

Research Article

Structural and Surface Compatibility Study of Modified Electrospun Poly(ϵ -caprolactone) (PCL) Composites for Skin Tissue Engineering

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Abstract. In this study, biodegradable poly(ϵ -caprolactone) (PCL) nanofibers (PCL-NF), collagen-coated PCL nanofibers (Col-c-PCL), and titanium dioxide-incorporated PCL (TiO₂-i-PCL) nanofibers were prepared by electrospinning technique to study the surface and structural compatibility of these scaffolds for skin tissue engineering. Collagen coating over the PCL nanofibers was done by electrospinning process. Morphology of PCL nanofibers in electrospinning was investigated at different voltages and at different concentrations of PCL. The morphology, interaction between different materials, surface property, and presence of TiO₂ were studied by scanning electron microscopy (SEM), Fourier transform IR spectroscopy (FTIR), contact angle measurement, energy dispersion X-ray spectroscopy (EDX), and X-ray photoelectron spectroscopy (XPS). MTT assay and cell adhesion study were done to check biocompatibility of these scaffolds. SEM study confirmed the formation of nanofibers without beads. FTIR proved presence of collagen on PCL scaffold, and contact angle study showed increment of hydrophilicity of Col-c-PCL and TiO₂-i-PCL due to collagen coating and incorporation of TiO₂, respectively. EDX and XPS studies revealed distribution of entrapped TiO₂ at molecular level. MTT assay and cell adhesion study using L929 fibroblast cell line proved viability of cells with attachment of fibroblasts over the scaffold. Thus, in a nutshell, we can conclude from the outcomes of our investigational works that such composite can be considered as a tissue engineered construct for skin wound healing.

KEY WORDS: compatibility study; composites; electrospinning; PCL; skin tissue engineering.

INTRODUCTION

Tissue engineered cell transplantations (1,2) are gaining importance for the patients suffering from tissue malfunctioning or loss due to some injuries, accidents, or other damages. Though tissue engineering emerged in early 1990s, still more researches are being continued to improve their functionality towards the cell transplantation (3). In tissue engineering, cells are initially seeded on a porous, degradable, mechanically strong, stable three-dimensional passive structure with biocompatible surface, known as scaffold (4–6). There are different methods for scaffold fabrication and electrospinning is one of the most easiest and economical process (7,8). Since 1930s (6), the electrospinning process is being widely used for scaffold preparation from different polymers. It produces micro- to nanofibrous-based scaffolds with desired porosity, biocompatibility, and enhanced specific surface area and also with proper mechanical stability (9). So structurally,

chemically, and mechanically, these electrospun scaffolds bear a resemblance-like extracellular matrix (ECM) of tissues (10). The different synthetic polymers (11,12) such as polyglycolide (PGA) (13), polyvinyl pyrrolidone (PVP) (14), and their copolymers poly(lactide-co-glycolide) (PLGA) (15), poly(ϵ -caprolactone) (PCL) (16), and polyurethane (PU) (17) have been widely used in electrospinning process. Other natural polymers such as collagen, fibronectin, laminin, alginic acid, and chitosan can be also electrospun for producing nanofibrous scaffolds (18,19).

PCL, a semi-crystalline aliphatic polyester, belongs to the group of degradable polymers due to the susceptibility to hydrolytic cleavage of the ester bond. This property, along with good compatibility and easy processing (melting point at 60°C), makes PCL an interesting substrate for tissue engineering (20–25). However, like other synthetic polymers, PCL also lacks surface wettability and functional surface groups improving the cell attachment that are essential in tissue engineering. Nowadays, concepts of hybrid scaffolds have been started to avoid such limitations and use of both natural and synthetic polymers together to combine their good properties. When natural polymers such as collagen, fibronectin, laminin, alginic acid, and chitosan are electrospun and used alone, they can exhibit properties of the extracellular matrix (26) with excellent biocompatible surface enhancing the cell adhesion (18,19). However, such scaffolds are mechanically weak. In hybrid approach, the scaffold produced from synthetic polymer is used as a physical

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support and its surface is hybridized with some natural polymer enhancing cell attachment (27–29). Zhang *et al.* already reported the preparation of PCL/collagen scaffolds by dipping PCL (Mw 80,000) nanofibers in collagen (type I from calf skin) solution (30). Such processes can cause clogging of the pores that are important for cell proliferation through the scaffold and also collagen dipping may not give efficient coating (30). Sometimes co-axial coating (30)/blending of PCL with collagen was also performed (31), but it may associate also some limitations. The limitations for co-axial coating/blending are that electrospinning processing parameters need to be optimized for either core (PCL; Mw 80,000)-coat solutions (collagen; type I) or co-polymer (PCL; Mw 80,000/collagen; type I) solution which might not be possible always and it may affect fiber morphology. Also, this will use more amount of collagen solution which is costly, not economical, and also mechanical strength of synthetic polymers; PCL may be compromised. Some researchers incorporated silver (Ag) or titanium dioxide (TiO₂) nanoparticles of average diameter 21 nm in the nanofibrous-based scaffolds which were used in tissue engineering for some specific applications like antimicrobial or UV protection (32–36). These structures are also referred as hybrid materials or nanocomposites. When using nanoparticles as a part of the nanofiber structure, it is necessary to investigate the biocompatibility of the resulting nanocomposites and their influence on cell attachment and growth. So it is very clear that scaffolds-surface chemical properties can regulate cell activities like cell adhesion and migration over the scaffold whereas biocompatibility is also governed by structural unit/building block of scaffold.

