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Improved infiltration of stem cells on electrospun nanofibers

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ABSTRACT

Nanofibrous scaffolds have been recently used in the field of tissue engineering because of their nano-size structure which promotes cell attachment, function, proliferation and infiltration. In this study, nanofibrous polyethersulfone (PES) scaffolds was prepared via electrospinning. The scaffolds were surface modified by plasma treatment and collagen grafting. The surface changes then investigated by contact angle measurements and FTIR-ATR. The results proved grafting of the collagen on nanofibers surface and increased hydrophilicity after plasma treatment and collagen grafting. The cell interaction study was done using stem cells because of their ability to differentiate to different kinds of cell lines. The cells had normal morphology on nanofibers and showed very high infiltration through collagen grafted PES nanofibers. This infiltration capability is very useful and needed to make 3D scaffolds in tissue engineering.

Introduction

Construction of biocompatible scaffolds is one of the most leading areas in the field of tissue engineering. An ideal scaffold should mimic the structure and biological function of native extracellular matrix (ECM) as much as possible, both in terms of chemical composition and physical structure. One of the most important properties of ECM is its nanoscale structure which promotes cell attachment and function [1,2]. Researchers have found that nanofibrous scaffolds play an important role in tissue engineering by providing a proper matrix for cell attachment, proliferation and differentiation [3–7].

Recently, electrospinning has gained popularity with the tissue engineering community as a potential means of producing scaffolds [1,8]. In the electrospinning process, a high electric field is generated between a polymer solution, which is held in a syringe and a collector. The droplets of the polymer solution from the syringe tip are converted into Taylor cones by an electric field. When the voltage reaches a critical value, the electric forces overcome the surface tension on the droplet and a jet of ultrafine fibers is produced from the tip of the Taylor cones and will be gathered on the collector surface [9]. A variety of polymers have been used as scaffolds in tissue engineering. Among them PES can be used in biomedical applications like hemodialysis, filtration and ultrafiltration due to its positive attributes of a biomaterial. Consequently, this polymer is receiving increasing attention for use in tissue engineering [10–13].

The scaffolds made via electrospinning have the pore size less than 10 μm so that the cells cannot easily infiltrate the nanofibers and make a 3D shape as exist in ECM [14,15] and rare works has been performed to increase the infiltration of the cells into electrospun nanofibers [16,17].

There are few Studies on the biocompatibility and tissue engineering applications of PES scaffolds. Lin et al. [18] produced PES nanofibers by gas/jet electrospinning and Chuaa et al. [19] studied the biocompatibility of PES and surface-aminated PES with hematopoietic stem cells. They showed that surface modified PES had the highest expansion efficiency of the cells and the biocompatibility was promoted after surface modification. Many polymers donot have the desired surface properties to be used as biomaterials in tissue engineering so surface treatment and modification is used to improve surface characteristics such as hydrophilicity, cell attachment, expansion, proliferation and infiltration [20–24]. Plasma treatment is one of the best ways to improve surface hydrophilicity [25,26]. Many studies have shown that protein grafting also improve surface properties of biomaterials. Collagen is one of the natural polymers that has been used for grafting in some works. It has many amino acid sequences which are very important in cell-scaffold interactions [27–30].

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In this study, a PES nanofibrous web, with modified surface properties was produced. Plasma treated and collagen grafted PES nanofibers were also investigated for their biocompatibility and application in tissue engineering.

In vivo studies are currently under way to determine modified PES application in tissue engineering.

Materials and methods

Materials. Polyethersulfone Ultrason E6020P with a weight average molecular weight of 58,000 Da was purchased from BASF (Germany). The solvent *N*,*N*-dimethylformamide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide(NHS) was obtained from Merck (Germany). All other chemicals were purchased from Sigma Co. (St Louis, MO, USA) and used as received unless stated otherwise.

Electrospinning. PES nanofiber mats were produced by an electrospinning method. Briefly, prepared PES solution (25%) in *N*,*N*-dimethylformamide (DMF) was fed into a blunted needle by using a syringe pump. The collector was a rotating cylindrical drum which was placed at a distance of 15 cm from the needle.

