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Improved proliferation and osteogenic differentiation of mesenchymal stem cells on polyaniline composited by polyethersulfone nanofibers

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ABSTRACT

Tissue engineering is a promising emerged method trying to reconstruct lost tissues that using synthetic and biomaterials and their combination with cells. The purpose of this study is increase osteoinductivity of polyethersulfone (PES) by using polyaniline (PANi). In this study, after fabrication of PES and composited PES–PANi scaffolds by electrospinning, scaffolds were characterized morphologically and mechanically. Then osteoinductivity of scaffolds was investigated by osteogenic differentiation of human mesenchymal stem cells (MSCs) cultured on the PES and PES–PANI in comparison to the tissue culture polystyrene as a control. The Osteogenic potential of MSCs was evaluated by Alizarin Red staining, ALP activity, calcium content assay and bone related gene expression assay. Scaffolds were smooth, bead free and in the scale of nanometers and PES mechanical stability was decreased significantly after composite with PANi. Highest growth, ALP activity and deposited calcium of cells were observed in the PES–PANi groups compared to the PES and TCPS. Significantly higher expression of collagen type one and osteonectin was also detected in the PES–PANi group. It can be concluded that PES–PANi construct has potential to be a good candidate as bone grafting substitute and using in tissue engineering applications.

1. Introduction

Actually the most common cause of bone fractures or loss; are accidents, trauma and osteoporosis and overuse that they can affect millions of people worldwide. Various treatments have been used for the bone loos for example; Autografts and allografts are commonly current strategies that used for bone repair surgically [1], but each possesses limitations, such as donor-site morbidity with the use of autograft and the risk of disease transmission with the use of allograft [2]. Another way of the treatment is tissue engineering that is an important emerging multidisciplinary field during 4 last decades, which is composed of 3 main parts such as: biological and synthetic materials as scaffold, cells and biomolecules that trying to reconstruct and repairing of lost tissues [3–5]. Due to the risks involved in using conventional approaches that attempt to reverse bone loss, such as bone autografts and allografts, bone tissue engineering strategies based on endogenous bone healing mechanisms have recently attracted the attention of the scientific community [6,7]. They stimulation of osteogenesis is considered equally important in bone tissue engineering and regeneration due to the fact that bone is a highly mineralized tissue maybe. Biologically active scaffolds are highly porous materials based on simple analogs of the extracellular matrix (ECM) that have induced synthesis of tissue and organs [8–10]. However, researchers often encounter an enormous variety of choices when selecting scaffolds for tissue engineering. In this study, the electrospinning technique was used for scaffold fabrication that it's a process of electro-hydrodynamic as a versatile and promising platform technology for producing electrospun materials on the nanometer scale from natural and synthetic polymers or composites of them [11–13]. This electrostatic processing strategy can be used mimics ECM and have been shown to promote the nutrition, improve the growth, direct the migration, maturation and cell fate decision of cells in *in vitro* [14]. Several studies have been shown that polyethersulfone (PES) nanofibrous membrane was useful for water and blood purification [15]. This reflects the low hydrophilic properties of PES and confirms it is not appropriate for biological research, but in recent years, many researches have been done on

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the PES scaffold with surface modifications, including: coating with gelatin, collagen or adding amide groups with plasma treatment [16–19]. Mesenchymal stem cells (MSCs) comprise a population of undifferentiated cells that has the ability to self-renewed throughout life and are capable of dividing into clones and also can differentiate into multiple connective tissue lineage such as muscle, cartilage and bone [20,21]. In this study, a PES nanofibers scaffold was fabricated by electrospinning technique and their structural and bio-physicochemical properties were modified by Polyaniline (PANi) polymer that led to an increase of the electrical conductivity of fabricated nanofibrous scaffold and also change other structural properties. PES is one of the most important biocompatible polymeric materials, in recent years, researchers have been attempting to modify by protein or other polymers. PANi is an oxidative polymeric product of aniline under acidic conditions and is commonly known as aniline black, and also known as a conductive polymer [22,23]. A range of applications for conducting polymers are currently being considered, such as the development of tissue engineered organs. Generally, polymers with loosely held electrons in their backbones can be called conducting polymers. In the past two decades, the ability of varying oxidative sate has allowed the conducting polymer PANi to be studied in a wide range of research fields, such as corrosion protection of metals as substrates for light-emitting device, but recently there has been evidence of PANi can support cell growth.

