CHEMICAL ENGINEERING TRANSACTIONS
VOL. 32, 2013
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-23-5; **ISSN** 1974-9791
DOI: 10.3303/CET1332182

Poly (α-Hydroxy Ester-Urethane) Based Electrospun Biomaterials for Tissue Engineering

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Generally fibrous mats are produced via electrospinning and utilized as tissue engineering scaffolds. This technique is widely used in tissue engineering studies due to unique properties of the resulting mats have, such as large surface area-to-volume ratios, high porosity and connected porous structure. In this study, alternatively, novel electrospun non-fibrous surfaces were prepared from poly (α-hydroxy ester-urethane) (PU). Low molecular weight α-hydroxy ester based polyurethane was synthesized from L-lactide, glycolide and dimethylol propionic acid (DMPA). Biodegradable and biocompatible PUs were then electrospun to obtain non-fibrous surfaces with high porosity and interconnectivity. Collagen blended PU was also prepared for improving cell adhesion and proliferation. 3T3 cells were seeded on electrospun non-fibrous scaffolds. The morphology of novel surfaces was characterized by environmental scanning electron microscope (ESEM). Biocompatibility of the scaffolds was examined by using MTT cytotoxicity assay. According to the results, electrospun scaffolds were non-toxic. 3T3 cells adhered to the electrospun scaffolds and settled into pores in the non-fibrous mesh.

1. Introduction

Tissue engineering, also called regenerative medicine has become a promising approach to generate new or substitute tissues for the replacement of defective or diseased tissues and organs (Agarwal, et al. 2008 and Baker, et al. 2009). Although a wide range of polymers have been investigated in tissue engineering studies, the most common synthetic biodegradable polymers used for tissue engineering applications are FDA approved, poly(alpha-hydroxy esters) including poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) (Li, et al. 2006).

Polyurethane (PU) based biomaterials are widely used in tissue engineering applications due to their biocompatibility and excellent mechanical properties and they are especially suitable for soft tissue engineering applications (Martina and Hutmacher, 2007; Frenot and Chronakis, 2003). Electrospinning is a novel and efficient fabrication technique that can be utilized to produce fibrous polymer mats composed of fiber diameters ranging from several microns down to fibers with diameter lower than100 nm and it are widely used for tissue engineering studies (Frenot and Chronakis, 2003).

In this technique an electric voltage between 0 and 50 kilovolts is applied to a polymer solution that is kept in a syringe or a glass pipet. Polymer solution can be fed to the tip of the capillary either by gravitational force or via a syringe pump. When a syringe pump is used, the flow rate can be controlled. This allows to obtain homogeneous electrospun fibers with narrow diameter distributions. The voltage is generally applied via electrodes which are attached to the tip of the syringe. The counter electrode is attached to the collector which is generally an aluminium foil. When the applied voltage reaches to a point, the droplet on the edge of the syringe becomes charged and the repulsions on the surface of the droplet work against the surface tension of the polymer solution. As a result of these forces the droplet is stretched and a cone shape begins to form. This shape is known as "*Taylor Cone*". A further increase in the voltage causes a polymer jet. While this jet travels to the collector, the solvent evaporates and the jet gets thinner. Finally micron or nano sized fibers are collected on the aluminium collector. There are several factors that affect the properties of these fibrous mats. The solution viscosity should be at a certain value in order to obtain beadless fibers. The viscosity of the polymer solution is related with the molecular weight of the polymer, its concentration and also the choice of the solvent. The increase in the molecular weight of the polymer increases the viscosity of the polymer solution and thus the spinnability of the polymer solution enhances.

Please cite this article as: Apohan N.K., Cakmakci E., Cigdem C., Kahraman M.V., Arslan M., Erdem Kuruca S., 2013, Poly ?-hydroxy ester-urethane) based electrospun biomaterials for tissue engineering, Chemical Engineering Transactions, 32, 1087-1092 DOI: 10.3303/CET1332182

On the other hand the increase in viscosity also increases the fiber diameters. Thus the concentration of the polymer solution should be precisely adjusted to a value that causes sufficient polymer viscosity for electrospinning and beadless morphology. Moreover the applied voltage, the distance between the collector and the syringe, temperature, atmosphere, humidity, additives, solution pH, diameter of the spinneret (needle gauage), solvent system and the collector type have decisive effects on the final polymer mat. The increase in voltage first decreases the fiber diameter than a further increase in voltage generally causes a reduction of the fiber diameter. Additives such as salts or acids increase the conductivity of the polymer solutions and the spinnability of the polymer solution improves. An increase in temperature increases the evaporation rate of the solvent and also decreases the viscosity of the polymer solution which in turn decreases the fiber diameter. The distance between the collector and the syringe can also be adjusted to control the diameter of the fibers. As this distance increases, the polymeric jet find much more time to get thinner and thus the diameter of the fibers is reduced. Also the evaporation of the solvent is much more effective when the distance is longer. (Çakmakçı, et al. 2012)

In recent years several modifications have been made to improve the properties of the polymer mats. Reactive electrospinning, coaxial electrospinning, emulsion electrospinning and melt electrospinning are only a small portion of the modified electrospinning techniques.