Thus, it is important to investigate how surface chemistry and structural unit of hybrid/composite scaffold can influence the cell transplantation process towards generation of new tissue or organ (37–39).

In this work, two different types of hybrid scaffolds over PCL-NF were prepared. These are collagen-coated PCL nanofibers (Col-c-PCL) and titanium dioxide-incorporated PCL (TiO₂-i-PCL). These were investigated for surface and structural biocompatibility using fibroblasts, respectively. The presence of electrospun collagen mesh by separate electrospinning over PCL nanofibers (Col-c-PCL) changed surface property like wettability, surface chemistry of PCL-NF. Incorporated antibacterial TiO₂ nanoparticles have been investigated here to check their biocompatibility and how they influence cell attachment and growth over PCL composite (TiO₂-i-PCL). MTT assay and cell adhesion assays were carried out for all scaffolds (PCL-NF, Col-c-PCL, TiO₂-i-PCL) to check biocompatibility towards tissue engineering. Detailed physicochemical investigations (scanning electron microscopy (SEM), Fourier transform IR spectroscopy (FTIR), contact angle, tensile strength, X-ray photoelectron spectroscopy (XPS), energy dispersion X-ray spectroscopy (EDX)) had been done to study morphology, interaction between polymer-polymer and polymer-nanoparticle, hydrophilicity, mechanical strength, and surface chemistry of these scaffolds.

MATERIALS AND METHODS

Materials

Reconstituted type I collagen of bovine skin was obtained from Central Leather Research Institute, Adyar, Chennai. PCL (Mw 80,000) was obtained from Sigma Aldrich, USA.

Glacial acetic acid, chloroform, and methanol in analytical grade were obtained from SRL, Mumbai. TiO₂ nanopowder of 20-nm size range was purchased from Sigma Aldrich.

Methods

Sample Preparation and Electrospinning

Different amounts of PCL (8, 11 and 13% w/v) were dissolved in 3:1 v/v chloroform/methanol mixture for obtaining PCL electrospinning solution. At first, weight amount of PCL was added in the required solvent and stirred for 2 h. Then the mixture was sonicated for 15 min and again stirred for another 12 h. Before electrospinning, solution was sonicated for 15 min and kept aside for another 30 min to remove all bubbles from the solution. The electrospinning apparatus (Holmarc, India) consisted of a 5-ml syringe with 0.84-mm internal diameter ID of a blunt end needle and integrated with a grounded electrode. The needle-to-collector distance was maintained at 12 cm. The applied voltages were 10 and 12 kV. The solution was fed at the rate of 2 ml/h and flow rate was precisely controlled by a syringe pumping system. Thin aluminum sheet attached over the fixed collector was used to collect fibers. The electrospinning was carried out at ambient temperature and pressure to produce dry, thin scaffolds in electrospinning. Thus, PCL-NF was obtained by electrospinning method.

The TiO₂-i-PCL composite was prepared by adding TiO₂ nanoparticles slowly to the PCL solution under continuous stirring, and then the solution was electrospun using the same electrospinning conditions as described above. Thus TiO₂-i-PCL was obtained. The PCL-NF and TiO₂-i-PCL were dried under vacuum at room temperature for 48 h.

Next, to get Col-c-PCL, collagen was used for coating of PCL-NF. PCL nanofibers will be abbreviated as PCL-NF. Collagen solution was prepared by dissolving in TFE (80 mg/ml).

Then, Col-c-PCL was prepared by following the method in Fig. 1.

SEM Study

At first, samples were prepared by sectioning in specific length and width for obtaining SEM images. Samples were coated by a platinum layer using a JEOL JFC 1600 Autofine coater. The images were obtained at 20 kV and then analyzed by software Digimizer® in order to calculate the fiber diameter.

EDX Study

EDX analysis of TiO₂-i-PCL was performed using the field-emission scanning electron microscope (FE-SEM) MIRA, manufactured by Tescan and equipped with the EDX add-on (Oxford Instruments). The Au coating with a thickness of 10 nm was deposited by RF magnetron sputtering prior to the imaging in order to compensate for a charging. The electrons were accelerated by a 15-kV high voltage, and the working distance was fixed at 15 mm in order to minimize the charging effect and to improve the resolution. The

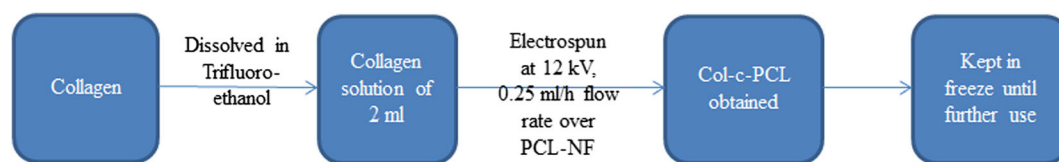


Fig. 1. Preparation of Col-c-PCL

quantification of the elemental composition was determined in the field of $43 \times 43 \mu\text{m}$ by the in-built software Aztec.

