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Comparative study between an electrospun fibrous copolymer and their single polymers: synthesis, characterization, and cell growth

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Abstract

Electrospun Fiber matrices fabricated from biopolymers have been shown too successfully to provide scaffolds for cell attachment and proliferation, so this study aimed to fabricate PCL, PLA scaffolds and their copolymer; PCL/PLA in the fiber form by using the electrospinning technique. Where, nanofiber (PCL/PLA) scaffold could be prepared by adding the dissolved solutions of 10% (PCL) to 10% (PLA) with a ratio 4:1 then applying this mixture into electrospinning to turn the dissolved solution into a fiber form. FTIR and XRD techniques were used to investigate the formation of electrospun nanofiber PCL/PLA (4:1) scaffold. Finally, scanning electron microscope images was used to observe the scaffold fibers before and after cell transplantation and it was concluded that PCL/PLA copolymer scaffold provided highest proliferation of Vero cells followed by PCL scaffolds then PLA that referred to its high biocompatibility with the cells compared to their single components PCL and PLA electrospun fiber scaffolds.

Key Words: electrospinning; nanofiber; scaffold; biocompatible polymers ; polycaprolactone; polylactic acid ; Vero cells.

1. Introduction

Recently, the demand for transplanted organs is increased and their complications accompanied by transplantation are increased so the importance of tissue engineering has also increased. It is possible to heal damaged tissues and return their functions by combining biodegradable scaffolds with cells. The basis of tissue engineering is synthesis scaffolds with good properties as biodegradability, biocompatibility and from materials that induce growth of cells on their surfaces. Commonly used and suggested materials to repair damaged sites in tissues comprise polymers, metals, and ceramics [**1**].

Scaffolds are desired mainly for tissue engineering. Their main function is to direct the proliferation of seeded cells inside their porous structure. Accordingly, it should possess appropriate intrinsic mechanical properties to allow the seeded cells to proliferate and produce considerable amounts of extracellular matrices. It should also be highly porous to induce cell attachment, proliferation and must be biocompatible with a suitable biodegradation rate, because when applied to tissue engineering in the body it should be totally replaced with cellular extracellular components, because if not fully degraded it will trigger deleterious inflammatory responses at the site, where it is applied **[2, 3, 4].**

Electrospinning was the used technique in this study, is a technique that consists of combination of two techniques (electrospraying and spinning). It is used to synthesize nano or micro fibrous scaffolds with polymer surfaces that mimic natural extracellular matrix (ECM) molecules as matrix proteins (Laminin, Fibronectin and Collagen, with diameter 5–500 nm) and proteoglycans (Hyaluronic acid, 450–1000 nm) that facilitate cell- material interaction to influence and control cell behavior; cell adhesion, migration and proliferation **[5].** It is a simple and versatile technique to fabricate 3D aligned nano fibrous scaffolds for biomedical applications.

In electrospinning, an electrically charged, viscous polymer jet is emitted from a spinneret through the air in the direction of a collector with opposite electrical potential where the fibers form either well-defined structures or chaotic mats depending on which electrospinning method is being used **[6].** By using a high-speed rotating nozzle to form a polymer jet which undergoes stretching before solidification, fiber diameter, web porosity and morphology can be controlled by changing the nozzle geometry, rotating speed and properties of the solution [**7**]. Disadvantages of the solution spinning process include the residual solvent in the fibers and uncontrolled fiber deposition are due to electrostatic forces that associated with increasing bending and deflecting of the polymer jet **[8, 9].** morphology, and web porosity can be controlled by varying the rotation speed, nozzle geometry, and solution properties [7]. Disadvantages of the solution spinning process include residual solvent in the fibers and only uncontrolled fiber placement due to electrostatic forces and associated increased bending and deflection of the polymer jet [8, 9].