Surface modification. PES surface modification was performed in two stages: (1) plasma treatment and (2) collagen grafting.

A microwave plasma generator of 2.45 GHz frequency with a cylindrical quartz reactor (Diener Electronics, Germany) was used. Pure oxygen was introduced into the reaction chamber at 0.4 mbar pressure and then the glow discharge was ignited for 10 min.

Plasma treated sheets were cut into 1 cm diameter punches and immersed in EDC/NHS solution (5 mg/ml) for 6 h. A collagen solution (1 mg/ml) was used to immerse scaffolds overnight. The scaffolds then rinsed with distilled water and used for following cell seeding and surface characterization.

Characterization of electrospun nanofibers. The fibers' morphology was characterized using a scanning electron microscope (SEM, Philips XL30, Netherlands) after specimens were coated with gold using a sputter coater. The fibers' diameter was determined from SEM images using image analysis software. Collagen grafting was investigated by FTIR-ATR. The spectra were recorded with a BOMEM Model BM-102 spectrometer equipped with a DTGS detector and a thallium bromoiodide ATR crystal.

Cell seeding. Unrestricted somatic stem cells (USSC) were used for cell seeding. They were isolated from human umbilical cord blood, cultured and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and a 1% antibiotic (10,000 U penicillin). USSCs were trypsinized and seeded on to the circular scaffolds with an initial cell density of 10^4 cm^{-1} and incubated in the culture medium.

Cell morphology. Cell morphology on the scaffold was investigated by scanning electron microscopy. The cell-loaded scaffolds were rinsed with PBS after 1 day and 7 days of cell seeding and fixed in glutaraldehyde 2.5% for 1 h. For dehydrating the scaffolds were placed in a series of gradient of alcohol concentration and then dried.

Contact angle measurement. To study the wettability of the nanofibers surface after surface modification, water contact angle was measured by the sessile drop method with a G10 Kruss contact angle goniometer at room temperature. A water droplet is placed on the scaffold surface and contact angle was measured after 10 s.

Results and discussion

PES nanofibers

SEM images of the PES, plasma treated and also collagen grafted PES is shown in Fig. 1. It is clearly obvious that nanofibrous PES scaffolds has a highly porous structure which makes it a good candidate to be used in tissue engineering. The diameters distributed in the range of 280–950 nm with an average of 565 ± 30 nm.

The natural microenvironment of the cells in human tissues like skin, ECM, is nanostructured and is composed of protein fibers which produce a 3D matrix for cells to attach, differentiate and proliferate. An ideal scaffold should mimic these natural properties of ECM like porosity and nanosized structure [1,2]. As the SEM images show, electrospun PES nanofibers are highly porous and nanostructured and has the potentiality to be used as a scaffold in tissue engineering.

Surface modification and characterization

Surface modification of the PES nanofibers was performed in two stages: (1) plasma treatment and (2) collagen grafting. According to the results, PES nanostructured webs physically mimic the ECM in an ideal manner because of its properties like high porosity and nanofibrous structure. According to the cell attachment and proliferation study, cells are neither able to attach to the surface of untreated PES scaffold in a proper amount nor infiltrate into the nanofibers. An ideal scaffold for tissue engineering must mimic the chemical properties of ECM as much as physical ones [30]. Many studies have stated that cell attachment and proliferation on the surface of biomaterials increases with the increase of the surface hydrophilicity [25–28].

Collagen is one of the native ECM proteins which have many attachment sites for the cells to attach and also improve cell proliferation [31]. In this study, collagen was used to improve cell behavior on the PES nanofibrous mats.

Fig. 2 shows the SEM images of the collagen grafted PES nanofibers. The image analyses of the SEM micrographs of collagen grafted nanofibers shows that the average fiber diameter has increased to 627 ± 94 nm. The surface roughness has been changed and existence of attached collagen is obvious in the images between the junctions of fibers.