FTIR Study

FTIR spectroscopic analysis of electrospun material was made using spectrum one (PerkinElmer, USA model). Attenuated total reflectance-FTIR (ATR-FTIR) spectra of PCL and Col-c-PCL and TiO_2 -i-PCL were recorded in the wavenumber range of $4000\text{--}600/\text{cm}$ at room conditions in ATR mode using 500 scans. The sample was kept on the diamond ATR top plate and through pressure arm; pressure was loaded on sample/crystal area before collecting FTIR spectra. PerkinElmer's revolutionary Spectrum™ FT-IR software helped to collect spectra.

XPS Study

XPS for a surface (2–3 nm) chemical characterization of nanofibers was carried out using an Omicron X-ray source (DAR400, output power 270 W) and an electron spectrometer (EA125) attached to a custom-built ultra-high vacuum system. The quantitative composition was determined from detailed spectra taken at the pass energy of 25 eV and the electron takeoff angle 50° . The maximum lateral dimension of the analyzed area was 1.5 mm. The quantification was carried out using XPS MultiQuant software.

Contact Angle Measurement

Contact angle measurement was done using contact angle meter (Holmarc Optomechatronics). For determination of hydrophilicity of samples, at first, the sample was kept on the flat bench of the instrument and then water droplet with size of $0.5 \mu\text{l}$ was set to fall on the sample. The contact angle was measured by a video contact angle system after 10 min of stabilizing, and five samples were measured for each test.

Mechanical Properties

For measuring tensile strength of the electrospun nanofibers, universal tensile machine (UTM) INSTRON 1408 was used and the measurement was carried out by following ASTM D 882 standard. Tensile testing was carried out using 500 N load cells at a speed of 1 mm/min onto the specimen. The samples were prepared with width of 5 mm, gauge length of 20 mm, and thickness of 0.2 mm. All the experiments were done for three samples for each specimen.

MTT Assay

For cell cultivation tests, L929 fibroblast cell lines were purchased from NCCS Pune. The cell line was maintained in Dulbecco's modified Eagle's media (HIMEDIA)

supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5% CO_2 (NBS, Eppendorf, Germany) in a humidified atmosphere in a CO_2 incubator.

For MTT assay, the cells were trypsinized (500 μl of 0.025% trypsin in PBS/0.5 mM EDTA solution (HIMEDIA)) for 2 min and then passaged to T flasks in complete aseptic conditions. Samples of 1 cm^2 from different formulations were sterilized and immersed in cell-free media for 24 h. Next day, trypsinized cells were added on to the surface of nanofibers and allowed to grow for 24 h followed by MTT assay. Optical density (OD) was read at 540 nm using DMSO as blank using an ELISA reader (LISASCAN, Erba). Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Cell Adhesion Study

At first, samples were cleaned ultrasonically and freeze dried in a lyophilizer. Then samples were sterilized using ethylene oxide (ETO) and stored aseptically. After overnight incubation in serum-supplemented DMEM cell culture media at 37°C in a CO_2 incubator, freshly trypsinized L929 cells were seeded on the surface of the scaffold and permitted to attach for 30 min followed by media supplementation. The cells were allowed to grow for 72 h. Cell adhesion was determined using acridine orange staining. After removing excess stain, morphology was assessed by fluorescent and phase contrast microscopy.

Cell adhesion study was carried out by following the method in Fig. 2.

Data and Statistical Analysis

Results are presented as mean \pm standard deviation (SD). Statistical comparisons were made at significance level of $p < 0.05$ using MS Excel software.

RESULTS AND DISCUSSION

SEM Study

Electrospinning is a way to generate fibrous scaffold from different synthetic and natural polymers. Voltage is one of the most important processing parameters to be set up for electrospinning. Electrostatic stress which pulled polymer as fiber from its solution will be obtained from electrospinning voltage. Fibers from charged polymer solution only will be obtained if voltage at threshold value is applied. This threshold voltage value differs for different polymer solutions. It depends on polymer concentration, viscosity of solution, humidity, temperature, etc. Fiber diameter and fiber morphology also can be affected by electrospinning voltage. Many research papers have shown fiber diameter increased

