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Research paper

Enhanced chondrogenesis of human bone marrow mesenchymal Stem Cell (BMSC) on nanofiber-based polyethersulfone (PES) scaffold

Hossein Mahboudi[a], Bahram Kazemi[a,d,*], Masoud Soleimani[b], Hana Hanaee-Ahvaz[c], Hossein Ghanbarian[a], Mojgan Bandehpour[a], Seyed Ehsan Enderami[c], Mousa Kehtari[c], Ghasem Barati[e]

[a] Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Hematology Department, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[c] Department of Stem Cell Biology, Stem Cell Technology Research Center, Tehran, Iran
[d] Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Department of Medical Biotechnology and Nanotechnology, School of Medicine, Zanjan University of Medical Science, Zanjan, Iran

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ABSTRACT

Mesenchymal stem cells (MSC) from bone marrow hold great potential as a cell source for cartilage repair. The objective of our study was differentiation of MSC toward chondrocyte by using Nanofiber-based polyethersulfone (PES) scaffold and also enhanced chondrogenic differentiation of BMSC in vitro. MSCs were harvested from bone marrow of human and PES scaffold was fabricated via Electrospinning. The isolated cells were cultured on the PES scaffold and scaffold free method. After 21 days, Real-time PCR was performed to evaluate the cartilage-specific genes in the mRNA levels. Also, in order to confirm our results, we have done immunocytochemistry and SEM imaging. Flowcytometry confirmed the nature of the isolated adherent cells. Immunocytochemistry and SEM imaging confirmed the differentiation of MSC toward chondrocyte. Also, real time PCR showed a significant increased gene expression of collagen type II and aggrecan on the PES scaffold method when compared to the mRNA levels measured in scaffold free method. Down regulation of Collagen type I was observed in PES scaffold compared to scaffold free at day 21. Also, both methods showed a similar pattern of expression of SOX9. Our results showed that PES scaffold maintains BMSC proliferation and differentiation, and can significantly enhance chondrogenic differentiation of BMSC. PES scaffold seeded BMSC showed the highest capacity for differentiation into chondrocyte-like cells.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease that is the most frequent cause of disability. The etiology for OA is unknown but multiple factors are thought to be involved. Because of the inability of the cartilage tissue to regenerate itself, OA and cartilage defects are two of the major health problems. Cartilage tissue is lack of blood supply, lymphatic system and nervous system within the tissue (Ham et al., 2015).

Different approaches have been studied to address this issue. Human mesenchymal stem cells (MSCs) are a promising alternative cell source for cartilage regeneration (Bulte et al., 2004) and recently have been applied for treatment of OA in clinical trials (Lai et al., 2015). MSCs are easily found in various tissue sources including bone marrow, adipose tissue, spleen, synovial fluid, and lungs. These cells can differentiate into several cell types (Ghorbani et al., 2015; Lai et al., 2015).

Nowadays, researchers use growth factors (GFs) for chondrogenesis including transforming growth factor-β (TGF-β), bone morphogenic proteins (BMPs), insulin- like growth factor (IGF), and fibroblast growth factor (FGF). Chondrocyte differentiation is usually done by members of the transforming growth factor-beta (TGF-β) superfamily (Watabe and Miyazono, 2009). Transforming growth factor (TGF)-betas and their families, including bone morphogenetic proteins (BMPs), are implicated in the development and maintenance of various organs, which

Abbreviations: BMSC, bone marrow mesenchymal Stem Cell; MSC, marrow mesenchymal Stem Cell; PES, polyethersulfone; OA, osteoarthritis; SEM, scanning electron microscope; TGF-β, transforming growth factor-β; FGF, fibroblast growth factor; BMP, bone morphogenetic proteins; GF, growth factors (GFs); PBS, phosphate-buffered saline (PBS); DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; TCPS, tissue culture polystyrene; COL1A1, collagen type I; COL2A1, collagen type II; COL10A1, collagen type X; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

* Corresponding author at: Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail address: bahram_14@yahoo.com (B. Kazemi).

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plays important roles in stem cells (Ng et al., 2008).

The application of appropriate biomaterials in combination with cells has attracted the attention of the research community for cartilage regeneration using a tissue engineering approach. In this method, stem or mature cells are loaded onto a scaffold and the capacity of this construct for the healing of cartilage defects is considered in vitro and in vivo (Shafiee et al., 2014).

Although some advancement has been made to generate differentiated cells using different stem cell types, the differentiation protocols are still time-consuming and costly (Enderami et al., 2016). Therefore, newer strategies need to be considered in chondrogenesis differentiation approaches.