To investigate the effect of plasma treatment and collagen grafting on the scaffolds surface chemistry, FTIR-ATR was performed and the results are shown in Fig. 3. According to the spectra A and B, the transmittance peak of OH groups appears newly near 3200 cm^{-1} and COOH groups near 1700 and 3200 cm^{-1}. The new

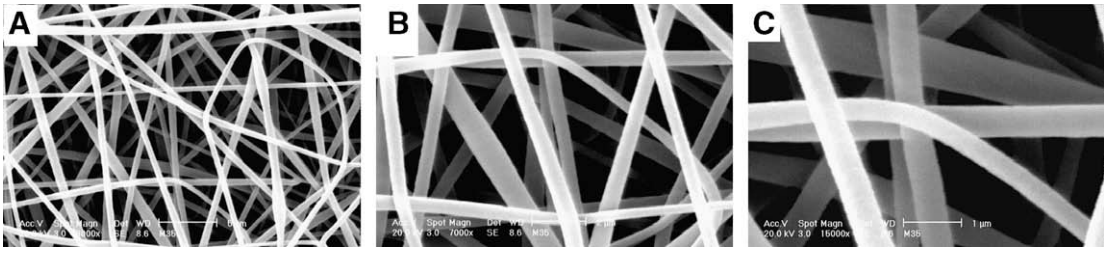

Fig. 1. SEM images of the PES nanofibers at different magnifications 3000× (A) 7000× (B) and 15,000× (C).

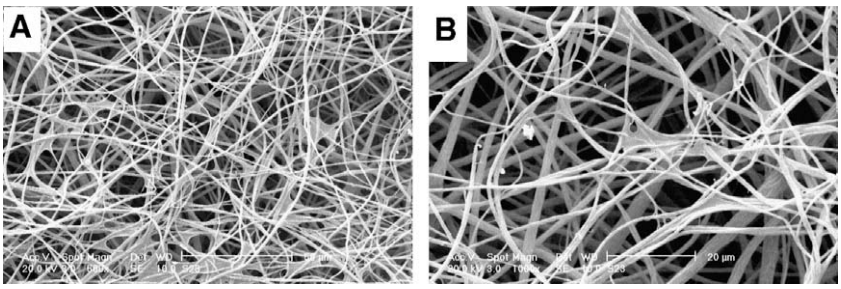

Fig. 2. Collagen grafted PES electrospun nanofibers at different magnifications 500× (A) and 1000× (B).

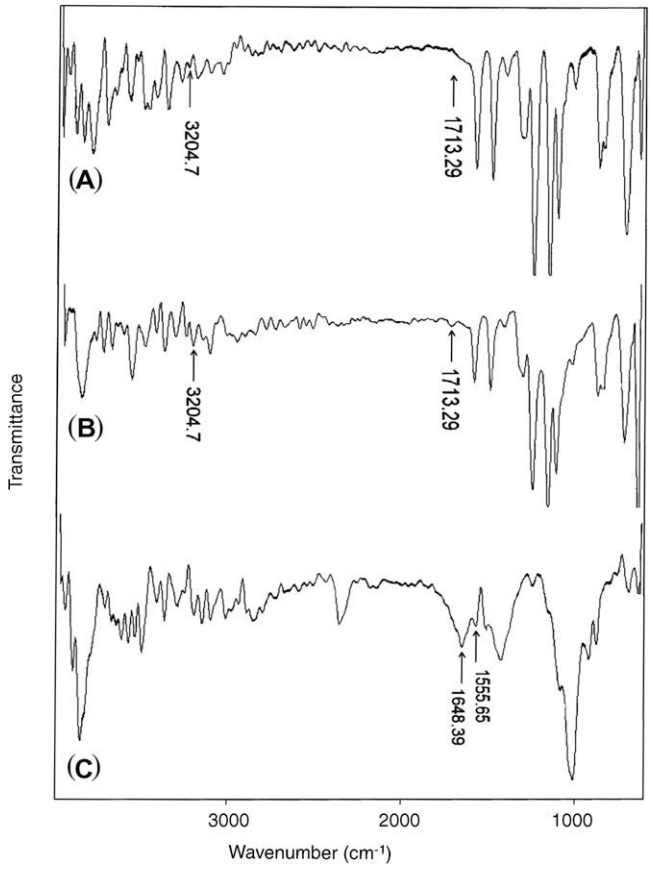

Fig. 3. FTIR-ATR spectra of pristine (A) plasma treated (B) and collagen grafted PES electrospun nanofibers (C).

chemical groups on the surface of the nanofibers increase surface hydrophilicity and serve as the reaction site for EDC/NHS chemistry. NH_2 groups on the amino acid side chains in collagen are cross-linked to the PES surface COOH groups via EDC [28].