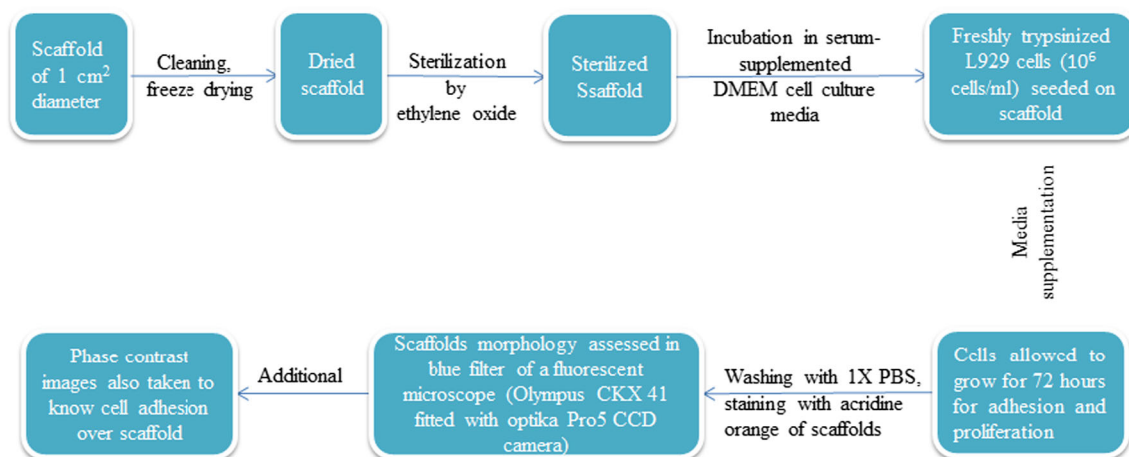


Fig. 2. Description of Cell adhesion study

with increased voltage (40,41) and many papers have reported reverse (42,43).

According to SEM, the fiber diameter for PCL at 11% concentration tended to decrease slightly with increased voltage from 10 to 12 kV. It can be explained by an electrostatic repulsive force on jet narrowing the fibers. Similar effects were observed when 13% PCL concentration was used (images are not shown). So the fiber diameter decreased for both 11 and 13% PCL concentrations with the increase of voltage. The ribbon-shaped nanofibers were observed at lower voltage (10 kV), whereas continuous nanofibers were obtained with increased voltage (Fig. 3a, b, respectively). Such effect can be explained by the fact that low voltage is not enough to stretch the polymer as continuous fiber from these higher polymer concentration solutions and bending instabilities occur (43). So polymer concentration should be optimized for obtaining nanofibers in electrospinning process. Here, PCL concentration was lowered to 8% and then electrospinning was

performed at 10 kV voltages. The continuous nanofibers with diameter distribution from 2.0 to 0.4 μm were produced without beads evidenced by SEM (Fig. 3c). Hence, 10-kV voltages were adequate to produce a continuous nanofiber from a lower polymer concentration. For carrying out further studies, PCL-NF obtained from 8% PCL at 10 kV was used for collagen coating and also for TiO_2 incorporation. Figure 3d represents Col-c-PCL. Here, some portions of the prepared fibers looked thicker as we obtained the fibers from nano to micro range.

EDX Spectra and XPS Analysis

The addition of the TiO_2 into the PCL solution led to electrospun nanofibers with sufficient amount of the embedded TiO_2 . Even addition of 1 wt.% of TiO_2 into the PCL solution led to the incorporation of the ~ 10 wt.% of Ti, as EDX analyses revealed (Fig. 4a, b). As shown in Fig. 4a, b, the

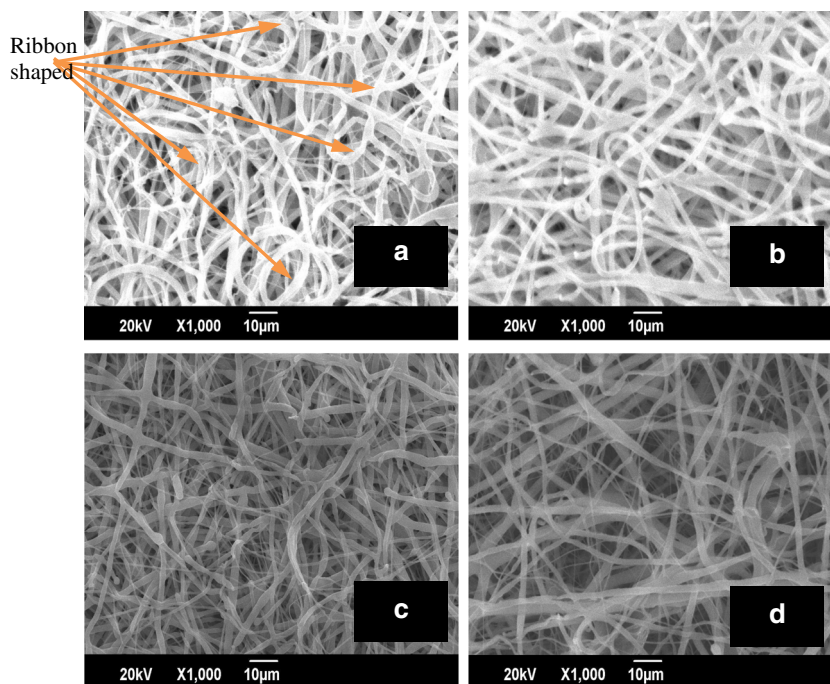


Fig. 3. SEM images of nanofibers. **a** 11% PCL, PCL-NF at 10 kV; **b** 11% PCL, PCL-NF at 12 kV; **c** 8% PCL, PCL-NF at 10 kV; and **d** Col-c-PCL

distribution of Ti is quite homogenous along the nanofibers. However, some clusters of TiO_2 were observed in Fig. 4a. To incorporate TiO_2 nanoparticle in PCL-NF, the nanoparticle was dispersed in the PCL solution. So during solution preparation, some titania may bind to PCL and clusters of titania of varying sizes are observed adhering to fibers (44). It is difficult to conclude from EDX if the TiO_2 is embedded inside the nanofibers or simply stuck to the surface. Thus, the surface-sensitive XPS analyses were performed on the same sample, and the survey scan is depicted in Fig. 4c. The XPS analyses revealed the presence of carbon and oxygen with the O/C ratio of 0.28. No even traces of Ti were visible in the spectra in spite of very high relative sensitivity of this element. Therefore, the Ti revealed by the EDX cartography is homogeneously embedded in the bulk of nanofibers and does not represent any heterogeneous nanoparticles trapped between the nanofibers.

