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An Investigation into Biocompatibility and Biodegradability of Electrospuned PLLA Nano-Scaffold

Elmira Pasban[*1], Shahrbanoo Oryan[2], Asadollah Asadi[3] and Akram Eidi[1]

1. Department of Biology, Science and Reaeach Branch, Islamic Azad University, Tehran, Iran
2. Department of Biology, Faculty of Biological Sciences,. Kharazmi University, Tehran, Iran
3. Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil,Iran

Corresponding author Email: Elmira.pasban87@gmail.com

ABSTRACT: Tissue engineering provides a new research field. In this new research field, cell scaffold and biomolecules are used to create an extended landscape in restoration, repair and maintenance of tissues. In this study, nano properties of polymer scaffolds for tissue engineering purposes are discussed. PLLA polymer scaffold was created using electrospinning technique, chloroform solvent and DMF. Then the surface of scaffold was treated to improve biodegradability. Scaffold was investigated in terms of surface morphological characteristics, biodegradablility, porosity and pore size. For this purpose, porosity measurements with Archimedes law, biodegradablility test and scanning electron microscopy study were performed. Degradation rate of scaffold is high because there are small pores and scaffold degradation rate is proportional to the time. The most important factor in the biodegradable scaffold is direct relationship between the amount of biodegradable scaffold and water absorption by the scaffold. Evaluation of cell growth and increased cell growth in the presence of nano-scaffold showed the confirmed the biocompatibility of nano-scaffold. It indicated high porosity of 90/75 percent and internal tissue growth.The overall results showed that PLLA nano-scaffold polymer synthesized with electrospinning technique is a good candidate for tissue engineering.

***Keywords*:** Nano-scaffolds, Electrospinning, Biocompatibility, Biodegradability

INTRODUCTION

In general, tissue engineering is development and transformation of in vitro growth of cells in a tissue to replace or repair damaged parts of the body (Rezwan et al., 2006). Three-dimensional cell growth and complex networksare developed to replace damaged tissue. Tissue engineering is a multi-disciplinary field that uses principles of engineering and biological sciences to generate biological alternatives (Marler et al., 1998). These alternatives can restore, maintain or improve function of a tissue or one organ. In fact, experts in biological sciences understand properly peculiarities of the environment encountered by cells in the body and several factors effective in the regulation of different cell behaviors (Fred et al., 1994). They can cooperate with experts in engineering to provide suitable conditions for cells to make certain tissues or organs. For tissue creation, it is necessary that cells to be embedded within the space of three-dimensional porous scaffold. This structure is purely temporary and is not a part of the final tissue (Peter et al., 2000). However, it is only a tool that allows cells to synthesize three-dimensional space, and gradually make the desired tissue under proper physical, chemical and mechanical conditions. Tissue engineering scaffolds play the role of the extracellular matrix (Ikada 2006).
Characteristics of an ideal scaffold are as follows:
1.Good mechanical strength, which is important in the engineering of hard tissues, size and porosity, scaffold fabrication methods and constituent materials are among the factors affecting the mechanical properties of scaffold.
2.Scaffold should be biocompatible and biodegradable.

3.Scaffold should have high porosity. Increased porosity rate leads to increased surface area ratio to volume ratio.
4.Scaffold should not induce toxic responses.
5.It should have the ability to provide the right size and shape in order to be replaced in the target tissue
6.It should have the ability of stem cells effects for proliferation
7.It should be capable of specific interactions with other cells (Chaignaud et al., 1996; Koh et al., 2004; Salgado et al., 2002).

Electrospinning

Electrospinning is among common applicable techniques in which nanotechnology techniques are used in the construction of scaffold. The mechanism of electrospinning system uses high-voltage electric field. It also includes a container in which polymer scaffold solution will be poured. This material is distributed under the high-voltage electric field (such as root fibers) and some nanoscale fibers would be created. The reason is that energy overcomes the surface tension of the liquid polymer. This material is obtained as a filament instead of spray droplets. The solvent is evaporated gradually and finally the product or fibers are collected on the collector (Janjanin et al., 2008; Pham et al., 2006).