Image C shows the FTIR-ATR spectrum of PES nanofibers after collagen grafting. The amide I band (1649 cm^{-1}) and amide II band (1565 cm^{-1}) can be observed. These peaks arise from molecular vibrations of C═O and N—H bonds in proteins.

Effects of surface modification on hydrophilicity of scaffolds

Surface collagen grafting of polymers improves surface characteristics, increases surface hydrophilicity and facilitates cell attachment and proliferation on the modified surface [27–30]. Plasma surface treatment of scaffolds with N_2, O_2 and NH_3 makes the polymer surface more hydrophilic, more polar and more bioadhesive [25,26]. In these studies, it has been shown that with hydrophilicity increase, the cell attachment and proliferation onto the surfaces will increase. In another word, the surface modification is used to improve the biocompatibility of the scaffolds. To compare the hydrophilicity of the surface of PES scaffolds, water contact angle measurement was done before and after surface modifications.

The water contact angles of PES, plasma treated PES and collagen grafted PES are 135.1, 95.5 and 13.2 degrees, respectively. It shows that the hydrophilicity of the surfaces have been increased after surface modification. After plasma treatment the results prove the role of hydrophilic groups (OH and COOH) to increase the hydrophilicity of the surface. The lowest water contact angle is observed after collagen grafting. It shows the high hydrophilic nature of the collagen and as previously said, we can predict that surface biocompatibility of the scaffolds has been also increased.

From this point of view, these measurements show that collagen grafted PES nanofibrous scaffolds are ideal for further studies in tissue engineering.

Cell morphology on the PES scaffolds and attachment study

In vivo and *in vitro* tests are used to investigate the applicability of a polymeric scaffold in tissue engineering. In *in vitro* investigation, the cells are cultured on the scaffold and cell-scaffold interactions are studied. With this method, the scaffold properties are evaluated and modifications are performed to improve tissue engineering related characteristics if needed [32,33].

Morphology of the cells on a scaffold is a criterion that shows their behavior, attachment and interaction with the surface of the scaffold. Cell growth, migration and differentiation are also other criteria that show the biocompatibility of nanofibers [34].

In this study, *in vitro* study was performed using unrestricted somatic stem cells (USSCs) which were cultured on the pure and treated PES and their morphology and attachment to the nanofibers were studied. Stem cells are highly potential to proliferate and differentiate to different types of cells in the body. One of the most promising areas in the field of tissue engineering is the differentiation of stem cells like USSCs to desired type of cells like neural, bone and cartilage cells attached to biomaterials and many studies have been done toward this goal [13,35–37].

The SEM micrographs of the scaffolds after 1 day and 7 days of cell culture are shown in Fig. 4. From the first view, we can see that the number of the cells on the treated PES scaffolds (A and B) are much more than untreated one (C) and the cells on the collagen grafted scaffolds (A) are the highest in the first day of culture. The cells on the collagen grafted scaffolds have a more normal shape and typical morphology of USSCs. The appropriate spreading of the cells on plasma treated and collagen grafted scaffolds prove their better biocompatibility and applicability in tissue engineering. The adhesive molecules in the membrane of the cells can attach to the hydrophilic surfaces better and more stable than hydrophobic ones because of their special molecular structure