It is thought that the electrospinning technique gives us an impression of being very simple and easily controlled technique for nanofiber production. But, actually this

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process is very complicated because of interaction of many parameters that affect this process. Each of these parameters could change the fibers morphology and diameter. These parameters were classified to; *a) Solution parameters*; concentration, viscosity, surface tension, conductivity and molecular weight. *b) Process factors*; gap (distance between the needle tip of the syringe and the collector), applied voltage and flow rate; *c) Ambient factors* such as temperature, air speed in the electrospinning cabinet and humidity **[10].**

Fibrous scaffolds produced from this technique usually have porous structures so the required sterilization method should have the ability to penetrate the scaffold pores without leaving residuals that may affect the ability of cells to proliferate and attach on the scaffold, so we used in this study Gamma irradiation as a highly penetrative and easily used sterilization method **[11]**.

This paper aimed to fabricate PCL, PLA scaffolds and their copolymer; PCL/PLA in the fiber form by using the electrospinning technique, then compare between them by using FT-IR, XRD and SEM techniques. Finally, we tested their biocompatibility by seeding Vero cells on the fabricated scaffolds and observed their growth by comparing SEM images before and after cell transplantation.

2. Materials and methods

2.1. Scaffolds preparation and cell culture

a) Electrospun scaffold preparation

The scaffolds were prepared by adding 10% Polycaprolactone (PCL) (Sigma Aldrich) to 10% Poly-lactic acid (PLA) (Sigma Aldrich) in a ratio 4:1. The solvent of PCL polymer was 1:1 chloroform (CF): N, N-Dimethyle formamide (DMF) and the PLA polymer solvent was chloroform only. Polymers dissolved slowly at room temperature so the dissolution process could be speeded up by stirring the solution by using a magnetic stirrer and by sonicating it by using an ultrasonic probe sonicator. Electrospinning process was done in the Nano Fiber Electrospinning Unit at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority. The process applied voltage was adjusted at 16 KV with a syringe pump speed 0.05mL/min and the collector to the syringe tip distance was 14cm. Fibers were composed on a foil of aluminum placed on the electrospinning unit collector.

b) Scaffold sterilization

to prepare scaffolds for cell culture procedure, they were cut and put into tissue culture plates then sterilized by Gamma ionizing radiation at NCRRT, Egyptian Atomic Energy Authority, at a dose level 25 kGy.

c) Cell culture

Vero cells were purchased from Center for Vaccination and Sera (Vacsera) – Egypt. These cells were derived from African green monkey kidney.

Cell cultures were prepared in a clean room facility of Tissue Culture Laboratory at NCRRT, Egyptian Atomic Energy Authority.

Vero cells were seeded into flasks (Falcon, Becton Dickinson Labware) and were distributed over the sterilized scaffolds with adding Culture medium; Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-glutamine, Fetal Bovine Serum (FBS), penicillin and streptomycin **[12]**.

2.2. Characterization techniques

2.2.1. Fourier-transform infrared spectroscopy (FT-IR)

Infrared spectra of all scaffolds were recorded by *Bruker Vertex 70 FTIR spectrometer (Germany).*

2.2.2. X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns of the samples were measured by Shimadzu XRD-6000 diffractometer (Germany), in a 2Θ range from 5 to 70°.

2.2.3. Scanning electron microscope (SEM)

Morphological observation of all scaffolds was made by SEM *(ZEISS EVO LS 15)* to examine objects on a very fine scale. Before SEM imaging, all specimens must be coated with an ultrathin coating of electrically conducting material of gold by using Sputter coater instrument *(Quorum Q150R S).*

For cells- scaffold fixation, 1.5% Glutaraldehyde (Fisher Scientific, US) was used for 30 minutes. Samples were exposed to 2% osmium tetroxide (Sigma-Aldrich, US) for 30 minutes then rinsing in distilled water. They were dehydrated through graded concentrations of ethanol (50, 70, 90, and 100%) for 2–5 min. Dehydration was completed in Hexamethyl Disilazane (Fluka, Germany) for 10 min. After air drying and gold sputter coating, the cell morphology on the scaffolds was assessed using (ZEISS EVO LS 15) scanning electron microscopy.

