Colloids and Surfaces B: Biointerfaces 113 (2014) 101–106

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

# Effects of plasma treatment to nanofibers on initial cell adhesion and cell morphology

Wei Liu[a,b], Jianchao Zhan[b,c], Yan Su[a,b], Tong Wu[b], Chunchen Wu[b], Seeram Ramakrishna[d], Xiumei Mo[a,b,*], Salem S. Al-Deyab[e], Mohamed El-Newehy[e,f]

[a] State Key Lab for Modification of Chemical Fiber & Polymer Materials, College of Material Science and Engineering, Donghua University, Shanghai 201620, PR China
[b] Biomaterials and Tissue Engineering Lab, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, PR China
[c] College of Materials and Textile Engineering, Jiaxing University, Zhejiang 314001, PR China
[d] HEM Laboratories, National University of Singapore, Nanoscience & Nanotechnology Initiative (NUSNNI), 2 Engineering Drive 3, 117576, Singapore
[e] Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[f] Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

ARTICLE INFO

*Article history:*
Received 10 April 2013
Received in revised form 8 August 2013
Accepted 26 August 2013
Available online 1 September 2013

*Keywords:*
Plasma treatment
Hydrophilicity
Initial cell adhesion
Electrospinning
Nanofibrous mat

ABSTRACT

Poly-L-lactic acid (PLLA) nanofibers were fabricated by electrospinning and treated with $O_2$ plasma. The surface properties of PLLA nanofibers before and after plasma treatment were characterized by water contact angle measurement and X-ray photoelectron spectroscopy (XPS). It was found that the hydrophilicity of PLLA nanofibers was improved and the amount of oxygen-containing groups increased after plasma treatment. Initial cell adhesion was evaluated by cell capture efficiency based on the cell count method. The results showed that initial porcine mesenchymal stem cells (pMSCs) adhesion to plasma-treated nanofibers was significantly enhanced. Moreover, the morphology of pMSCs on PLLA nanofibers (PLLA NFS) and plasma-treated PLLA nanofibers (P-PLLA NFS) were observed by scanning electron microscope (SEM) after 10 min, 20 min, 30 min and 60 min cell adhesion. It was found that plasma treatment to electrospun nanofibers had a great effect on pMSCs morphology at earlier time points. Therefore, plasma treatment is an efficient surface modification strategy to improve cell adhesion in earlier culture time intervals. It may be a promising method in the design of novel tissue-engineered scaffolds.


## 1. Introduction

Large quantities of polymeric biomaterials employed in tissue engineering have been designed and synthesized in recent years. Synthetic polymeric biomaterials such as polycaprolactone (PCL) and Poly(L-lactic acid) (PLLA) have been known to have poor hydrophilicity and weak biocompatibility when compared to natural polymeric biomaterials. Plasma treatment is a promising method to modify the surface of materials and has been employed in tissue engineering to make suitable implants and scaffolds in recent years [1,2]. Different functional chemical groups could be introduced onto the surface of materials by different plasma modifying strategies, and the surface properties of materials such as wettability, surface energy and surface roughness were changed [3,4].

Poly(L-lactic acid) (PLLA) is a synthetic biodegradable polymer which has been approved by US Food and Drug Administration for clinical use. But scaffolds derived from PLLA lack bioactive signals for cell recognition and the surface of PLLA scaffold is hydrophobic [5]. There are many surface modification methods to solve these problems; plasma treatment is one such promising approach [6]. The effect of plasma-treated PLLA surface on cell adhesion has been widely investigated [7]. Different plasma strategies and cells were employed in those studies, but the results were similar. It was found that plasma-treated PLLA scaffolds showed improved surface hydrophilicity and better biocompatibility [8–11].

It has been confirmed that cell adhesion to materials surface (cell capture efficiency) played an important role in cell growth, spreading, proliferation and differentiation [12,13]. As cell adhesion on material surface is crucial for tissue formation *in vivo*, it is important to know how cells can interact with material surface *in vitro*. A better understanding of cell adhesion on material surface at the initial adhesion stage is helpful to the design of ideal scaffold for tissue engineering. Moreover, the study of initial cell adhesion can explore the possibility of an attractive therapeutic approach in which scaffold with an enriched population of target cells could be

* Corresponding author at: Biomaterials and Tissue Engineering Lab, College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, PR China. Tel.: +86 2167792653.
*E-mail address:* xmm@dhu.edu.cn (X. Mo).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.08.031

achieved. The hope of seeding the cells directly on scaffold *in situ* at the operating theater may be realized.

But as of current literature on cell adhesion to scaffolds after the cells have been seeded on scaffolds for hours or longer culture time [14,15], there are no reports on cell adhesion of plasma-treated materials, and cell morphology in an earlier time period such as in 10 min or 20 min. As it has been widely investigated that cell adhesion to plasma-treated surface was improved in hours or longer culture time, the aim of this work is to investigate mesenchymal stem cell (MSC) adhesion behaviors on electrospun PLLA nanofibers treated with plasma at earlier time interval. The surface properties of electrospun nanofibers were characterized by water contact angle and X-ray photoelectron spectroscopy (XPS). Initial cell adhesion was evaluated by the cell count method and the morphology of pMSCs on plasma-treated nanofibers was observed by scanning electron microscope.

