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Gelatin coated PCL nanofibers for biomedical applications

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ABSTRACT: With increasing interest in nanotechnology, development of nanofiberous scaffolds prepared by the technique of electrospinning is having a new momentum. Poly caprolactone (PCL) is a semi crystalline linear hydrophobic polymer, FDA approved, the electrospun PCL fibers mimic the identity of extracellular matrix (ECM) in living tissues, while its poor hydrophilicity caused a reduction in the cell-interactive behavior. To enable cell attachement and proliferation, gelatin as a biopolymer is a good choice for coating PCL nanofibers. In the present study, the surface of electrospun PCL nanofibers was modified to improve their cell biocompatibility. For the preparation of PCL nanofibers, electrospining technique was introduced first, followed by spin coating of gelatin. Both coated and uncoated materials were characterized using scanning electron microscopy, static contact angle measurements, and attenuated total reflection infrared spectroscopy. The results indicated that gelatin coating has changed the hydrophobic nature of PCL nanofibers and provided a hydrophilic surface on it for cell adhesion and growth in vitro. The hydrophilicity of the electrospun PCL nanofibers was increased by gelatin coating, as confirmed by contact angle measurements.

KEY WORDS: Electrospinning, Nanofibers, PCL, gelatin.

I. INTRODUCTION

Extracellular matrix (ECM) plays an important role in controlling cell growth. Nanofibrous scaffolds are a good candidate for treatments based on tissue regeneration because of its suitable environment for cell attachment, and proliferation due to similarity to physical properties of natural ECM.
Electrospinning is a technique in which a nano-sized continuous fibers can be obtained from different polymers (1) , which provides very high specific surface areas. Therefore, electrospun nanofibers are very useful for developing nanofibrous scaffolds for tissue regeneration. From the biological point of view, almost all of the tissues and organs, such as bone, nerve, blood vessel, and cartilage, are synthesized and hierarchically organized into fibrous form with fiber dimensions down to nanometer scale [2,3].
Various polymers have been employed for scaffold fabrication for tissue regeneration [4, 5]. Among these, Polycaprolactone (PCL), which is a synthetic polymer, has a slow degradation rate and good mechanical properties [4, 6]. Nanofibrous PCL scaffolds, because of their native tissue like properties are widely used for various tissue regeneration [7,8]. Although PCL has good mechanical strength, it shows poor cell adhesion due to its hydrophobic nature.
Recently, Surface modification of biomaterials to improve the biocompatibility and the cell-interactive behavior of implants has gained increasing interest [9]. Since the success or failure of an implant mainly depends on the

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interactions that occur at the tissue-implant interface, gelatin was selected as the polymer coating in order to improve the surface properties of the different implant materials.

Gelatin is a natural biopolymer derived from collagen which is biodegradable, biocompatible and has been widely used in the pharmaceutical and medical fields [10]. Therefore, gelatin can be coated on PCL nanofibers to obtain a scaffold with desired cell adhesion and degradation properties.

In the present study, PCL nanofibers prepared by electrospinning method and applied with gelatin polymer as a coating on it. Changes in the surface characteristics of PCL- gelatin coated nanofibers including chemical composition, morphological changes and hydrophilicity have been examined using FTIR spectroscopy, SEM and contact angle. Biological activity and degradation rate were also investigated.

II. EXPERIMENTAL DETAILS

Materials:

PCL (Mn 80 KDa), gelatin (type B, from bovine skin), N,Ndimethylformamide (DMF), dichloromethane (DCM), glutaraldehyde (GA), were purchased from Sigma-Aldrich.
HSF:Human Skin Fibroblast was obtained from Nawah Scientific Inc., (Mokatam,Cairo,Egypt).

Preparation of PCL nanofiber mat

First, PCL polymer was dissolved in a mixture of DMF and DCM (40:60% v/v) at room temperature.
For the process of electrospinning, solution of PCL (10 wt.%) were placed in a plastic syringe electrospining using NANON-01A electrospining system (MECC Co., LTD). A voltage of 18 kV was applied, with a distance of 15 cm between needle and collector. The nanofibers were collected on flat aluminum foil.

Preparation of multilayered biocomposite scaffold

The multilayer structure was prepared by incorporation of PCL nanofiber layer between two layers of gelatin. So, first 10 wt.% of gelatin powder was dissolved in distilled water at 60ºC. Then, After fabricating the PCL nanofiberous structure, it was coated from both sides with gelatin layer using spin-coating.
Then, the prepared samples were crosslinked using glutaraldehyde (GA) vapor by fixing the dried fabricated multilayer composite on a porous shelf in well-sealed container filled with GA at room temperature. The crosslinked samples were transferred to desiccator to remove the residual GA.