Results and discussion

3.1. FTIR Characterization:

FTIR was used to distinguish functional groups in the fibers to ensure the presence of the scaffold components in addition to observe any chemical interactions or modification. FTIR spectrum of electrospun PCL/PLA fibrous scaffold was recorded in the 400–4000 cm^{-1} region. Major FTIR absorption peaks for this copolymer could be found in Figure 1.

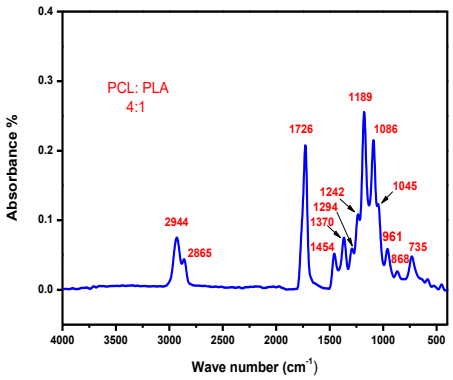

Figure 1: FTIR spectra of the electrospun PCL/PLA (4:1) copolymer Nano fiber scaffold.

Figure 1 shows the FTIR spectra of the electrospun PCL/PLA nanofiber scaffold. Its characteristic peaks appears at 2944, 2865, 1726, 1454, 1370, 1294, 1242, 1189, 1086, 1045, 961, 868, 735 cm^{-1}. Asymmetrical and symmetrical stretching vibrations of methylene groups observed at 2944 cm^{-1} and 2865 cm^{-1}, respectively. As well as the carbonyl group of esters (O-C=O) stretching mode was detected at 1726 cm^{-1}. Bands at 1454 cm^{-1} and 1370 cm^{-1} referred to the-CH2- asymmetrical and symmetrical deformation mode. Additionally, bands at 1294 cm^{-1} and

1242 cm^{-1} are corresponding to C-O stretching vibrations. Bands at 1189 cm^{-1} and 1086 cm^{-1} are corresponding to the asymmetric and symmetric stretching of C-O-C bonds. Other low intensity peaks were assigned as following: the bands 1045 cm^{-1} and 961cm^{-1}corresponded to -C-CH3 stretching and -CH_2- rocking mode, respectively. The bands 868 cm^{-1} and 735 cm^{-1} referred to –C-COO stretching and -C-C- stretching, respectively**[13]**.

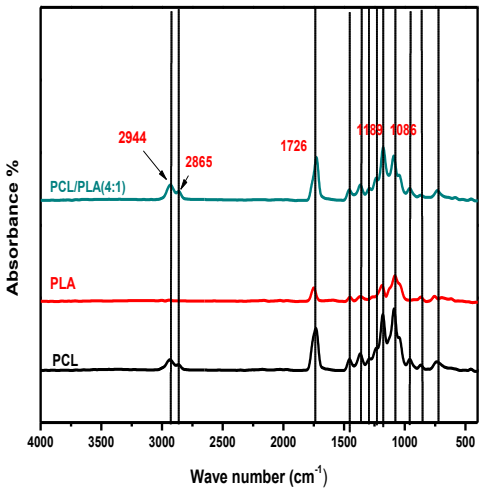

Figure 2: A comparative FTIR spectra for electrospun PCL, PLA and PCL/PLA (4:1) nanofiber scaffolds.

As shown in figure 2, PCL scaffold and PCL/PLA scaffold almost have the same characteristic peaks because the amount of PCL was larger than PLA amount in the scaffold with ratio 4:1.

3.2. XRD Characterization

X-ray diffraction was carried out to determine the crystal structures in electrospun polymer fibers.

As shown in Figure 3, The X-ray diffraction patterns of the PCL fiber scaffold showed existence of two main peaks around 21.5° and 23.9°.Moreover, these peaks were distinct and sharp which reflected its high degree of crystallinity [**14,15].**

XRD pattern of PLA electrospun fiber scaffold showed presence of one broad peak at (16.8°). This peak indicates that sample belong to materials with a strong amorphous structure [**16]**. The broad pattern for PLA scaffold is reflecting its amorphous structure referring to evaporate the solvent through electrospinning process is very fast not permitting crystallization of the polymer **[17]**.

