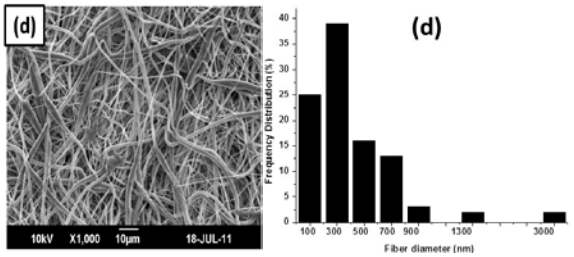


Figure 3: SEM micrographs and corresponding distribution curves of electrospun PCL/TiO2 nanocomposite fibers with an applied DC voltage of 15KV, Feed-Rate -1mL/hr, Tip-Collector distance – 14cm, and 15 wt% of PCL. a) 0 wt% TiO_2 b) 0.06 wt% TiO_2 c) 0.6 wt% TiO_2 d) 3wt% TiO_2.

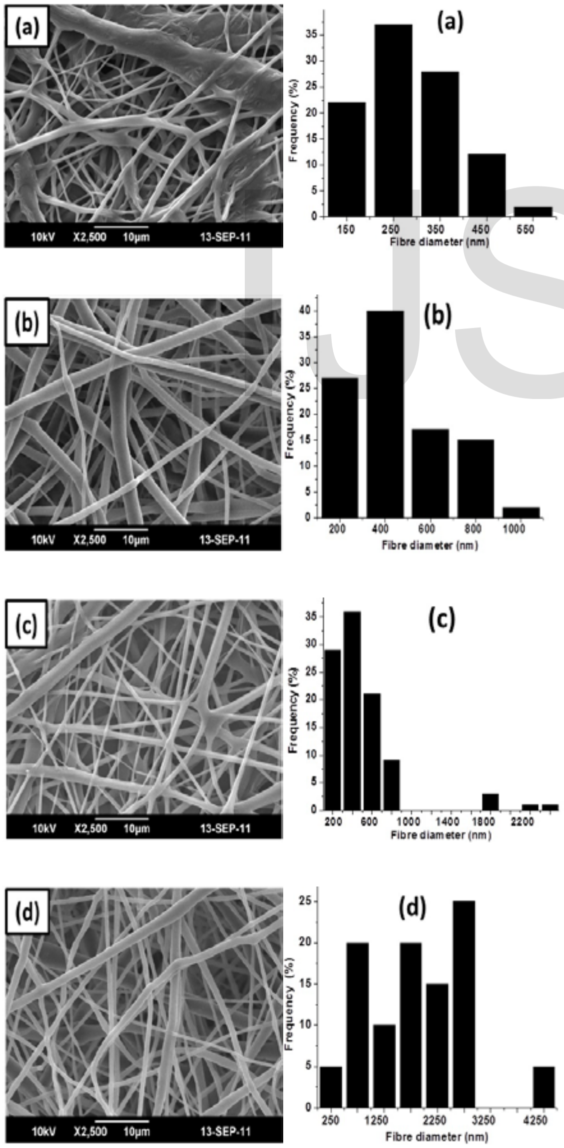


Figure 4: SEM micrographs and corresponding distribution curves of electrospun PCL/TiO2 nanocomposite fibers with an applied DC voltage of 15KV, Feed-Rate -3 mL/hr, Tip-Collector distance – 14cm, and 15 wt% of PCL. a) 0 wt% TiO_2 b) 0.06 wt% TiO_2 c) 0.6 wt% TiO_2 d) 3wt% TiO_2.