## 2. Experimental

### 2.1. Materials

The material PLLA and the solvent 1,1,1,3,3,3-hexafluoro-2-isopropyl alcohol (HFIP) were purchased from Sigma–Aldrich Chemical Company (St. Louis, Missouri, USA). Essential Medium Alpha medium (α-MEM), fetal bovine serum, penicillin & streptomycin and 4′, 6-diamidino-2-phenylindole (DAPI) were purchased from Invitrogen Corporation (USA). All products were used without further purification.

### 2.2. Preparation of PLLA nanofibers

The PLLA nanofibers (PLLA NFS) were prepared by the electrospinning process. The electrospinning solution of PLLA was prepared by dissolving a 3% (w/v) PLLA solution in HFIP. Electrospinning was done by using a 5 mL standard syringe with a blunt-ended needle. The syringe was located in a syringe pump (789100C, Cole-Parmer, USA) and dispensed at a rate of 1.0 mL/h. A voltage of 20 kV was applied. The distance between the collector and needle was 12 cm. The nanofibrous membranes were dried under vacuum at room temperature overnight.

### 2.3. Plasma treatment of PLLA nanofibers

Electrospun PLLA nanofibrous scaffolds were treated 60 s using PDC-002 plasma (USA) in the presence of oxygen. The chamber was evacuated to less than 10 mTorr before it was filled with gas, followed by generation of glow-discharged plasma for a predetermined time.

### 2.4. Scanning electron microscopy

The morphology of PLLA NFS and plasma-treated PLLA nanofibers (P-PLLA NFS) was characterized by a JSM-5600 scanning electronic microscope (SEM) (Japan). The samples were mounted on aluminum holders before sputter-coated with gold platinum for better conductivity, and then the SEM images were taken.

### 2.5. X-ray photoelectron spectroscopy measurement

X-ray photoelectron spectroscopy (XPS) was performed with a PH1 1500C Spectrometer (Japan) in order to determine the surface chemical components of PLLA NFS and P-PLLA NFS. The X-ray source was a magnesium anode at 15 kV and 400 W. The relative amounts of differently bound carbons were determined from high

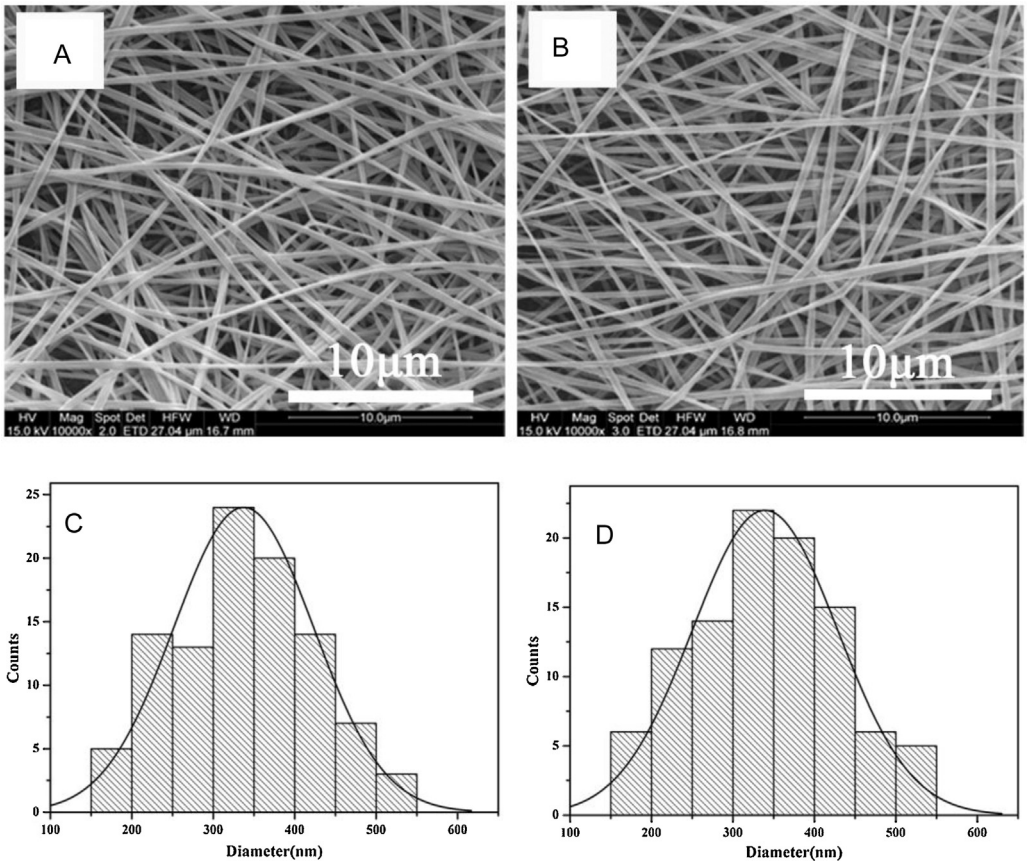


**Fig. 1.** SEM images of PLLA NFS (A) and P-PLLA NFS (B); The diameter distribution of PLLA NFS (C) and P-PLLA NFS (D).