Contact Angle Measurements

The contact angle value of PCL-NF was about 88° (Fig. 5). The contact angle values (45°) were lower for collagen-coated PCL-NF due to the hydrophilicity of the collagen. When liquid was dropped over these nanofibrous scaffold, the convex shape of the drop became more flat and measured values were seen 40° for Col-c-PCL after 10 min. TiO_2 -i-PCL nanofibrous scaffold also showed decrement of contact angles but in a very small extent. The value decreased to 80° . These contact angle determination was performed for three specimens in each sample.

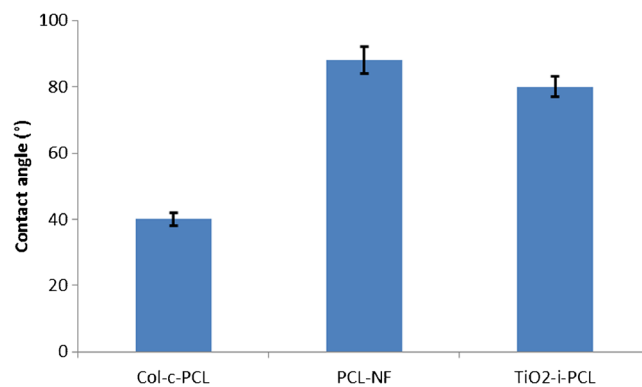


Fig. 5. Contact angle values with standard deviations for scaffolds

Generally, contact angle value is used to know whether matrix/nanofibrous mat used is hydrophilic or hydrophobic. Contact angle values of 0 to 30° present hydrophilic surface, 30 to 90° contact angle value present less hydrophilic surface, and more than 90° values present hydrophobic surface. So we can see that PCL matrix alone produces hydrophobic surface. But presence of collagen mess or TiO_2 incorporation enhanced its hydrophilicity. Amine as a functional group is present in collagen structure in large numbers and this enhanced hydrophilicity of Col-c-PCL might be attributed to this functional group. As TiO_2 nanoparticles were well dispersed throughout the matrix and also enhanced surface to volume ratio of electrospun matrix, they increased hydrophilicity of TiO_2 -i-PCL matrix at some extent. Many other research groups have shown that blending of polymers or presence of

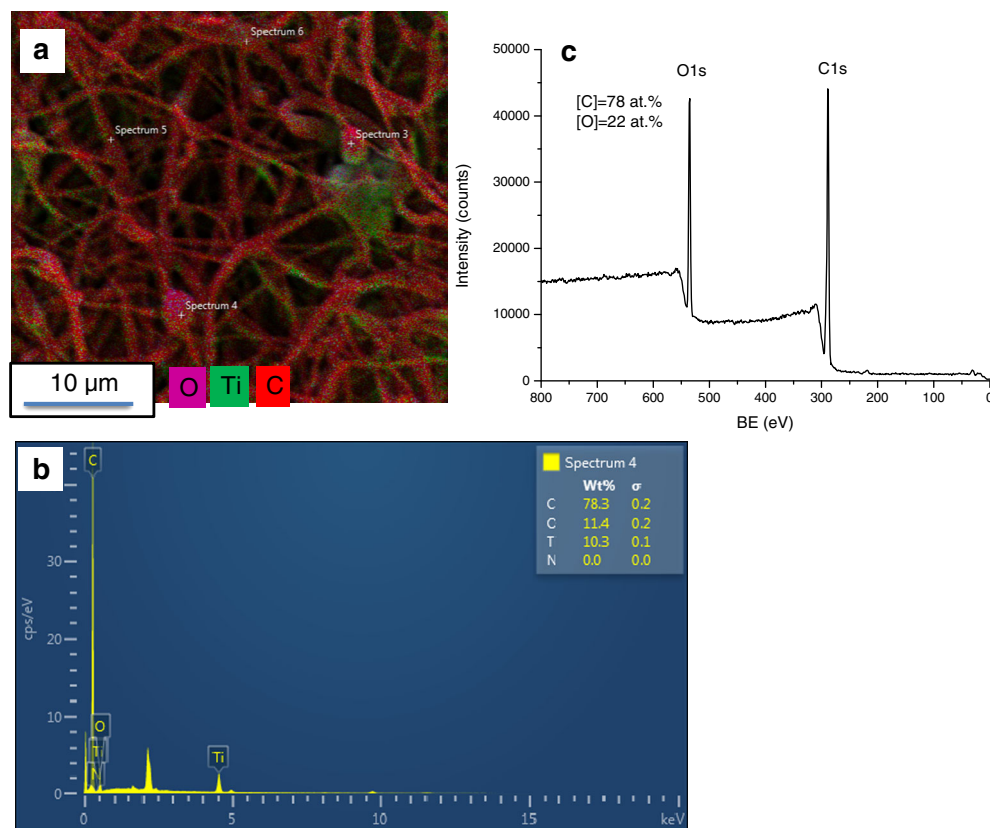


Fig. 4. Effects of TiO_2 addition. **a** EDX mapping of TiO_2 -i-PCL. **b** The representative EDX spectrum of TiO_2 -i-PCL. **c** XPS survey scan of TiO_2 -i-PCL