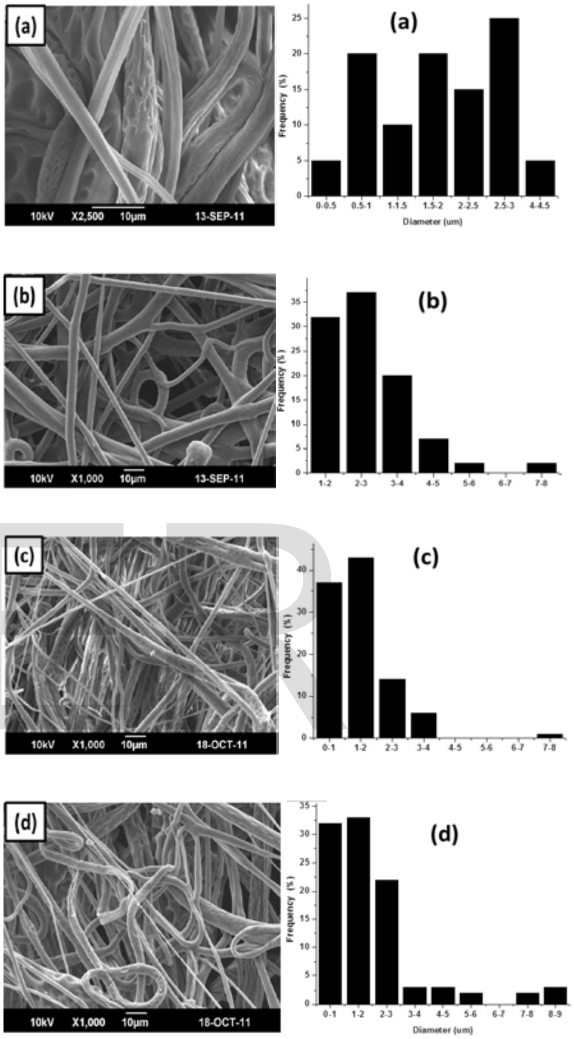


Figure 5: SEM micrographs and corresponding distribution curves of electrospun PCL/TiO_2 nanocomposite fibers with an applied DC voltage of 15KV, Feed-Rate -5 mL/hr, Tip-Collector distance – 14cm, and 15 wt% of PCL. a) 0 wt% TiO_2 b) 0.06 wt% TiO_2 c) 0.6 wt% TiO_2 d) 3wt% TiO_2.

3.4 Effect of TiO_2 nanoparticle concentration on fiber diameter

Fig. 6 shows the diameter of the fibers decreases with the concentrations of TiO_2 nanoparticles.

Addition of TiO_2 nanoparticles into the polymer solution results in a higher charge density of the surface of the solution jet during the electrospinning, bringing more electric charges to the jet and the charges carried by the jet increased, higher elongation forces were imposed to the jet under the electric field, which results in reduced beads and thinner fibers [21].

Both XRD and DSC data showed high degree of crystallinity at optimum loading of nanoparticles. Electrospinning was not able to perform for the solution with 5% and above TiO2 nanoparticle concentrations on account of strong agglomeration process of the nanoparticles in the PCL matrix.

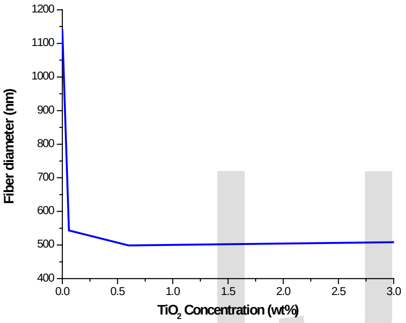


Figure 6: Graph showing the variation in fiber diameter with TiO_2 nanoparticle concentration at an applied DC voltage of 15 kV, feed rate of 1mL/hr, PCL concentration of 15 wt% and a tip – collector distance of 14cm.

3.5 Effect of applied DC voltage on fiber diameter

Applied electric potential is also an important parameter which affects the fiber diameter significantly. Lower electric potential such as 10 kV and 12 kV were not sufficient enough to elongate the solution jet to form thinner fibers. However, at higher the applied voltage (18 kV and 21 kV), more fluid eject from the jet which cause larger diameter and bead formation which is evident from the Fig. 7. The optimized electric field to get thinner fibers is 15 kV.

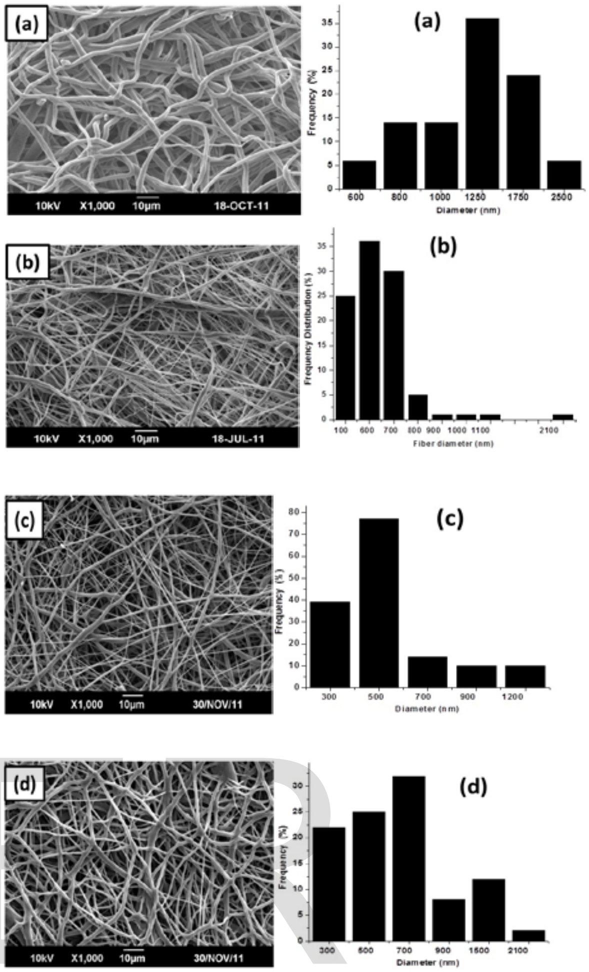


Figure 7: SEM micrographs and corresponding distribution graph of electrospun PCL/TiO_2 nanocomposite fibers with varying applied DC voltages. a) 12 kV, b) 15 kV, c) 18 kV, d) 21 kV, Feed-Rate -1 mL/hr, Tip-Collector distance – 14 cm, and 15 wt% of PCL and 0.6 wt% of TiO_2.

