Increasing biocompatibility of scaffold made of Polyethersulfone (PES) through combining with polyaniline(PANI)

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ABSTRACT

Today's tissue engineering is an important and interesting method of treatment in bone lesions; hence, tissue engineering specialists and orthopedic surgeons are forced to serve as a safe alternative to current methods. The introduction of a scaffold that has the best efficiency is a major challenge to scientists and researchers in this field. The aim of this study is to investigate the polyaniline (PANI) influence on biocompatibility of Polyethersulfone (PES) nanofibrous scaffold. Fabricated scaffold was structurally characterized using SEM microscope and tensile assay. To investigate biocompatibility of nanofibers by MTT assay and DAPI staining, human mesenchymal stem cells were seeded on the surface of PES, PES-PANI and tissue culture polystyrene (TCPS) as control. SEM images demonstrated that the scaffolds were flat and the average diameters of the nanofibers were 286 ± 588. Moreover, the results of MTT assay and DAPI staining revealed that the highest proliferation rate of cells was observed in PES-PANI scaffold compared to the PES and TCPS as control. It can be concluded that PANI improves the three-dimensional structure of PES nanofibers and increases the biocompatibility of PES through support proliferation and penetration of MSCs in the PES nanofibers and can also be a good candidate for introduction and use in the bone tissue engineering application.

Keywords: Scaffold, Polyethersulfone, Polyaniline, Mesenchymal stem cells

INTRODUCTION

Recently, due to the growing therapeutic needs for a variety of bone lesions, trauma or fractures, bone tissue engineering has attracted the attention of a great number researchers and orthopedic surgeons[1,2]. Full disability or loss of tissue is one of the most destructive and expensive medical problems, making an approximate cost of 400 billion dollars each year for health care, together with millions of lost working days. Tissue engineering, using living cells or their extracellular matrix components and natural or synthetic scaffolds provide the link that leads to the recovery or restoration of tissue function[3]. When specific organ or tissue is required for transplant, two problems may arise: 1. they may not be accessible. they may be rejected by the mmunity system[4]. Two important characteristics of tissue engineering scaffolds are biocompatibility and biodegradability. So far, very few studies have been done on the improvement of polyethersulfone (PES) biocompatibility to better the application of tissue engineering. In a study reported by Chuaet al., PES showed normal biocompatibility with different functional groups examined and it was shown that increased amino groups led to the increased proliferation and growth of cells were seeded on the scaffold, resulting in increased biocompatibility. PES is a commercially available material with unique properties[5] and it has been commonly used as blood purification membranes.Over the last decade, many studies were reported over this polymer due to its special properties, including mechanical, hydrolytic, thermal and oxidative stability; yet, one of the most important challenges is its low biocompatibility [6,9]. On the other hand, Polyaniline (PANI) is a conductive polymer which has been an interesting material in tissue engineering and regenerative

medicine research domain in the past two decades [10,12]. Conductive polymers in the last two decades have been an interesting research area; the most common of these polymers were polythiophene, polyacetylenes, polyaniline, polypyrrole and poly-p-phenylene. Among conducting polymers, aniline has attracted special attention due to its features such as easy synthesis, low cost, wide application and high efficiency polymerization. This combination was discovered in the 1980s and was highly regarded due to its high electrical conductivity and is among the conductive polymers and has a high electrical conductivity[13] over which plenty of studies have been performed. Oxidation-reduction reactions in this polymer causes the transformation among different species of polyaniline [14].In the present study we try to increase biocompatibility properties of PES nanofibrous scaffolds through its combination with PANI.

MATERIALS AND METHODS

Electrospining For the production of nanofiberous scaffold used in this project, PANI (100 mg) in an amount equal to CPSA was poured and then was dissolved in 10 mL of DMSO for 24 hours. At this stage, it was centrifuged at a speed of 1000RPMisolated, and then added to the PES solution at a concentration of 20% in N, Ndimethylformamide as solvent (for 4 hours at 35 ° C). Then, this solution was poured into a syringe and the syringe was connected via a tube to the nozzle at a distance of 15 cm from the cylindrical form.

Plasma surface modification

Plasma surface treatment was used to increase hydrophilicity of fabricated PES-PANI composite scaffolds. For plasma treatment, 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.