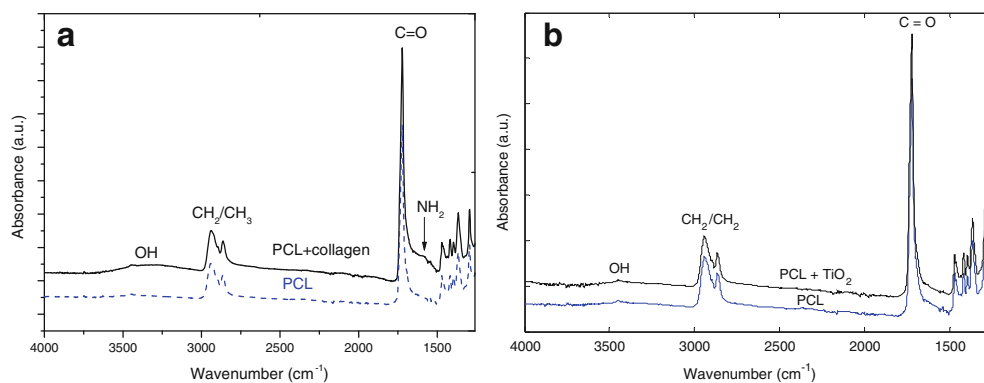


Fig. 6. ATR-FTIR spectra of **a** Col-c-PCL and PCL-NF and **b** TiO₂-i-PCL and PCL-NF

nanoparticle may modify surface properties of nanofibrous matrix. Shalumona *et al.* (2011) (46) showed also enhancement of hydrophilicity of PCL nanofibrous matrix by blending PCL with chitosan. Similar work carried out by Zhang *et al.* (2005) (47) showed blending of gelatin with PCL improved its hydrophilicity. Pant *et al.* (48) used TiO₂ nanoparticles in nylon-6 nanofibrous membrane, and hydrophilicity of this matrix was improved significantly. Enhanced hydrophilicity for scaffold used in tissue engineering is desirable for initial cell adhesion and cell migration. Hydrophobic surface shows lower cell adhesion (49).

FTIR Data

FTIR analyses of PCL-NF and Col-c-PCL revealed peaks at 2991, 2900, and 2878/cm for CH₂ vibrations. The intense sharp peak at 1720 cm for C=O vibrations; CH₂ bending vibrations at around 1490, 1450, and 1362 cm; and COO vibrations at around 1250 were attributed to PCL structure (Fig. 6a). Then at around 1570/cm, one peak was visible only in the Col-c-PCL and was attributed to NH bending vibrations of collagen. The presence of amide group in FTIR spectroscopy of Col-c-PCL nanofibrous scaffolds indicates that the PCL chain was chemically attached to collagen and it may improve biocompatibility of PCL-NF scaffold in tissue engineering. From Fig. 6b, it is evident that incorporation of TiO₂ was quite compatible with PCL as all the corresponding peaks of PCL were clearly observed in FTIR spectra.

Mechanical Properties

The incorporation of TiO₂ has increased mechanical strength of PCL-NF, as shown in Table I. The collagen-coated PCL-NF was not used for tensile strength study due to high cost of collagen. It can be assumed that additional coating with collagen may slightly increase the tensile strength of PCL-NF, as Lee

et al. reported before (28). Guo *et al.* has shown that use of functionalized alumina nanoparticle in polymer nanocomposite increased both modulus and tensile strength (50). The enhanced mechanical property was due to interfacial attachment of functionalized alumina nanoparticle with vinyl-ester resin. In another study, silver nanoparticles were embedded uniformly in nylon nanocomposite and enhancement of mechanical strength of nylon fibers (51) was noticed. Similar results were observed for PCL-PU nanocomposite. Use of silver nanoparticles in PCL-PU nanocomposite improved tensile strength and tensile modulus of this composite due to more fiber packing (52). So overall, use of nanoparticle has increased mechanical strength of nanocomposite. In the present study, mechanical characterization results of PCL nanocomposite are quite consistent with other reported results. It can be assumed that the mostly homogenous distribution of TiO₂ nanoparticles in PCL nanofiber at lower concentration helped to distribute stress uniformly, minimized formation of stress-concentration centers, and further helped to increase interfacial area for stress transfer from the polymer matrix to the fillers. Indeed, EDX spectra confirmed that TiO₂ was mostly homogeneously dispersed in nanofiber structure and, therefore, it closely interacted with PCL and as a result, the mechanical strength was improved. Li *et al.* (53) have investigated that nanoparticles at lower percentage has strong reinforcing effects leading to production of nanofibers with increased mechanical strength. But increased concentration of nanoparticle may decrease tensile strength and modulus of composite. As concentration of nanoparticle is increased more, nanoparticles may aggregate and, as a result, effective interfacial interactive area between nanoparticle and nanofiber is decreased. So optimization of nanoparticle in a concern to mechanical property of nanocomposite is very requisite. Further exploration such as optimization of mechanical strength of PCL composite using different nanoparticle concentration will be carried out in a future study to get PCL composite scaffold as a three-dimensional fibrous skin tissue engineered construct providing desirable mechanical property.