3.6 Effect of the tip to collector distance on fiber diameter

Another important electrospinning parameter which affects the fiber diameter is tip to collector distance. In Fig. 8, it is observed that at higher tip to collector distance the fiber diameter get increased. When the tip to collector distance is high, the solution jet has to travel a long distance which causes the multiple jet formation and leads to the accumulation of the fibers together to form thicker diameter. The average diameter of the electrospun fiber at 14 cm tip to collector distance was found 543 nm and at 24cm tip to collector distance, it was

1.9 μm. The tip to collector distance of 12 cm to 15 cm is optimum to get thinner fibers.

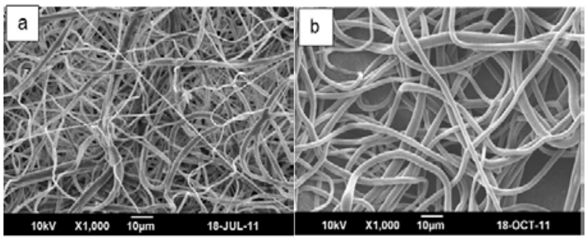


Figure 8: Representative SEM micrographs of PCL/TiO2 electrospun nanofibers electrospun at 15 kV applied voltage, 1 mL/hr feed-rate, 15 wt% PCL concentration and 0.06 wt% of TiO_2 nanoparticle concentration at (a) 14 cm tip to collector distance and (b) 24 cm tip to collector distance.

3.7 XRD analysis

The XRD spectra of bare PCL membrane, pure TiO_2 nanoparticles and PCL/TiO_2 nanocomposite membranes are given in Fig. 9. In the spectrum of pure PCL membrane and PCL/TiO_2 nanocomposite membrane, there are two strong diffraction peaks of PCL crystalline phases. This could be observed at 21.4° and 23.75° in the (110) and (200) plane respectively [25, 26]. It was observed that the addition of TiO2 nanoparticles did not significantly affect the crystalline structure of PCL. The degree of crystallinity of neat PCL fiber was 53.67% and that has increased up to 67.33% and 77.07% at 0.06% and 0.6% of TiO_2 nanoparticle concentrations, respectively. However, a reduction in the degree of crystallinity, that is, 71.77% has observed at 3% of TiO_2 nanoparticle concentration which might be due to the poor dispersion of nanoparticles in the polymer matrix that might disturb the crystallization process.

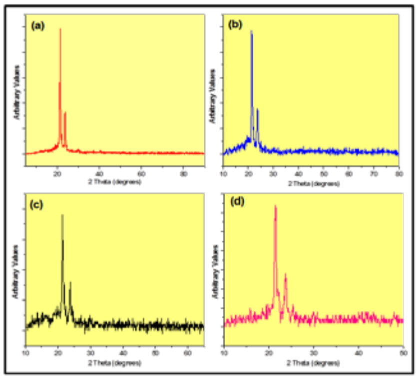


Figure 9: XRD pattern of electrospun PCL membranes (a) and PCL/TiO_2 nanocomposite membranes with 0.06 (b), 0.6 (c) and 3 (d) wt% TiO_2 nanoparticles.

3.8 DSC analysis

Fig. 10 shows the DSC thermograms of neat PCL membrane and PCL/TiO_2 nanocomposite membrane with various concentrations of TiO_2 nanoparticles. From DSC thermograms, the melting point and crystallization temperature have shifted to a higher value for 0.06% and 0.6% TiO_2 nanoparticle concentrations which shows the good dispersion and the reinforcing effect of the nanoparticles in the polymer matrix. At these concentrations, nanoparticles act as nucleating agents which leads to the increase in the degree of crystallinity. At 3% of TiO_2 concentration the peak temperature gets decreased which shows the poor dispersion of nanoparticles with the polymer. Table 1 shows the melting peaks and corresponding enthalpy values and crystallinity of the nanocomposite fibers. It is observed that the crystallinity from both DSC and XRD measurements following the same trend.

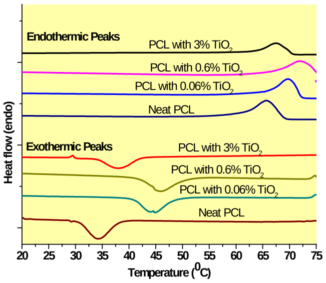


Figure 10: DSC Thermograms of electrospun PCL / TiO_2 nanocomposite fibers.