Mechanical characterization

In order to evaluate the mechanical properties of fabricated nanofibrous mats, a tensile tester (SANTAM Stress machine, Iran) was used. Nanofibrous mat samples were cut into 10 mm × 60 mm × 0.11 mm and placed in the machine for testing with the speed rate of 50 mm/min at RT.

Cell cultur

Prior to cell seeding, the scaffolds were cut into 1.5 cm diameter circular scaffolds, which were placed in 24-well tissue culture polystyrene (TCPS) and sterilized in 70% ethanol. Then the scaffolds were incubated overnight with Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Fetal bovine serum (FBS), penicillin, streptomycin, and amphotericin B to prevent from yeast growth and ensure sterilization and enhance cell attachment after seeding. An initial density of 5×10^3cells per well were suspended in 500 μL of medium and seeded onto the PES-PANI and PES scaffolds and TCPS as control. After one day, the scaffolds were transferred to new wells and the basal medium (DMEM supplemented with 10% FBS) was added to the wells with and without scaffolds. Culture medium was replaced every 2 days during the period of study

SEM

For SEM investigation, cell-seeded scaffolds were fixed with 1.5% glutaraldehyde in 0.14 M sodium cacodylate buffer, then dehydrated in graded alcohols, critical point dried, sputter-coated with carbon and analyzed in scanning electron microscope equipped with an X-ray energy dispersive spectroscopy (EDX) microanalysis capability. To study nanofibrous scaffolds morphology, the different scaffolds were coated with gold, using sputter coater and then observed by a scanning electron microscope.

MTT assay

The proliferation rate of stem cells cultured on PES, PES-PANI and TCPS was evaluated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.

Sterilized nanofiber membranes were placed in a 96-well culture plate, seeded with a cell density of 1×10^3cells per cm^2, and incubated at 37°C, 5 % CO2for4 h. During 5 days (Days 1 to 5) of cell seeding, 50 μL of MTT solution (5 mg/ml DMEM) was added to each well. The formazan product of the MTT accumulates as an insoluble precipitate inside cells as well as being deposited near the cell surface and in the culture medium. The formazan must be solubilized prior to recording absorbance

readings by appropriate solvent. The optical density was read at a wavelength of 570 nm in a micro-plate reader (BioTek Instruments, USA). The same procedure was performed for cultured cells in TCPS as control.

DAPI staining Test

Stem cell seeded scaffolds were fixed with 4% paraformaldehyde at 4°C for 30 min on days 1,3, and 5 after cell seeding. Then, samples were washed twice with PBS, incubated with 4', 6-diamidino-2-phenylindole (DAPI) (Sigma Chemical Co, USA) for 30 seconds to label nuclei of the cells, and rinsed twice with PBS. The immunofluorescence images were obtained through using fluorescence microscope.

Statistical analysis

All experiments were conducted for at least three times. Data were reported as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the results. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS software, version 11.0 (SPSS, Chicago, IL, USA).

RESULTS

Characterization of Nanofibers

The morphology of fabricated nanofibers was evaluated using a SEM imaging and results revealed that scaffolds were quite porous and free from defects and the fiber was quite flat with the average diameter of 550 ± 149 nm (Fig 1A). SEM was also used to evaluate biocompatibility of PES-PANI nanofiber after 10 days of cell seeding (Fig 1B). PES and PES-PANI nanofibers showed tensile strength of 1.01±0.403 and 1.85 ± 0.365 MPa and an elongation break of 30.964 and 54.54 % respectively which showed no significant change after plasma surface treatment.

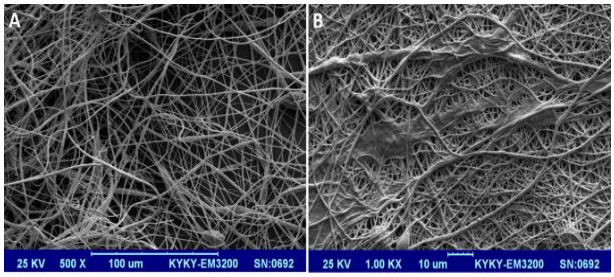

Figure 1. Morphology of electrospunfabricated nanofibers. PES nanofibers without (A), and with AT-MSCs after 10 days at two magnifications (×500 and ×1000).