Characterization

Morphology analysis

Surface morphology of the developed PCL NFs and coated samples were examined by a scanning electron microscope, SEM (Nova Nano SEM, FEI, USA).

Attenuated Total Reflectance Infrared (ATR-IR) Spectroscopy

FTIR analysis of the plain Nf and coated was performed using a Frontier Perkin Elmer FT-IR spectrometer with universal ATR module (Diamond/KRS-5 crystal). The instrument was operated with a resolution of 4 $cm^{-1}$and 32 scans with frequency range of 600–4000 cm^{-1}.

Contact angle measurement

The hydrophobicity of the samples was evaluated by contact angle measurements. The contact angle was measured after dropping a water drop on the surface of the samples, and the angle between the liquid surface and sample surface was measured.

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Cell biocombitibility evaluation

SRB assay was performed for the evaluation of biocombitability and biological behavior of PCL and PCL/gelatin composite. Aliquots of 100µL fibroblast cells suspension ($5x10^3$cells) were in 96 well plates and incubated for 24h. PCL nanofibers after sterilization, were placed in the well culture plate and seeded with the cells then incubated at 37ºC , 5% CO_2. After 72h of incubation, cells were fixed by replacing media with 150µL of 10% TCA and incubated at 4°C for 1h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70µL SRB solution (0.4%w/v) were added and incubated in a dark place at room temperature for 10min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry over night. Then, 150µL of TRIS (10mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540nm using a BMGLABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany).

III. RESULTS AND DISCUSSION

Figure 1 presents a digital image of an electrospun nanofiber scaffold of poly(ε-caprolactone) (PCL) and gelatin coated nanofibers.

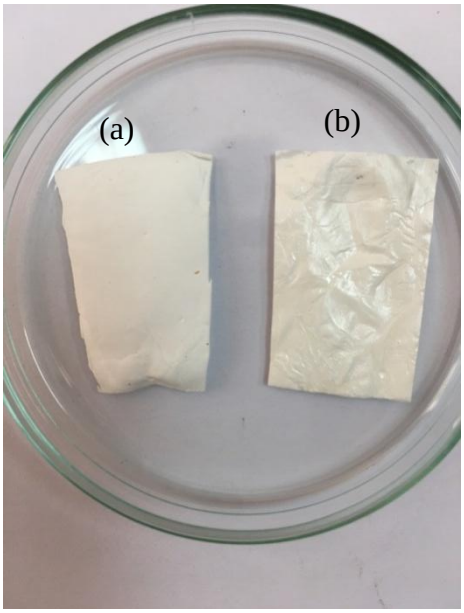

Fig. (1): Digital images of PCL nanofibers (a) and gelatin coated PCL composite (b).

Morphological characterization

Morphology of the developed uncoated nanofibers and the coated biocomposite was examined using SEM (figure 2). Figure 2(a), shows that the PCL NFs demonstrated a relatively smooth surface of randomly oriented NFs, and confirms that defect-free smooth fiber mates without bead formation were obtained. As depicted in Figure 2b, the gelatin coatings were obtained as a smooth surface, with a good covering to the PCL nanofibers without any cracks on the surface.

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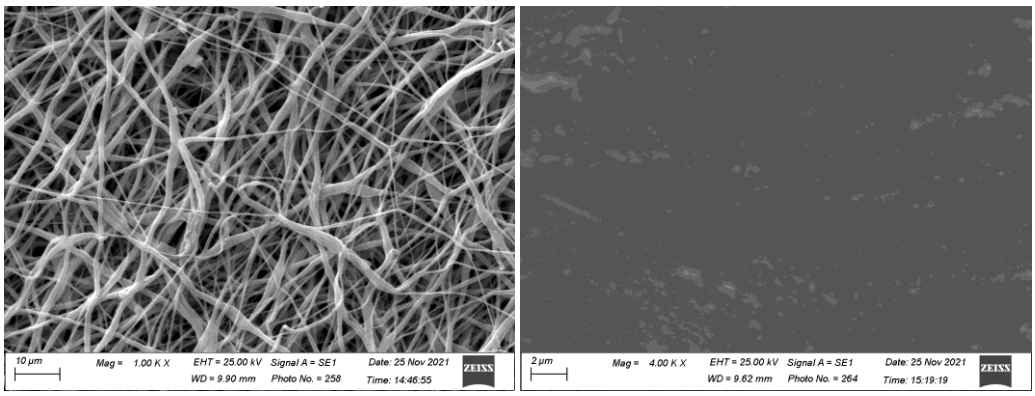