Besides tissue engineering, electrospinning has a wide range of uses ranging from textile applications to medical applications, from sensors to catalysts, from filters to composites, from cosmetics to military applications.

PLGA or PLA based systems have the advantage of controllable degradation profile and controllable surface hydrophilicity. These two factors have an enormous effect on the cell adhesion and the proliferation of the seeded cells. The hydrophilicity and the degradation profile of PLGA can be adjusted by con trolling the ratio of lactide to glycolide. On the other hand the surface of both PLGA and PLA can be modified to increase their hydrophilicity which enhances cell adhesion. Moreover these polymer can be blended with other polymers to control these features. In literature there are several studies that focus on the preparation of PLGA or PLA based electrospun nanofibrous scaffolds. In most of these studies, fibrous surfaces are generated. Contrary, in the present work, highly porous non-fibrous novel PU scaffolds based on poly(α-hydroxy ester) were prepared by electrospinning. Collagen coated PU scaffolds were also prepared to improve the cell adhesion. In this study we prepared electrospun scaffolds from low molecular weight PLGA based polyurethanes. This allowed us to obtain biodegradable porous scaffolds with novel morphologies.

2. Experimental

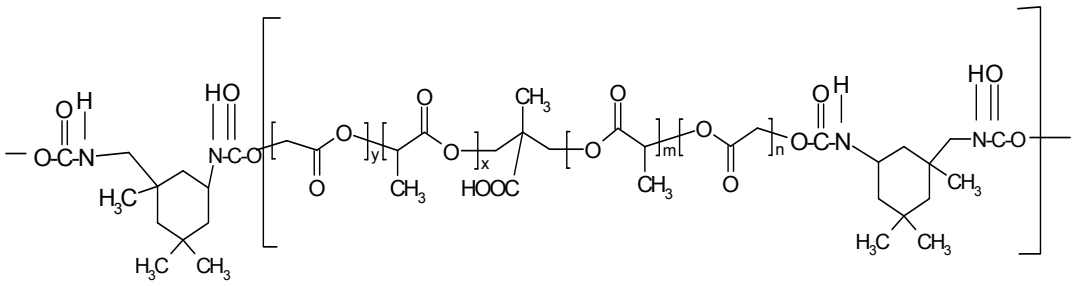
2.1 Materials

Dibutyltin dilaurate and tin 2-ethylhexanoate were kindly supplied by Henkel. L-Lactide (LA), glycolide (GA) and isophorone diisocyanate (IPDI) were purchased from Alfa Aesar. LA was recrystallized from dry benzene and dried under vacuum at room temperature before use (Akdemir, et al. 2011). GA was also dried under vacuum prior to use. Dimethylolpropionic acid was obtained from Perstorp and dried before use. Collagen was obtained from Calbiochem and used by dissolving in acetic acid (0.1 M). All other chemicals and solvents were of analytical grade and were purchased from Merck AG. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Aldrich. Double distilled water was used throughout the experimental work.

2.2 Synthesis of polyurethane

Polyurethane (PU) was synthesized from the reaction of IPDI with poly (α-hydroxy ester). First, dimethylol propionic acid initiated poly(L-lactide-co-glycolide) copolymer (D-PLGA) (lactide/glycolide = 75:25 (wt./wt.) was prepared by ring-opening copolymerization. Briefly, calculated amounts of monomers and DMPA were charged into a flame dried three-neck round bottom flask in a nitrogen atmosphere. Then, tin 2-ethylhexanoate was added as a catalyst. The reaction was carried out at 110 ˚C for 24 h. The resulting polymer was purified using anhydrous chloroform and after complete dissolution, the mixture was precipitated in excess methanol and then filtered and dried at 40˚C under vacuum for 24 h. The reaction yield was 89 % for D-PLGA, on the basis of the mass of precipitated and purified D-PLGA .