Obviously, the XRD pattern of PCL/PLA copolymer has the characteristics of both PCL and PLA, namely: H. Broad amorphous scattering and distinct peaks, but the PLA peaks do not absorb as strongly compared to the PCL peaks.

By adding PLA to PCL polymer, the crystallinity of its fibrous copolymer decreased sharply. This low crystallinity can reduce the time of degradation and solve the long degradation time proplem of polycaprolactone [**18].**

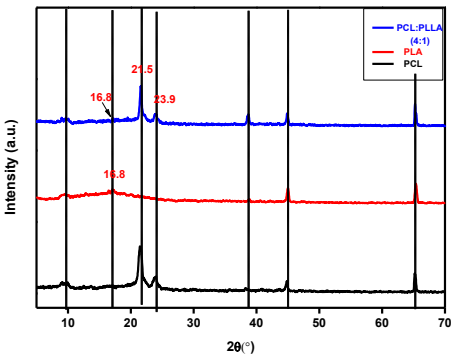

Figure 3: Comparative XRD pattern for the electrospun PCL, PLA and PCL/PLA (4:1) nanofiber scaffolds.

3.3. Scanning electron microscopy (SEM):

SEM images for the prepared fibrous scaffolds before cell transplantation are in Figure 4.

Figure (4a) Showed non uniform interconnected fibers for PCL polymer with small fiber diameter ranged between 100 nm to almost 1 µm and a high surface area-to-volume ratio. Obviously, there are very low quantities of thicker fibers and beads that accurately reflect a nanofiber scaffold with lower defects. On the other hand figure 4b showed a PLA nanofiber scaffold with higher defects where there are enormous amount of huge beads that almost cover the whole surface of the scaffold and give it very bad mechanical properties that it could not be removed from the aluminum foil and disrupted.

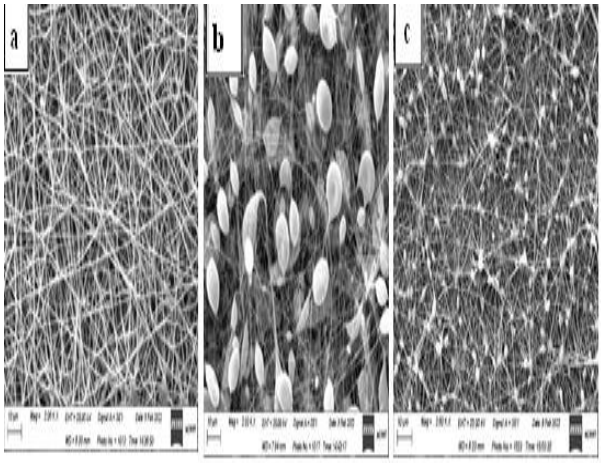

Figure 4: SEM images for electrospun PCL, PLA, and PCL/ PLA copolymernanofiber scaffolds, respectively.

Scaffold in figure 4C was formed from good mixing solutions of 10% PCL and 10% PLA with a ratio 4:1, respectively then electrospinning it to make an electrospun PCL/PLA copolymer to combine the properties of both polymers. Therefore, this figure showed a nanofiber scaffold with very fine interconnected fibers almost less than 100 nm and an average number of small beads. It is well known that the finer fibers have greater strength properties and Young's moduli than fibers with large diameters, but beads that cover the fiber surface weaken the nanostructure and reduce its mechanical properties. Presence of beads may be resulted in

the low viscosity of solutions or owing to thermodynamic immiscibility between the two polymers [**19**].
SEM images for PCL, PLA and PCL/PLA nanofibrous scaffolds before and after 2 & 4 days from Vero cell transplantation are found in Figure 5.