Table I. Mechanical Strength of Nanofibers

Formulation code	Ultimate MPa	Break stress MPa	Yield stress MPa	Modulus MPa
PCL-NF	1.343 ± 0.01	0.73 ± 0.06	1.273 ± 0.04	3.80 ± 0.02
TiO ₂ -i-PCL	1.531 ± 0.003	1.507 ± 0.01	1.390 ± 0.01	4.103 ± 0.11

PCL-NF poly(ϵ -caprolactone) (PCL) nanofibers, TiO₂-i-PCL titanium dioxide-incorporated PCL

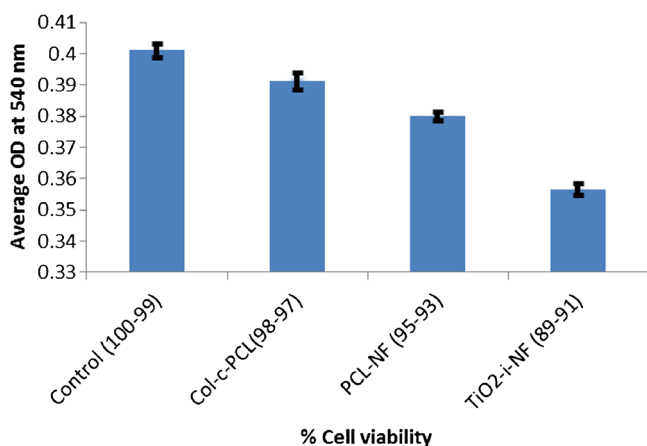


Fig. 7. MTT assay of nanofibers

MTT Assay

MTT assays had shown cell viability in different types of PCL nanofibrous matrix. The result is shown in Fig. 7. Cells were allowed to grow in different polymer nanofibrous matrix solutions for 24 h and then by absorbance values, % viability of cells had been estimated. It was clear that cell metabolism activity over electrospun nanofibers established cytocompatibility. Degradation products released from the scaffold were non-toxic and did not hamper cell growth. However, slight reduction of % viability in TiO₂-i-PCL sample was observed due to presence of TiO₂ nanoparticles. However, still approximately 90% of cells were viable over the matrix which was good for assuming it as appropriate scaffold for tissue engineering application. To get more clear ideas, cell viability over PCL-NF matrix containing different percentage ratios of these antibacterial nanoparticles would be performed in the next possible study. Col-c-PCL promotes

more cell growth than PCL-NF, because coating of collagen over any synthetic polymer nanofibers could enhance their biocompatibility (19). In this study, the % viability of cells in the presence of different electrospun matrixes was compared and calculated as the percentage relative to the untreated control cells (cells were not added in any nanofibrous matrix solution).

Cell Adhesion Study

Fluorescence images and phase contrast images were taken to see the attachment of L929 fibroblast cells over the scaffold. Additionally, these tests helped to know whether there was any change in the morphology of cells due to contact with the different types of nanofibrous scaffold.

Initially, cell adhesion and spreading over the scaffold would be considered as essential step for cell growth required for wound healing and restoration of the tissues. To investigate fibroblasts adhesion and spreading on the surface of scaffold, fluorescence and phase microscopy images were acquired. All images (Figs. 8 and 9) clearly proved that normal morphology of cells was maintained even after 3 days for different types of nanofibrous scaffold. Spindle-like shapes are for live cells and rounded cell are for dead cells. Hence, the initial requirements for being an essential scaffold (cell morphology maintenance, attachment and spreading of cells) were fulfilled. However, higher numbers of dead cells have been observed on TiO₂-i-PCL scaffold due to nanoparticle toxicity.

Fluorescence images also proved that cells are adhering through nanofibers. Live cells were shown by green nucleus, whereas the dead cells were presented with an orange color. Figures 8 and 9 showed presence and adhesion of higher number of cells over the Col-c-PCL nanofibrous scaffold and higher numbers of dead cells were seen over TiO₂-i-PCL nanofibers scaffold. It is clear that presence of collagen would