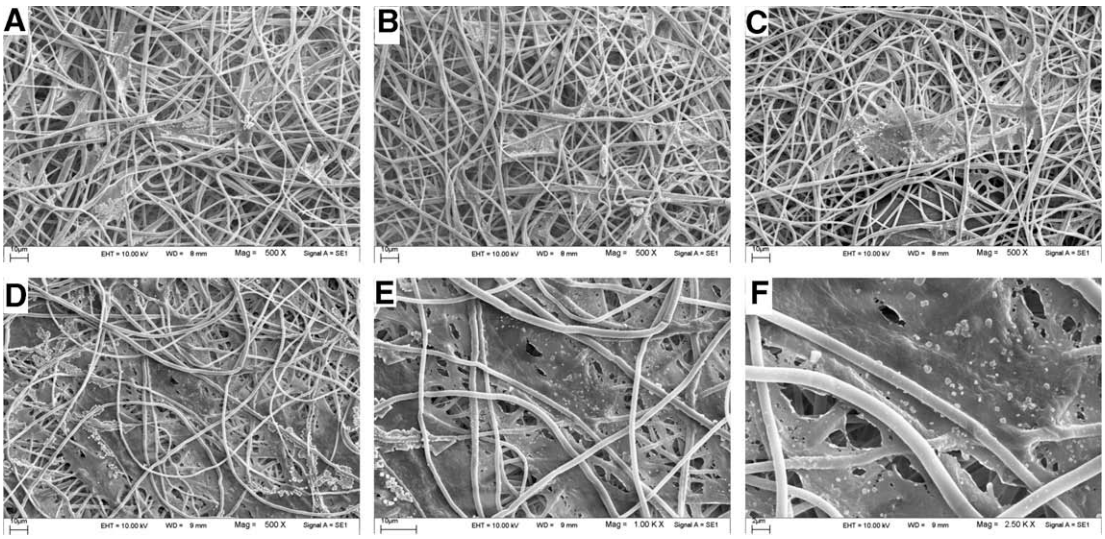

Fig. 4. SEM micrographs of cell seeded PES scaffolds after 1 day at 500× (A–C), collagen grafted (A), plasma treated (B) and pristine (C) PES nanofibers and collagen grafted PES scaffolds after 7 days (D–F), at 500× (D), 1000× (E) and 2500× (F).

[33]. After surface modification by plasma treatment or collagen grafting, the hydrophilicity will increase as shown in water contact angle measurements. The higher attachment and better morphology of the cells on the treated surfaces are because of their higher hydrophilicity and make the scaffolds a better candidate for *in vivo* test and further investigations in tissue engineering.

Also it can be seen that the USSCs have better morphology on the collagen grafted scaffolds than plasma treated ones. Collagen is a key natural polymer in ECM and has many locations and sequences on its structure for cell attachment [27–30].

The cells prefer to attach these sites rather than the surface of the PES nanofibrous scaffolds. The collagen on the scaffold surface creates a natural microenvironment for USSCs to attach and proliferate. The amount of proliferation can qualitatively be observed from images (A) and (D).

One amazing result that can be observed in micrographs (D), (E) and (F), is the infiltration of the cells into the collagen grafted nanofibers. Low infiltration of the cells is one of the problems of nanofibrous scaffolds made via electrospinning. To overcome this problem, some innovations have been performed by researchers but none has resulted in higher cell infiltration into scaffolds [16,17]. As it is shown, the cells have passed the surface nanofibers after 7 days and continue to proliferate through the pores among nanofibers. According to literature review done by authors on interactions between cells and nanofibrous scaffolds, this high infiltration has been rare. This observation introduces collagen grafted PES nanofibers as a potential scaffold to be used as 3D matrix in tissue engineering applications.

Conclusion

In this study, a novel nanofibrous scaffold was prepared using electrospinning process. Non-woven PES mats was constructed and then surface modified by plasma treatment and collagen grafting. Contact angle measurements showed that surface hydrophilicity increased after surface treatment. The results from FTIR-ATR proved the appearance of COOH and OH group after plasma treatment.

The unique protein bands (amide I and II) were also observed after collagen grafting which shows efficient grafting of collagen on nanofibers' surface. Cell attachment examination showed that collagen grafted scaffold promote cell attachment and natural morphology of the cells. Infiltration of stem cells into the collagen grafted nanofibers was observed after 7 days of cell culture. This shows that collagen on the nanofibers has attracted cells to infiltrate. This rare observation makes collagen grafted PES nanofibers an ideal candidate to form 3D structures in tissue engineering.

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