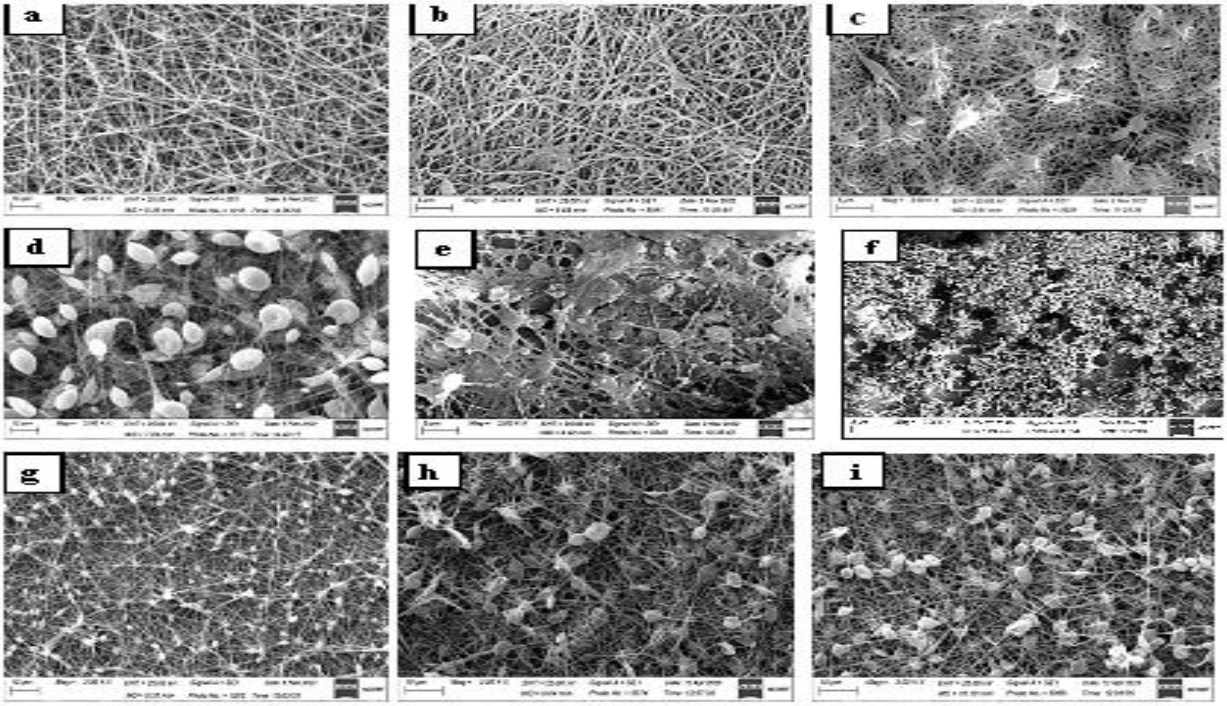

Figure 5: SEM images for PCL, PLA and PCL/PLA Nano fibrous scaffolds before and after 2& 4 days from VERO cell transplantation

It is shown from figure 5, images (a, b, c) represent PCL fibrous scaffold before and after 2 & 4 days from Vero cell transplantation that reflect the biocompatibility of cells on PCL scaffold. images (d, e, f) represent PLA fibrous scaffold before and after 2 & 4 days from Vero cell transplantation but the fiber form was hidden completely after 4 days due to its highly degradation rate. images (g, h, i) represent PCL/PLA fibrous copolymer before and after 2 & 4 days from Vero cells transplantation that reflect good spreading of seeded cells over this scaffold.

Briefly, by comparing images (c, f, i) that represents Vero cells over different scaffolds after 4 days, we could deduce that fibrous copolymer PCL/PLA scaffold have better properties and have highly biocompatibility with the cells than their single components PCL and PLA electrospun fiber scaffold. All the scaffolds under investigation were prepared by electrospinning technique.