MTT assay

In addition to the SEM analysis, MTT assay and DAPI staining was used in order to investigate the biocompatibility of PES scaffolds before and after blending with PANI in comparison to the TCPS as control groups. MTT assay indicated that there was no difference between groups until day 3, but after that phase, proliferation rate of MSCs cultured on the surface of PES-PANI showed a significant increase compared to the other groups during days 4 and 5 (Fig 2). However, stem cells seeded on scaffolds showed enhanced proliferation rate in comparison to theseededcellsonTCPS

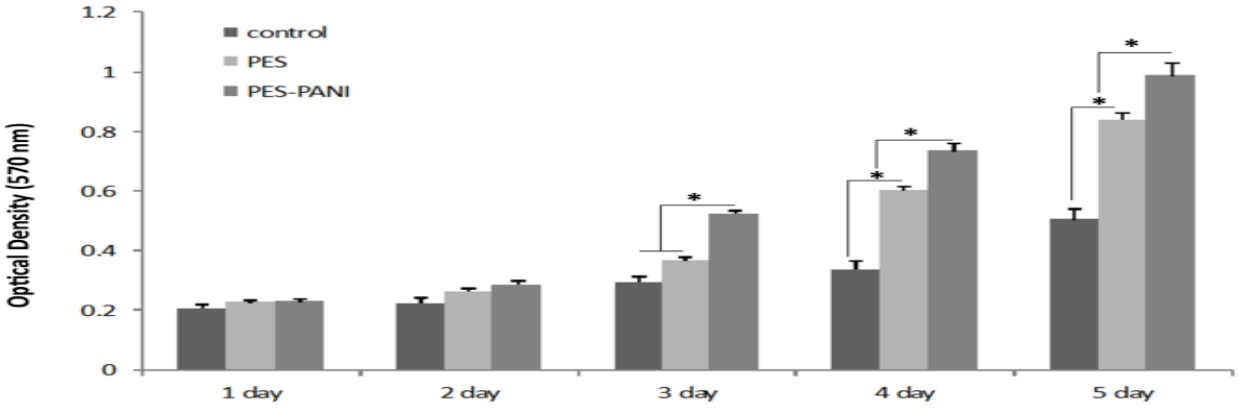

Figure 2. Proliferation rate of AT-MSCs on tissue culture polystyrene (TCPS) as control, PES and PES-PANI nanofibrous scaffolds during 5 days culture period (A, asterisks significant difference between the groups at P<0.05).

DAPI staining

For more conformation, DAPI staining nucleus of cells was used to evaluate proliferation rate of stem cells seeded on PES, PES-PANI and TCPS. As showed in Fig 3, no significant change was observed on day one after cell seeding but most stem cells were observed in PES-PANI groups compared to the PES and TCPS groups on days 3 and 5. During days 3 to 5 stem cells seeded on PES was also increased in comparison to the TCPS.