In the second stage polyurethane (PU) was synthesized with a ratio of NCO to hydroxyl of 0.75. D-PLGA was dissolved in chloroform. Then the temperature was raised to 70 ˚C and IPDI was added drop by drop in two hours. After two hours, dibutyltin dilaurate was added as a catalyst and the reaction was further continued for 1 h. The reaction system was then cooled to room temperature and D-PLGAPU was dissolved in chloroform. The product was then precipitated in excess methanol, filtered and dried under vacuum at 40 ˚C for 1 day. The structure of D-PLGAPU is presented in Figure 1.



D-PLGAPU

Figure 1: Structure of D-PLGAPU

2.3 Electrospinning

For the electrospinning experiments Nano FMG NE100 laboratory type electrospinning unit was used. 15 wt % poly (α-hydroxy ester) based polyurethanes containing solutions were prepared by dissolving the required amount of polymer in a mixture of THF-DMF (3:1, v/v). The applied voltage was 21 kV and the distance between the collector and the syringe tip was 13 cm. A syringe pump was used to feed the polymer solution to the tip of the syringe with a feeding rate of 1 mL/ h. Fibers were collected on aluminum sheets. Also in order to enhance cell attachment and cell proliferation collagen blended samples were prepared by adding 1 mL of 0.1 M collagen solution in acetic acid to polymer solutions in THF-DMF mixture.

2.4 Cell culture studies

Cell culture and cell seeding experiments were conducted as described in our previous studies (Mosmann, 1983; Akdemir et al., 2011; Çakmakçı et al., 2012).

2.5 Characterization

FT-IR spectrum was recorded on Perkin Elmer Spectrum100 ATR-FTIR spectrophotometer. ^{1}H-NMR was performed in CDCl3 400 MHz Mercury-VX 400 BB model NMR spectrometer. The molecular weight of the polymers was determined by gel-permeation chromatography (GPC) using a Waters pump, a refractive-index detector (model 2414) and Waters Styragel columns placed in series (HR 5, HR 4E and HR3). THF was used as eluent at a flow rate of 0.6 mL/min. Polystyrene standards were used for calibration. All measurements were performed at 40 °C.DSC measurements were performed using Pyris Diamond DSC. Samples were run from 30 to 200 °C with a heating rate of 10 °C / min. Glass transition temperatures were obtained from the second heating scan. The morphology of the electrospun fibers were investigated by using an environmental scanning electron microscope (Philips XL30 ESEM-FEG/EDAX).

3. Results and Discussion

The goal of this study was to prepare novel tissue engineering scaffolds via electrospinning technique. Generally electrospun fibrous mats are used for tissue engineering studies. Alternatively, we aimed to prepare novel electrospun non-fibrous surfaces with high porosity.

The molecular weight and thermal properties are summarized in Table 1 for the polymers. It can be seen from the table that the Mn of D-PLGAPU was 9.000 g/mol. It can be seen that PU polymer was synthesized with narrow polydispersity. Thermal properties of the synthesized polymers were investigated by DSC. As seen from Figure 2, the glass transition temperature was 45 °C for D-PLGAPU.

Table 1: Molecular weights and thermal properties of synthesized polymers

Sample Design :	D-PLGA	D-PLGAPU
Mn x 10^{-3} (g/mol)	6000	9000
Mw/Mn	1.23	1.17
Tg (°C)	47	45