The two investigated synthetic biopolymers are PCL and PLA. Pure PLA has good biocompatibility, high degradation rate, its degradation final products are (CO_2 + H_2O) and the intermediate products are (lactic acid + hydroxy acid) that are completely accepted by the body [**18**]. Pure poly PLA has good tensile strength, brittle and lack of toughness. Degradation properties decrease rapidly when high strength fibers have to be added **[20]**. Electrospun PLA fibers have a positive effect on angiogenesis, inflammation and collagen deposition and have the ability to accelerate the rate of wound healing compared to commercial gauzes **[21].** PCL is important biodegradable polyester emerging into biomedical applications because of its good mechanical properties, biocompatibility, biodegradability, chemical and thermal stability but its degradation rate is slower than PLA **[22]**. It is an aliphatic semi-crystalline polyester polymer accepted by the US Food and Drug Administration (FDA) for biomedical uses. In addition to good biocompatibility, PCL also has the characteristics of good solubility in various organic solvents, low melting point near 60 ℃ and remarkable mixture compatibility, which make it an ideal candidate for tissue engineering research **[23].** Although PCL is biocompatible and very easy to handle and shape, its use in biological applications is limited due to its hydrophobicity and lack of active sites. This will allow it to immobilize or bind biomolecules that can actively interact with cells to enhance their tissue engineering and drug delivery properties **[24].**

Biodegradable polymers or copolymers may be the best materials for fabricating supports for biomedical application. For example, a Synthetic biodegradable PCL/PLA copolymer may be used as a biomedical scaffold because PCL, PLA and their copolymers are the best promising materials used for biomedical applications. Briefly, PCL/PLA copolymer has better properties compared to single component like PCL or PLA [**18**]. Both PLA and PCL are Synthetic materials. However, PLA is more elastic than PCL and is more biodegradable than PCL [**25, 26].** Blending both scaffolds together produces a scaffold with desired mechanical properties and longer degradation time **[27**]. PCL/PLA blend scaffolds are

characterized by tunable porosity, controlled degradability, and successful cell-material interaction **[28**].

In this study, Vero cells were used to demonstrate the efficiency of the two fabricated electrospun scaffolds PCL, PLA and PCL/PLA combined together. Vero is a continuous cell line that derived from kidney of the normal African green monkey. Vero cell has been shown to have the size that ranges between 403-369 microns **[29]**

Cell culture is generally applied for testing biomaterials in general since it allows for an immediate evaluation of the biological performance of biocompatible polymers. It provides a prediction of the possible reactions to these polymers *in vivo* when utilized as substitute parts in the human body for stimulating restoration of damaged tissues without consuming experimental animals **[30].** Cell culture is also a very important methodology for biomaterials research because it permits a fast evaluation of the biological performance of the biopolymers. Thus, it is possible to search quickly for the best materials to be easily used in experimental animals. Our goal is the biological evaluation for different electrospun scaffolds to stimulate cell adhesion, growth, and differentiation in vitro. It is important to evaluate the cell–biomaterial interaction for the prediction of possible reactions to these polymers in vivo when used as substitute body parts or to stimulate the regeneration of damaged tissues **[30].** Although using animals in experimental tests is essential, such experiments are expensive, and do not allow the evaluation of the direct effects of biomaterials on the stimulation of important parameters such as cell adhesion, growth, and differentiation. However, adhesion of mammalian cells to polymer scaffolds is one of the important issues in tissue engineering, which depends on the ability to direct specific cell to grow, migrate, and make physiological behaviors in to produce a cellular architecture that can perform functions of the desired tissue **[31].** In the present study it is evident that Vero cells were successfully attached to PLA, PCL and PCL/PLA successfully. However, best adhesion is observed with the PCL/PLA blend.

4. Conclusion

It is concluded from this study that the electrospun nanofiber (PCL/PLA) copolymer applied a good spreading for the seeded Vero cells grown over it that referred to its high biocompatibility with the cells compared to their single components PCL and PLA electrospun fibrous scaffolds.

Conflicts of interest

The authors declare that they have no conflict of interest.

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