MATERIALS AND METHODS

Fabrication of nano-polymer scaffold

PLLA polymer with intrinsic viscosity of 1 is dissolved in the chloroform solvent and DMF. The prepared solution was entered to the 5 mL syringe. Electrospinning is a high voltage source with 2 electrodes in which one electrode is connected to the needle and another one is connected to the surface of aluminum plate (collector plate). Needle distance from plate collector is 15 cm. Injection began after connections. Injection rate was 0/4 ml/h . High voltage was equal to 15 kV. Nanofibers formed on the aluminum foil of in the collector plate were collected. Scaffold fabrication was performed in vitro at 25 ° C over 6 h. Then the scaffold was optimized with plasma treatment. Plasma treatment was performed (Huang et al., 2003). Weight of the samples was measured in the dry state(Wi).
They put in PH or PBS ,7.4, for 40 days. Wet weight (Ww) was measured followed by dry wet (Wd). PBS buffer was also measured (El-Kady et al., 2010).
Determination of weight loss and water absorption of the samples was obtained using the following formula.

weight loss % = $\left[w_i - w_d / w_i\right]\times 100$

water absorption%= $\left[w_i - w_w / w_w\right]\times 100$

Measurement of nano-Porosity scaffold

Porous structure of scaffold can directly affect the stimulation of cells, transfer of nutrients and oxygen. In this measurement, initially dry scaffold and submerged scaffold weighted in two fluids (Water and ethanol). The density of ethanol and water was calculated based on weight-to-volume ratio. Initial volume of scaffold (V1) calculated based on considering parameters of length (a), width (b) and thickness (c). Next, second volume (V2) was calculated using weight of scaffold immersion in water and ethanol proportional to density of ethanol-water (Yang et al., 2003). Finally, scaffold porosity was calculated by the following formula:

$$p_e = \frac{m_e}{v_e} \qquad p_w = \frac{m_w}{v_w}$$

$$v_1 = abc \qquad v_2 = \frac{w_w - w_e}{p_e g - p_w g} \quad \varepsilon = \frac{v_1 - v_2}{v_1}\times 100$$

Scanning electron microscopy test

There must be an interconnection between scaffold pores for transfer of nutrients, oxygen, cell motility, and removal of wastes. Pore size and scaffold porosity were determined by analysis of gold coated nano-scaffold electron microscopy images. (Hong et al., 2007).

Nano-porosity structure of scaffold

The porosity size of the scaffold (scaffold pores) was measured by electron microscopy images with specific magnification scale using Image J software.

Biocompatibility of nano-scaffold

It is a colorimetric method based on tetrazolium reduction and breakdown of yellow crystals by succinate and hydrogenase in formation of insoluble blue crystals. Unlike the other methods, steps of washing and removal of the cell (causing a loss of some cells) are omitted. All stages, from planting to reading photometer results are performed in a plate (Falak et al., 2007). Generally, cells in medium are of two forms. They are either attached to scaffold or attached to the bottom plate. Initially, appropriate number of cells to be cultured in 24 plate in each block. Cells are allowed to attach to the bottom plate or connect to the scaffold. The appropriate amount of the substance or drug will be tested to incubate the time required to effect the desired material. After incubation, the supernatant medium will be discarded. MMT ½ mg ml will be added to each block of the plate in the medium contains. Then it put in CO2 incubator in the in 37 ° C. During MTT incubation by succinate dehydrogenase (One of mitochondrial respiratory chain enzymes), restoration and breaking of this cycle leads to production of blue crystals of Formazan that can be observed under the microscope. Before coloimetric method, crystals are dissolved using DMSO. Finally, optical absorption in a wavelength of 570 nm in solution can be read. To investigate the cell survival and cellular biology, specified values of of the cells in 24-plate are cultured with or without scaffold and transferred in to 5% CO2 incubator. MMT test was performed on first, third and seventh days after the test. Diagrams plotted using obtained optical absorption values. To evaluate the biocompatibility of scaffold, first scaffold components put in alcohol and then placed under ultraviolet light for 10 min for sterilization. Scaffolds were incubated using PBS buffer in 37^0C. Cells at a concentration of 1×10^5 poured on the scaffold in one milliliter, put in 5% CO2 incubator in in 37 ° C to connect cells to bottom plate and scaffold. After mentioned time, DMEM medium along with 20% FBS and one percent of antibiotics was added to samples. Tests of biocompatibility were performed during a week in, first, third,and seventh days. RPMI medium and MMT color were added and incubated. Next, an evacuated medium and DMSO added and put in the incubator (Helgason et al., 2007). Samples transferred in to cubits and ELISA reader at a wavelength of 570 was used to measure light absorption.