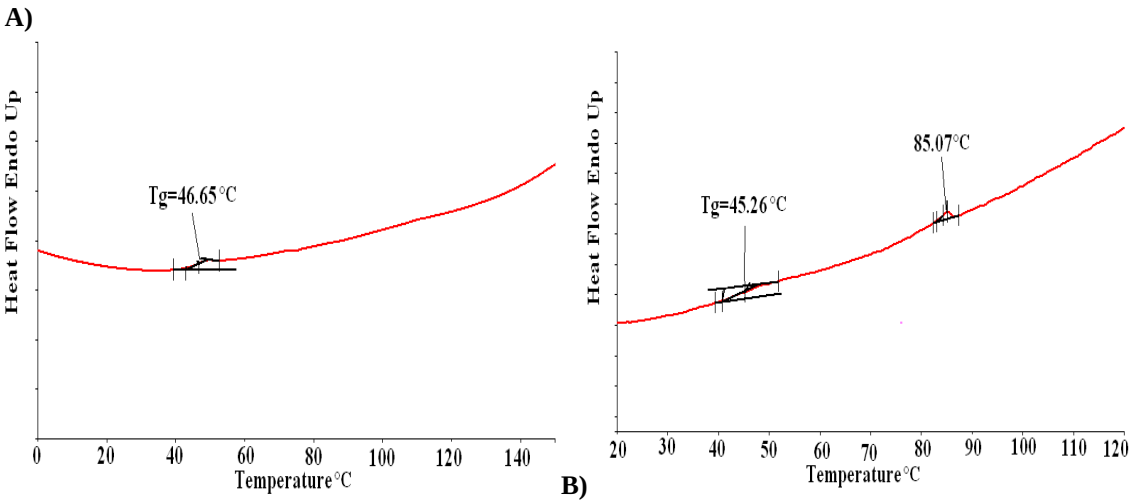


Figure 2. DSC thermograms of a) D-PLGA b) D-PLGAPU

The morphological characterization of the electrospun biodegradable mats was determined by ESEM analysis. Figure 3 shows the ESEM micrographs of PU electrospun mats with and without collagen. It is clearly seen from these micrographs that the general morphology of the electrospun mats were non-fibrous and highly porous. Mats were composed of very tiny fibers with nano sized beads on them which were uniformly distributed. This novel morphology can be attributed to the low molecular weight of the polymers used. As discussed earlier, the viscosity and the concentration of the electrospinning solutions have a decisive effect on the morphology of the electrospun surfaces. In this study the use of relatively low molecular weight polyurethanes caused lower viscosities, which in turn prevented the formation of fibers. Thus, by using these low molecular weight polymers we obtained non-fibrous and highly porous electrospun surfaces in line with our goal to prepare novel structures for tissue engineering applications.

The in vitro biocompatibility of the electrospun scaffolds was tested by MTT assay. In our study, 3T3 mouse fibroblasts cells can attach on all scaffold materials. The cell viability of electrospun mats can be seen in Figure4.To sum up it can be stated that the electrospun mats were non-cytotoxic and supporting cell growth to fibroblast cells. 3T3 cells adhered to the electrospun scaffold and settled into pores in the non-fibrous mesh. As can be seen, the scaffolds improved the interconnectivity and also provided a unique physical support that allows the cell growth.

Electrospun mats that were used in cell culture were subjected to SEM analysis. Figure 5 shows the SEM micrographs 3T3 cells seeded D-PLGAPU and D-PLGAPU-C. It can be seen from these micrographs that cells were well attached and uniformly distributed all over the surface. It was observed that the cells did not vanish or lose their original structural shapes, and they also adhered to the surface of the electrospun mats. This is strong proof that poly (α-hydroxy ester) based polyurethanes are biocompatible materials and do not exhibit a toxic property.