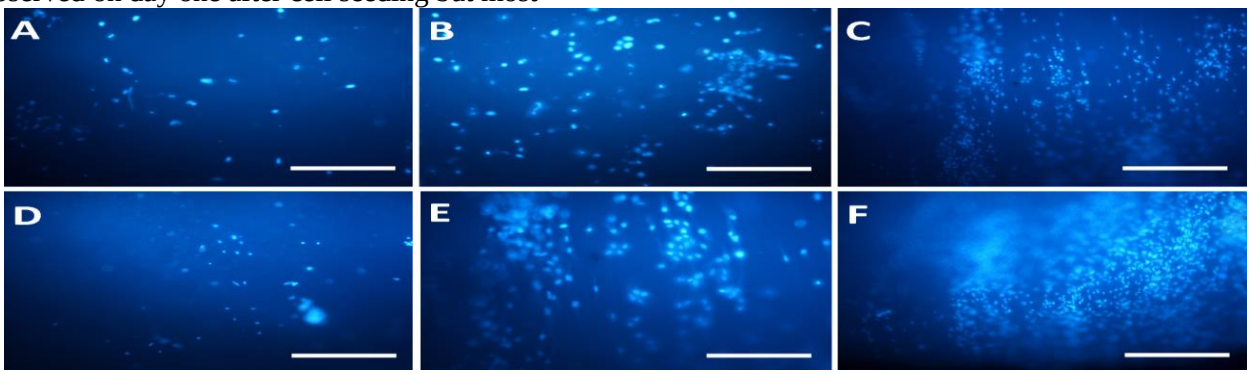

Figure 3.DAPI stained nanofibers on days 1 (A), 3 (B) and 5 (F) for PES seeded AT-MSCsand on days 1 (D), 3 (E) and 5 (F) for PES-PANI seeded AT-MSCs, (magnifications ×10).

DISCUSSION

In the present study, biocompatibility of PES scaffolds in combination with PANI was investigated by culturing AT-MSCs on PES, PES-PANI and TCPS as control group for the purpose introducing a new appropriate substrate for bone tissue engineering,. These cells are multi-potent and adult stem type, which have the ability of differentiating many of cell types. MSCs were isolated from the bone marrow for the first time. However, in recent years researchers have found that cells from other tissues, such as adipose tissue have also been isolated and identified[15, 16].The biomimetic approach is an efficient way of introducing and developing new scaffolds for bone tissue engineering applications[17]. In bone tissue engineering and regenerative medicine, stem cells from appropriate and available sources are seeded on the surface of biocompatible and mimetic substrate and this construct can be used for bone lesions and defects treatment[18,19]. The mimicking of the physiochemical structure of native bone tissue ECM is one of the most important characteristics for scaffold introduction and preparation which has attracted the attention of the tissue engineering researchers and orthopedic surgeons during recent years. We also performed several studies about PES biocompatibility and application in bone tissue engineering [20,22]. In the present study, we tried to increase biocompatibility of PES nanofibers by composing to PANI.PANI is one of the most well-known conducting polymers and there are many reports about its highly electrochemical activity, controllable conductivity, good biocompatibility, low cost and ease of preparation[27]. Prabhakaran et al. cultured rat NSCs on the surface of PLLA/PANi scaffolds and the results showed that in vitro electrical stimulation increased neurite growth compared to the cells grown on non-stimulated scaffolds[28]. In another study, Ghasemi-Mobarakeh et al. showed that NSCs cultured on PANi/PCL/gelatin scaffolds is a very good candidate in the nerve tissue regeneration and nobody reported PES-PANI composite biocompatibility or tissue engineering application[10]. In this study we showed that PES nanofibers had good biocompatibility after plasma treatment and stem cells proliferation rate was better than TCPS as control group. But proliferation rate of stem cells was significantly increased in PES-PANI group and showed biocompatibility of PES nanofibers was enhanced in combination with PANI. In the other hands we tried to depict proliferation rate increase of stem cells seeded on PES-PANI in comparison to the PES and TCPS using DAPI

staining during 5 days and our results showed enhanced PES-PANI stem cells proliferation rate in comparison to the PES and TCPS seeded cells. McKeon et al., in their study, manifested that when primary rat muscle cells were cultured on electrospunpolyaniline and poly(D,L-lactide) (PANi/PDLA) mixtures at different weight percents, the cellular proliferation measurements showed no significant difference between the scaffolds groups and control group[29]. But in another study, Yan et al. reported that L-929 cells demonstrated growing trend on graphene-PANI hybrid papers during 4 days[30]. During this research scaffolds as extracellular matrix play an important role and make a good anchorage for mesenchymal cells, and also provide a suitable environment in order to produce tissue; consequently, enhancing cell adhesion seems crucial in the process of tissue engineering. In this study, we tried to show enhanced biocompatibility of PES nanofibers through blending with PANI via three methods such as SEM, MTT assay and DAPI staining. All results showed that the proliferation rate of MSCs was increased significantly after blending PES with PANI as showed in result section.

CONCLUSIONS

It can be concluded that the PANI nanofibers can increase biocompatibility of PES nanofibers and also support the proliferation of MSCs, resulting in a better stem cell differentiation and can be a good candidate for use in the treatment of bone defects with tissue engineering.

"The authors declare no conflict of interest"

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