Concentrations		Melting Point Tm °C	Enthalpy Value ΔHm J/g	Crystallization Point Tc °C	Crystallinity %	
PCL	TiO_2				DSC	XRD
15%	0%	65.67	73.78	34.24	52.89	53.67
	0.06%	69.74	89.70	44.91	64.30	67.33
	0.6%	72.00	93.67	45.84	67.15	77.07
	3%	67.31	88.54	37.98	63.47	71.77

Table 1: Data obtained from the DSC measurements.

3.9 Antimicrobial Studies

The antibacterial activity of the fabricated membranes against MRSA was evaluated by disc diffusion method and the zone of inhibition is given in Table 2. When gentamicin sulphate was loaded in to the PCL/TiO_2 fiber membranes, there was noticeable antibacterial activity on drug resistant S. aureus. Among the gentamicin loaded polymeric membrane except PCL membrane containing 3% TiO_2 nanoparticles all the other membranes showed similar zone of inhibition (8mm). The formation of zone of growth inhibition around the membranes loaded with gentamicin showed that PCL/TiO_2 fiber membranes can be a good system to be used as drug loaded wound dressings which can control staphylococcal infections.

Table 2: Zone of growth inhibition by the PCL/TiO2 electrospun samples with and without gentamicin

Concentrations of PCL/TiO_2 electrospun fibers			Inhibition zone diameter (mm)
PCL	TiO_2		
15%	0%		5
15%	0%	Gentamicin loaded	8
15%	0.06%		5
15%	0.06%	Gentamicin loaded	8
15%	0.6%		5
15%	0.6%	Gentamicin loaded	9
15%	3%		5
15%	3%	Gentamicin loaded	11

*diameter of the membrane disc is 5 mm.

4. Discussion

Bactericidal effects of PCL/TiO_2 electrospun membranes on MRSA showed synergistic effect with gentamicin. Compared to neat PCL membrane, PCL membrane containing 0.06 and 0.6% TiO_2 nanoparticles, PCL membrane containing 3% TiO_2 nanoparticles along with gentamicin has shown higher inhibitory effect. Since the nanoparticles are entrapped in PCL matrix, a lower concentration will not be able to provide an effective release rate to inhibit bacterial growth and consequently the lower concentrations of TiO_2 could not show any antibacterial activity. Such synergistic effect of silver nanoparticles with gentamicin on *E.coli* [27] and *Staphylococcus aureus* [28] was already reported. It was concluded that PCL/TiO_2 electrospun membranes have potential as a combination therapeutic agent for the eradication of wound infections.

5. Conclusion

Poly (ε-carprolactone) / Titanium dioxide nanoparticles nanocomposite fibers were prepared through electrospinning by varying several parameters such as nanoparticle concentration, feed-rate, applied electric field and the distance between tip and collector. While introducing TiO_2 nanoparticles to PCL, the fiber becomes thinner and if the concentration increased further, beyond a critical concentration, the diameter increases. The fiber diameter increases as the feed-rate increases. At lower-voltages the fiber diameter was high and as the applied voltage increases up to a certain extent the diameter becomes reduced and again increased with further increase in the applied voltage. In order to get the thinner fibers, feed-rate should be less than or equal to 1mL/hr, the applied voltage should be in between 12KV and 15KV, and the distance should be 12cm to 15cm range. It is also observed that the interstitial pore diameter also follows the same pattern as that of fiber diameter with varying TiO_2 nanoparticle concentration and electrospinning parameters. The FT-IR spectra showed every characteristic peaks of PCL in the electrospun nanocomposite fibers and the absorbance of PCL increase as the TiO_2 nanoparticles increases. The addition of TiO_2 nanoparticles into PCL, did not significantly affect the structure of PCL, but an increase in the degree of crystallinity was shown at 0.06% and 0.6% of TiO_2 nanoparticles filled PCL fiber. The crystallinity was decreased at 3% of TiO_2 nanoparticle concentration as copared to the other filled systems. DSC thermograms also support the XRD spectra and showed the nucleating action TiO_2 nanoparticles in the PCL matrix. Interestingly the melting peak temperature shifted to a higher values and the crystallization temperature was increased due to nucleation effect. It is proposed that as prepared non-woven fiber mat can be used as scaffold for bone tissue engineering due to its controlled porous nature which might enhance the cell growth. The anti-staphylococcal activity of the gentamicin loaded PCL/TiO_2 fiber membrane may open up new doors in the new era interventional strategies adopted in wound treatment also. The applicability of the drug loaded composites can be instituted only after assessing the issues related to toxicity and cyto compatibility in mammalian cell culture or by animal experiments.

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Conflict of Interest

The authors declare no conflict of interest.

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