Fig. (2): SEM images of uncoated PCL nanofibers (internal layer) (a) and coated surface with Gelatin (external layer) (b).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was used to study the difference in the chemical composition of the PCL nanofibers and the developed structure of PCL nanofibers coated with gelatin. The spectrum of plain PCL nanofibers exhibit all the characteristic bands of polycaprolactone, in particular two peaks centered around 2941 cm^{-1}, 2865 cm^{-1} due to asymmetric and symmetric C-H stretching. Also, an intense peak at 1723 cm^{-1} corresponding to carbonyl stretching. Finally, two peaks at 1238 cm^{-1} and 1171 cm^{-1} characteristic to stretching of carbon-oxygen bond within the ester moieties are shown. FTIR spectrum of PCL/gelatin displayed PCL characteristic peaks in addition to characteristic bands of gelatin at 3292 cm^{-1} attributed to stretching of O-H bond, band at 1630 cm^{-1} due to C=O bond, and also band at 1537 cm^{-1} due to C-N-H bending.

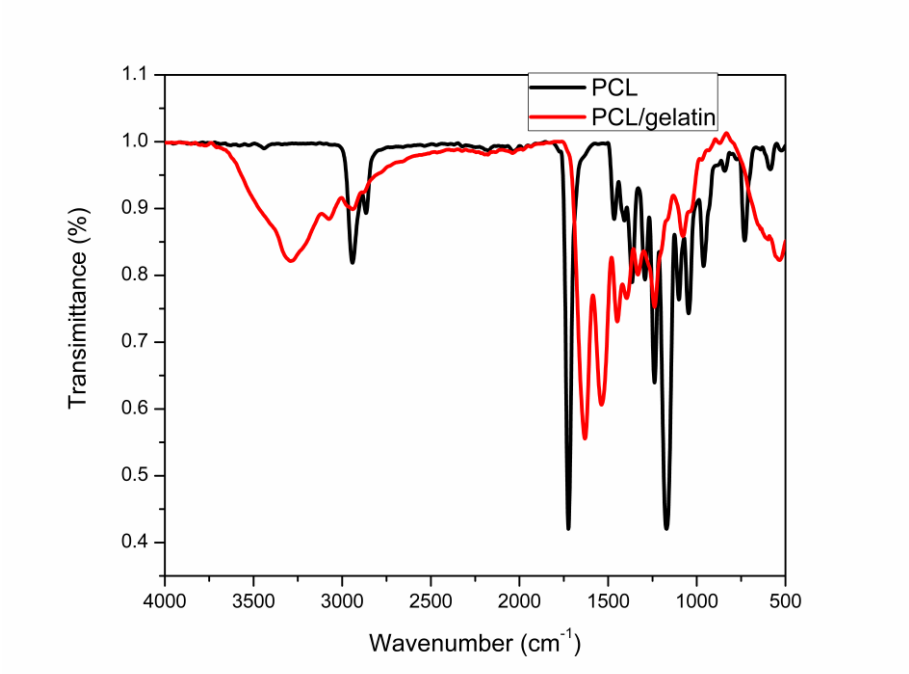

Fig. (3): ATR-FTIR spectra of electrospun neat PCL and its composite with gelatin.

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Hydrophilicity evaluation

Natural polymers reveal better biocompatibility, cell interactions and superior hydrophilicity, which has an effect on cell adhesion and proliferation compared to synthetic ones. Due to the biological source, proper biodegradability, low cost, and commercial availability, gelatin has been widely applied in tissue engineering application, specifically in the combination with PCL. Contact angle is a measure of the degree of wettability revealing the surface state (hydrophilic or hydrophobic). In the present study, it was found that PCL NFs has the highest value of contact angle (120°) which confirm the hydrophobicity of PCL nanofibers. Coating of PCL nanofiber with gelatin layer has decreased the contact angle to 35.8° which can be attributed to the hydrophilic property of gelatin.

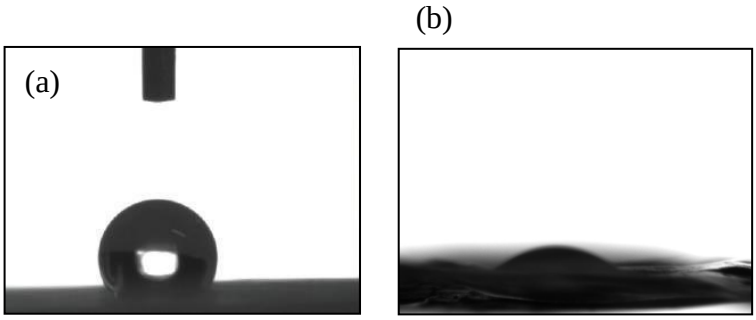

Fig. (4): Static contact angle measurements of (a) uncoated PCL nanofibers, and (b) gelatin-coated nanofibers..