2. Materials and methods

2.1. Electrospinning

For the production of nanofibrous scaffold used in this project, 100 mg emeraldine base PANi and 100 mg CPSA were dissolved in 10 ml DMSO with stirring at room temperature for 24 h, followed by filtering through a regular qualitative filter paper. Thus, the concentration of the pure CPSA-PANi solution was 3% (w/v) and PES solution 20% (w/v). Finally products were collected into electrospinning setup (Nano-Model, IRAN). Flow rate and voltage were adjusted at 0.4 mL/h and 23 kV, respectively. Also, the distance between nozzle and collector and the nozzle angle were selected 24 cm and 25°.

2.2. Mechanical assay and contact angle measurement

Mechanical characterization of the PES and PES/PANi nanofiber samples were done through tensile test on an Instron universal testing machine (Model STM-20, SANTAM, Iran), with sample sizes of 60 mm × 10 mm and a loading velocity of 50 mm/min. The results are based on four samples punched from electrospun sheets. The machine recording data were applied to plot the tensile stress–strain curves. To study the hydrophilicity of the nanofiber surface followed by surface treatment, the water contact angle was done by the sessile drop procedure with a G10 Kruss contact angle goniometer at room temperature. A water droplet was placed on the scaffold surface and the contact angle was evaluated after 10 s.

2.3. Plasma treatment

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

2.4. Cell culture

Before to cell seeding, scaffolds were cut into 1.5 cm diameter circular scaffolds, which were placed in 24-well and sterilized in 70% ethanol, then the scaffolds were incubated with basal medium overnight to facilitate cell attachment, Adipose derived MSCs (AT-MSCs) were isolated from the adipose tissue exactly previously reported [24]. MSCs were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l glucose supplemented with 25 units/ml penicillin/streptomycin and 10% fetal bovine serum under standard culture conditions (37 °C, 5% CO_2). After one day, the scaffolds were transferred to new wells and the osteogenic medium (DMEM contains FBS 10%, 10 mM dexamethasone, 50 μg per ml ascorbic acid and 10 mM beta-glycerophosphate) was used to culture of AT-MSCs with 2 × 104 per cm^2 cell density on both scaffolds and tissue culture polystyrene (TCPS). Osteogenic medium was replaced every 2days during 21 days.

2.5. MTT assay

Proliferation of MSCs on scaffold was determined using the MTT assay. After cutting the scaffold, they were transferred into a new 96-wells plate, seeded with a cell density of 2000 cells per well under basal medium in 5% CO_2/95% air atmosphere. At days 1–5, every day 10 μL of (3-[4, 5-dimethyl-thiazolyl-2]-2, 5- diphenol tetrazolium bromide) solution (5 mg/ml in DMEM) was added into each well. After incubation at 37 °C for 4 h, then supernatant was removed and the precipitated formazan was dissolved in 100 μL of DMSO. The quantity of formazan is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer (BioTek Instruments, USA). The same procedure was performed for cultured cells in TCPS as a control.

2.6. Alizarin red S histochemical staining

Alizarin red S was used to assess matrix mineralization on day 21 after cell seeding. Alizarin red S is used for histological characterization of calcium deposited, the cell washed with PBS and then incubated in cold 4% paraformaldehyde for 30 min at 4 °C. Then, these cells were washed with PBS. The fixed cells were stained with 2% Alizarin red pH 7.2, after 5–10 min at 37 °C, the cell were washed with PBS three times and observed under the light microscope.

2.7. Alkaline phosphatase (ALP) activity and Ca content assay

For ALP activity measurement on days 7, 14 and 21 in cultured stem cells under osteogenic media, total protein of cells was extracted using 200 μl radio immune precipitation (RIPA) lysis buffer followed by shaking for 4 h at 4 °C and then centrifuged at 15,000 RPM at 4 °C, 15 min and supernatant was collected for ALP activity assay. ALP activity was measured with an ALP assay kit (PARS-AZMOON, Tehran, Iran) and the plate was read at 405 nm in a micro-plate reader (BioTek Instruments, USA). The activity of enzyme (IU/l) was normalized equal the total amount of protein (mg). The amount of Ca^2 deposited on stem cells under osteogenic induction was measured using a calcium content assay kit (PARS-AZMOON, Tehran, Iran), Ca^2 was extracted from differentiated cells with 0.6 N HCl (Merck) followed by shaking for 4 h at 4 °C. After the addition of the reagent to calcium solutions, optical density (OD) was measured at 570 nm in a micro-plate reader (BioTek Instruments, USA). Calcium content was obtained from the standard curve of OD versus a serial dilution of calcium concentrations.