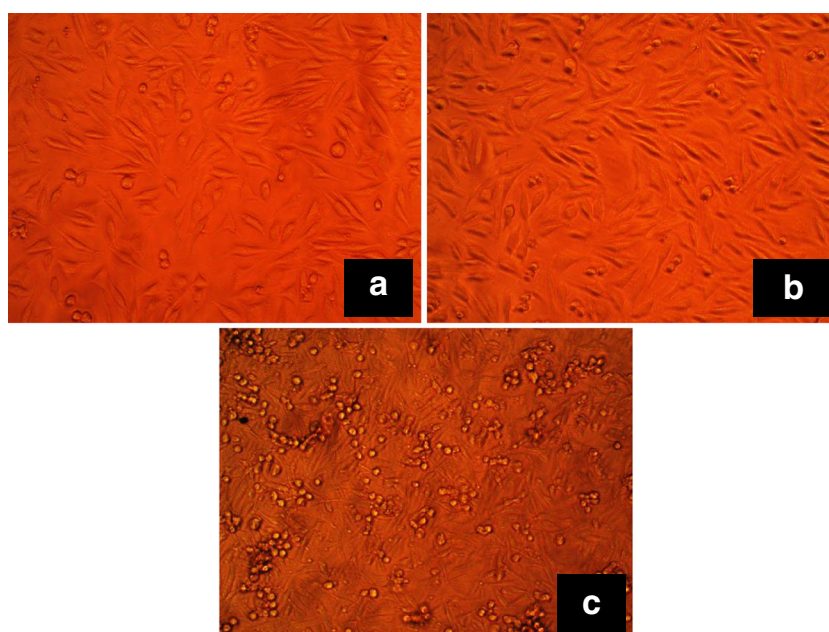


Fig. 8. Phase contrast images for cell adhesion study over **a** PCL-NF, **b** Col-c-PCL, and **c** TiO₂-i-PCL

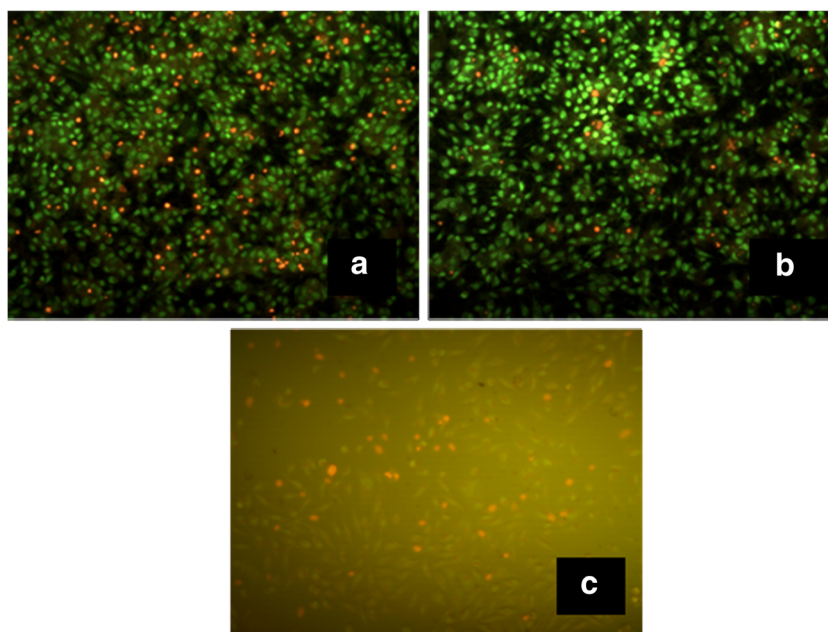


Fig. 9. Fluorescence images for cell adhesion study over **a** PCL-NF, **b** Col-c-PCL, and **c** TiO₂-i-PCL

mimic as extracellular matrix and enhanced the cell adhesion. However, optimized TiO₂ nanoparticle concentration and collagen coating will help to engineer PCL-based scaffold favoring wound tissue formation with antimicrobial property.

Zhang *et al.* (30) showed in their study that co-axial blending of collagen over PCL mimicked as ECM due to widespread presence of biological samples like collagen and influenced cell-scaffold interaction. This kind of scaffold showed significant cell migration (31.8% in 6 days) than the scaffold which was hybridized by dipping PCL nanofibers in collagen solution (21.0% in 6 days). But, it was certain that inert PCL needs coatings of bioactive samples. So our approach towards coating of PCL by biologically active sample (collagen) via electrospinning was beneficial for this kind of scaffold of skin tissue engineering. From Fig. 3, scaffold confirmed porous structure which assisted transportation of nutrition and metabolic waste, thus regulates cell-scaffold interaction. Though porosity was not measured here, we will try to perform in a future study.

CONCLUSION

PCL nanofibers with collagen coating and entrapped TiO₂ nanoparticles were successfully synthesized. Polymer concentrations and working voltages in electrospinning technique had a significant effect on the fiber morphology. The hydrophilicity of the electrospun nanofibers could be significantly enhanced by collagen coating and slight modification was also possible with TiO₂ insertion. According to EDX spectra, the TiO₂ homogeneously distributed in the nanofibrous mesh. No traces of Ti were visible in the XPS spectra, and therefore, all TiO₂ that was revealed by EDX was embedded inside the nanofibers. The incorporation of the TiO₂ nanoparticles had increased mechanical strength of the nanocomposite, but the presence of this material slightly decreases the viability of cells. The MTT assay and the cell

adhesion study indicated these nanocomposites as a suitable biomaterial for skin tissue engineering, though the optimization of nanoparticle concentration needs to be further addressed. Further investigations will be carried out in a future study to get maximum antibacterial property from TiO₂ with minimization of its toxic property.

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