FTIR test

Samples FTIR was measured to investigate Nano-scaffold structure, structure and chemical bonds and the peaks (Rezwan et al., 2006). Structural changes of the samples were within the spectrum range of 400-4000cm -1.

RESULTS AND DISCUSSION

Confirmation of the synthesis of nano-scaffold

Absorption peaks in area of 860 cm -1, 1182 cm -1 and 1752cm -1 are related to the presence of carboxyl groups while absorption peaks in area of 1452 cm -1, 2991cm -1 are attributed to the presence of hydroxyl groups (fig 1).

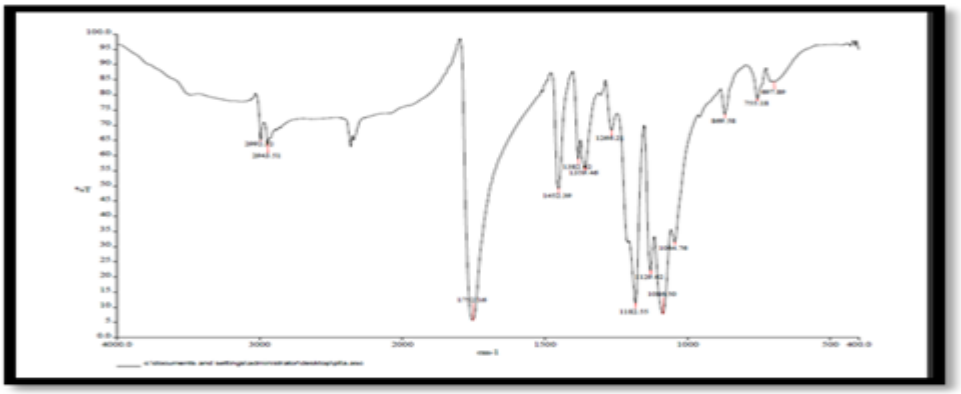
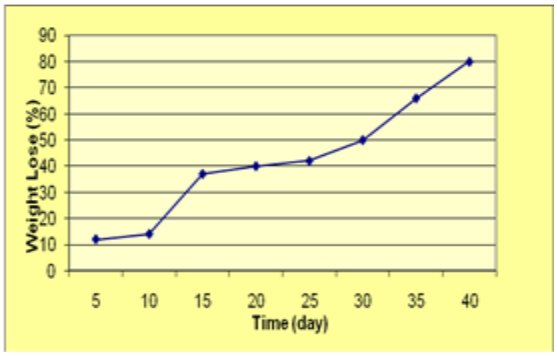


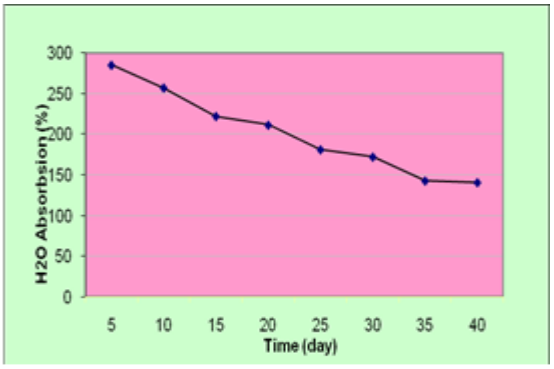
Figure 1. FTIR spectra for PLLA nano-scaffold