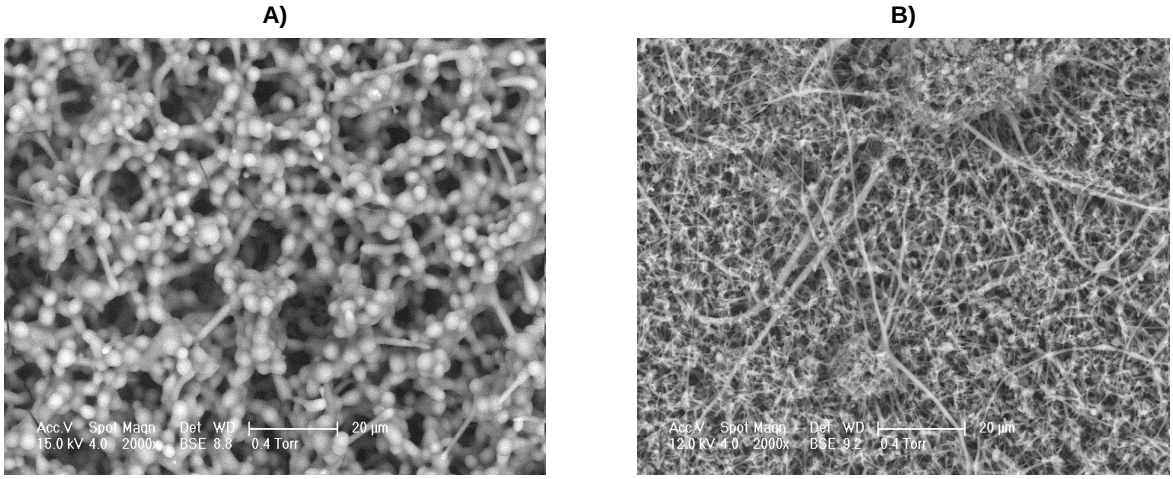


Figure 3: ESEM micrographs of a) D-PLGAPU b) D-PLGAPUC

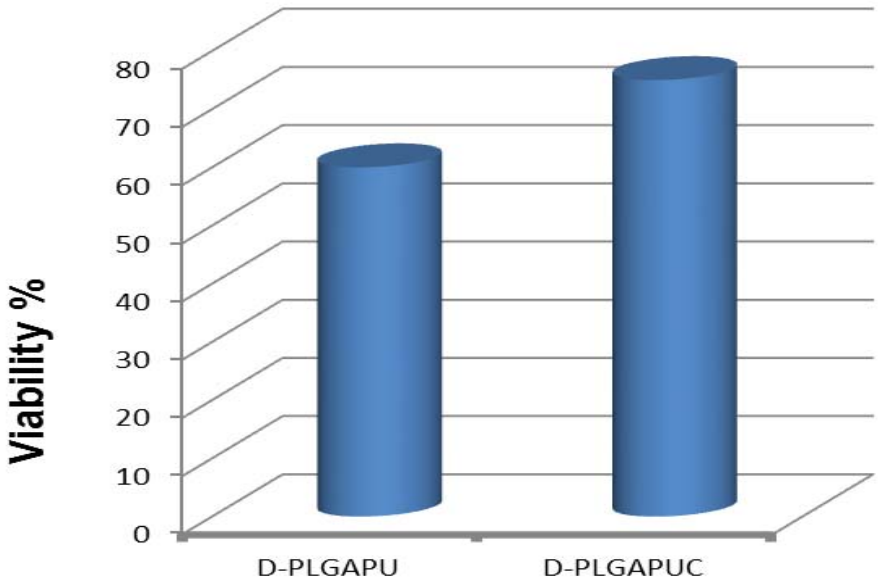
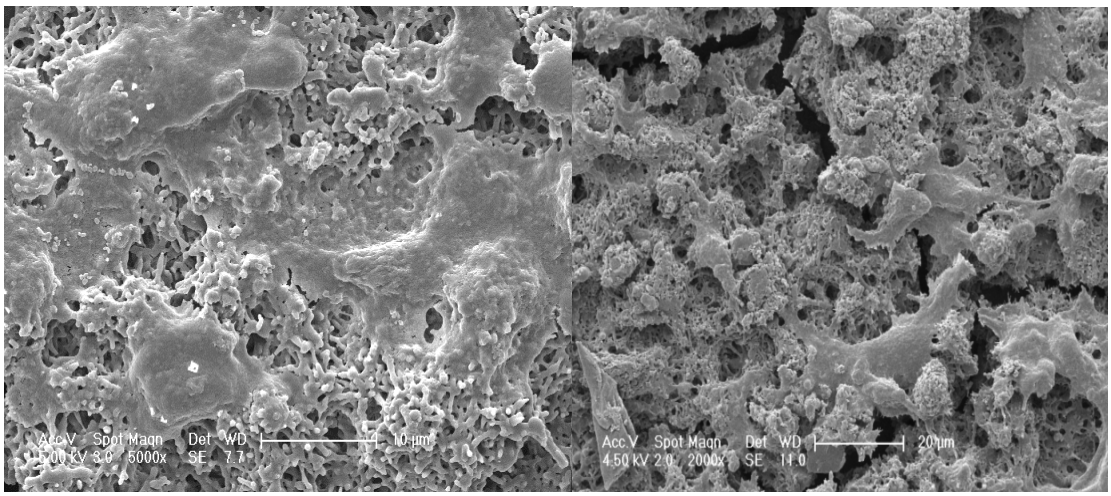


Figure 4: The % viability of 3T3 fibroblast cells cultured on novel scaffolds determined with MTT



A) ***B)***

Figure 5. SEM micrographs of 3T3 cells seeded electrospun scaffolds: a) D-PLGAPU b)D-PLGAPU-C

4. Conclusions

In literature there are several PLGA or PLA based studies where fibrous surfaces are generated using the electrospinning technique. In the present work, highly porous non-fibrous novel PU scaffolds based on low molecular weight poly(α-hydroxy ester) were prepared by electrospinning. The aim of this study was to develop alternative scaffoldings for tissue engineering. Therefore (α-hydroxy ester) based polyurethane (PU) with low molecular weight was prepared and then non-fibrous, highly porous and biocompatible scaffolds were obtained by electrospinning. Collagen containing and collagen free PU scaffolds were used in cell growing experiments. 3T3 cells were growth on electrospun scaffolds. The morphology of novel surfaces was characterized by environmental scanning electron microscope (ESEM). Biocompatibility of the scaffolds was examined by using MTT cytotoxicity assay. SEM images showed that the 3T3 cells spread very well and demonstrate strong extensive interconnection between themselves and the scaffolds. It can be concluded that the scaffolds improved the interconnectivity and also provided a unique physical support that allows cell growth and cell infiltration.

Acknowledgements:

This work was supported by Marmara University, Commission of Scientific Research Project (M.U.BAPKO) under grant FEN-A-080410-0078.

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