Degradation in PBS

The rate of scaffolds degradation should be synchronized with the rate of new tissue formation. Figure (5) shows the in-vitro biodegradation performance of the coated sample compared with plain PCL nanofibers incubated in PBS for 7 days at 37°C. before proceeding to cell biocombitability test, the chemical stability of the specimens in phosphate buffer saline (PBS) should investigated. The behavior of the plain fibers and coated fibers was evaluated in terms of variation of dry weight and pH value of PBS.

As shown in figure 5a, PCL nanofiber didn't show any significant change in the dry weight over the whole time of experiment. The hydrolytic degradation of PCL is known to have very slow kinetics, so it stays stable for years in saline solution due to the hydrophobic nature of PCL. As shown from the figure gelatin coated sample showed no significant change in weight loss in the first week. From figure 5b there is no relevant variation in p value of PBS.

From the degradation test, it is confirmed that both PCL and PCL/gelatin are stable in physiological buffer saline solution and are suitable for cell testing.

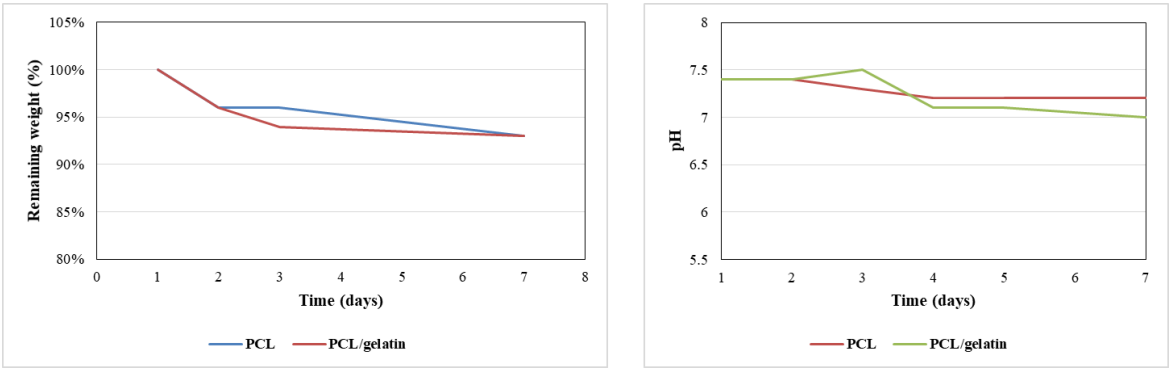

Fig. (5): The stability of PCL nanofibers and gelatin coated fibers (a) degradation of samples over 1 week, and (b) pH value of PBS over 1 week.

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Cell biocombitability

Samples of plain PCL nanofibers and gelatin coated sample, were biologically characterized using human fibroblasts in order to evaluate the cytocompatibility of the materials after the addition of gelatin. Accoding to figure 6, the cell viability.increased from 80% to 95% after coating PCL nanofibers with gelatin. In particular, As already reported, gelatin was found to be exceptionally cytocompatible, characterized by values of cell viability, measured by means of SRB.

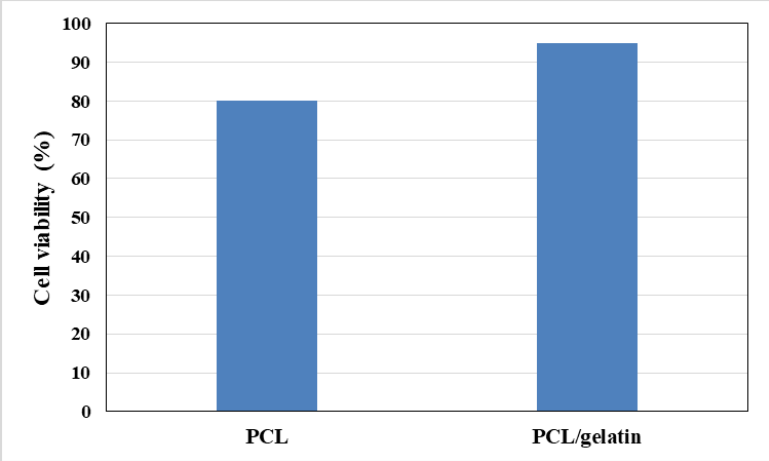

Fig. (6): The cytotoxicity of PCL nanofibers and gelatin coated fibers.

IV. CONCLUSION

In the present work, from the morphological characterization, electrospun PCL nanofibers was surface-functionalized with gelatin with the aim to increase their cell-interactive properties, and this will lead to a good integration with the surrounding tissue which is essential to realize a successful *in vivo* biological performance.

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