Nano-scaffold biodegradability

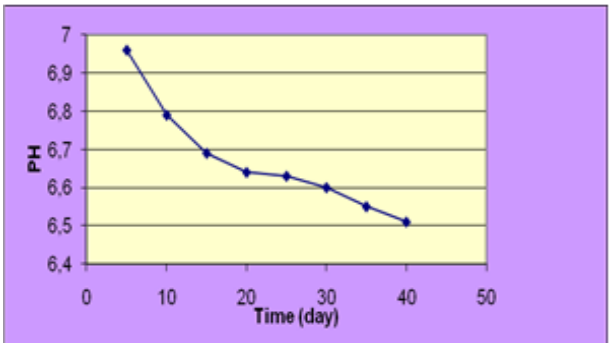
According to obtained results, it can be said degradation rate of scaffold is high because there are small pores and scaffold degradation rate is proportional to the time. About 12% scaffold degradation occurs during the first 5 days , 40% after 20 days and 80% after 40 days (fig 2: A). Moreover, an important factor in the biodegradable scaffold is direct relationship between the amount of biodegradable scaffold and water absorption by the scaffold because high water absorption can facilitate scaffold hydrolysis through interactions between water molecules and the polymer matrix. Results of water absorption by scaffold showed that 210 percent of water absorption was obtained on the twentieth day. At the end of 40th day, 80 percent of the scaffold was degraded (fig 2: B). Water absorption at 145% level of evaluated buffer PH (in which scaffold degredation was occurred) indicated decreased PH value. The largest decrease was observed at the end of the day 10. After 40 days, the damage was minimal (fig 2: C). This decrease indicates the presence of monomer acid lactic in polymer synthesis. Polymer is isolated to the monomer maker.



(A)



(B)



(C)

Figure 2. weight loss in nano-scaffold biodegradability (A) and water absorption (B) and pH value (C)

Nano-porosity scaffold

The results show that the synthesized scaffold porosity was 90/75%. This high porosity increases tissue growth. Thus, it influences the transfer of nutrients and oxygen in the three-dimensional matrix. It can directly affect the cells stimulation. Optimal porosity is over 90 percent that enables the maximum cell change and conversion required for tissue fabrication.

Table 1. percent of prosity PLLA nano-scaffold

Persent of Porosity (%)(ε)	density of ethanol (p_e) g/cm^3	density of water (p_w) g/cm^3	Initial volume of scaffold (V1) cm^3	second volume of scaffold (V2) cm^3
90.75	0.7488	0.908	0.04	0.0037

Scaffold pore size and evaluation of the morphology of samples by scanning electron images

Porosity and porosity measurements are important parameters in determining the mechanical properties. Thirty samples of scaffold pores were measured by scanning electron microscopy images and the pore size was determined. Maximum pore size was in the range of 20-60 micrometers. Small pore size is considered as one of

the most important factors of tissue engineering. Images indicate internal relationship between porosity plays an important role in supplying the cell and disposal of cell wastes. According to images, it was observed that samples have a porous structure (fig 3).

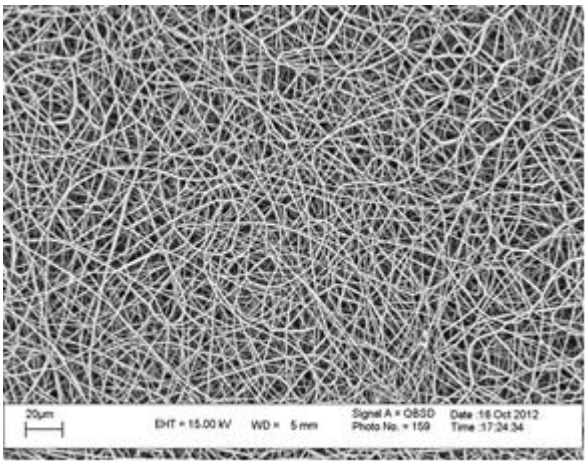


Figure 3. scanning electron microscopy image from PLLA nano-scaffold (500X)

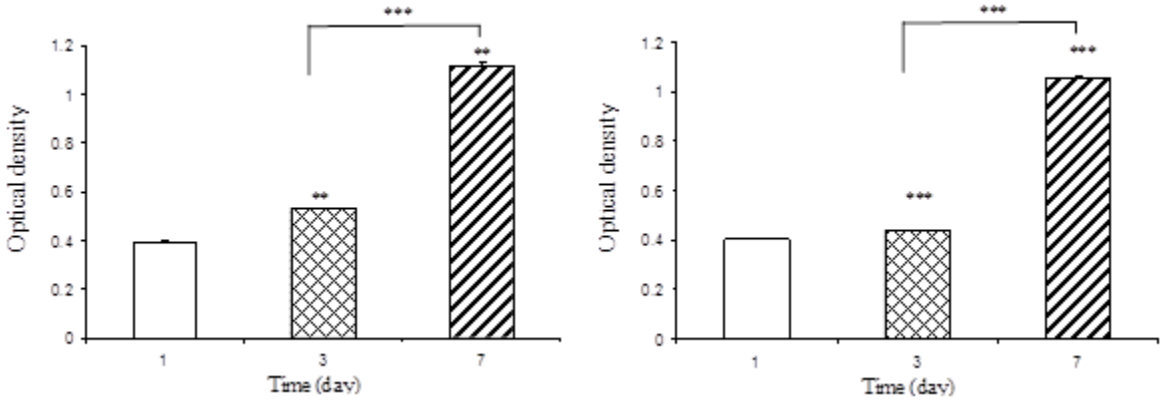


Figure 4. Biocompatibility of nano-scaffold, diagram of cell bidimentional growth on day 1, 3 and 7 (A) and cell attached to scaffold on day 1, 3 and 7

CONCLUSION

Recently, the electrospinning technique has been widely provided to prepare micro pour biodegradable and biocompatible scaffolds. This technique is economical and easy to use tool to generate nano -fiber scaffolds (Vail et al., 1999). It is obtained by a variety of biodegradable polymers which is able to mimic the natural extracellular matrix structure and biological functions (Langer and Vacanti, 1993). PLLA polymer scaffold synthesized using electrospinning technique, chloroform solvent and DMF. In order to perform surface modification for this study, scaffold surface plasma was optimized with treatment method. This technique was performed by CO2 gas. This treatment improved the quality of scaffold. It means that condition was optimized for growth, proliferation, and differentiation. According to importance of relationship inside the pore in cell migration, transfer of nutrients to scaffold depth and removal of waste products from scaffold , one cutting was made in the scaffold. This cutting was investigated in terms of internal relationship between pores. Therefore, scaffold was cut with gold. Evaluation of scanning electron microscope images of the surface of the scaffold indicated highly porosity of scaffold. The cross-section images of scaffold confirmed high level of interrelationship of the pores (El-Kady et al 2010). Pores size was determined by measuring 30 porous samples. Maximum pore size was in the range of 20-60 micrometers. Biodegradable test were performed in phosphate buffer for biodegradable nano-scaffold. Based on the results obtained it can be said that degradation rate of scaffold is high because there are small pores and scaffold degradation rate is proportional to the time (Do Kim et al., 2004). This leads to cell growth, transport of

nutrients and wastes. The most important factor in the biodegradable scaffold is direct relationship between the amount of biodegradable scaffold and water absorption by the scaffold. Moreover, an important factor in the biodegradable scaffold is direct relationship between the amount of biodegradable scaffold and water absorption by the scaffold because high water absorption can facilitate scaffold hydrolysis through interactions between water molecules and the polymer matrix (Freed et al., 1999). Reduced water absorption indicates a direct relation between absorption of water by scaffold and biodegradability of scaffold. Scaffold porosity measurement was performed by the Archimedes technique. Results indicated that the synthesized scaffold porosity was 90/75%. This high porosity increases tissue growth. Thus, it influences the transfer of nutrients and oxygen in the three-dimensional matrix. It can directly affect the cells stimulation. Optimal porosity is over 90 percent that enables the maximum cell change and conversion required for tissue fabrication (Helgason et al., 2003). To evaluate the biocompatibility of scaffold, cells were cultured on the scaffold and growth value was determined using light absorption measurement. The cells growth was investigated under conditions without scaffold in 7 days. MMT test was performed on first, third and seventh days. Cells in the 3 and 7 days showed significant growth compared to the first day. Under conditions of cells attached to the scaffold, in the days 3 and 7 with significant growth compared to the first day (Wang et al., 2010). Cells growth in the presence of scaffold is relatively more than absence of scaffold. Confirmation of biocompatible scaffold is the most important experiment to confirm scaffold application in the engineering field (Heyman et al., 2007). Conclusion of this study showed that PLLA nano-polymer scaffold synthesized using electrospinning is an appropriate candidate for tissue engineering.

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