In this study we used nanofibrous scaffolds for enhanced chondrogenesis of human bone marrow mesenchymal Stem Cell (BMSC). Nanofibrous scaffolds have been used in the field of tissue engineering because of their nano-size structure which promotes cell attachment, function, proliferation and infiltration. Different types of polymers have been used as scaffolds in tissue engineering. Among these polymers, polyethersulfone (PES) can be used in biomedical applications like hemodialysis, filtration and ultrafiltration due to its positive attributes of a biomaterial. There are few studies on the biocompatibility and tissue engineering applications of PES scaffolds. Consequently, there is increasing attention to the use of this polymer in tissue engineering (Christopherson et al., 2009; Shabani et al., 2009; Yoshimoto et al., 2003). PES scaffold as a nanofibrous scaffolds is prepared via electrospinning and its surface is modified by plasma treatment and collagen grafting. (Mahboudi et al., 2017; Shabani et al., 2009).

In our study, we have investigated the differentiation potential of human BMSC into chondrocyte cells using biocompatible nanofibrous PES scaffold.

2. Material and methods

2.1. Bone marrow harvest, cell isolation and expansion

The bone marrow mesenchymal Stem Cells (BMSCs) were isolated from bone marrow samples collected from healthy volunteers (mean age 45 ± 5, Taleghani Hospital, Tehran, Iran) after informed consent according to guidelines of the Medical Ethics Committee, Shahid Beheshti University of Medical Sciences and Health services (Tehran, Iran). Briefly, the bone marrow samples (8–10 ml) are diluted with 20 ml phosphate-buffered saline (PBS). Cells are then fractionated on a Lymphoprep density gradient by centrifugation at 500*g* for 20 min. The interfacial mononuclear cells are collected and washed with low-glucose Dulbecco's modified Eagle medium (LG-DMEM) supplemented with 10% (v/v) FBS. The BMSC primary cultures seeded at a density of $10^6/cm^2$ in LG-DMEM with 10% FBS in 25 cm^2 flasks. The cells are incubated at 37 °C under 5% CO2. Non-adherent cells are removed after 48 h by changing the medium. Thereafter, the medium changes every 3 days. Typically, cultures reach 80–90% confluency by day 14. In this stage, the cells trypsinized from the culture dishes using 0.25% trypsin containing 0.53 mM EDTA, are counted and plated again. Cells from the 3rd passage have been used for all experiments. In P0, cells show the typical fibroblast-like morphology of primary BMSC. During culture propagation up to P3, cells are slightly flattened but still have fibroblast-like morphology. In P3, BMSC verification is performed by flow-cytometric analysis from surface marker profile.

2.2. Flow cytometry analysis

The human BMSCs were detached after 2 weeks in vitro with trypsin/EDTA and about 2×10^5 cells were divided into aliquots and centrifuged at 1200 RPM for 5 min at RT. The pellet was resuspended in human serum and incubated for 30 min on ice. After centrifugation at 1000 rpm for 5 min, the pellet was resuspended in 3% (v/v) human serum albumin (HSA)/PBS and incubated with appropriate antibodies including fluorescent isothiocyanate (FITC)-conjugated mouse anti-human CD45, Phycoerythrin (PE)-conjugated CD106, CD34, CD44 and CD10 (Biosciences, USA) for 1 h on ice, washed twice in PBS and centrifuged for 5 min. The cells were resuspended in 100 ml of PBS and studied by a Coulter Epics-XL flow cytometer (Beckman Coulter, CA, USA). An isotype control with FITC- or PE-labeled antibodies was included in each experiment, and specific staining was measured from the cross point of the isotype using a specific antibody graph. The corresponding histograms were created by Win MDI 2.8 software (Scripps Institute, CA, USA).

Table 1
Primers used for real-time PCR.

Gene (accession no.)	Primer sequence	Product size (in base pairs)
GAPDH (NM_002046)	5-AGAAAAACCTGCCAAATATGATGAC-3 5-TGGGTGTCGCTGTTGAAGTC-3	126
Collagen I (NM_000088)	5-CAGCCGCTTCACCTACAGC-3 5-TTTTGTATTCAATCACTGTCTTGCC-3	79
Aggrecan (NM_013227.2)	5-TGCATTCCACGAAGCTAACCTT-3 5-GACGCCTCGCCTTCTTGAA-3	70
Collagen II (NM_033150)	5-GGCAATAGCAGGTTCACGTACA-3 5-CGATAACAGTCTTGCCCCACTT-3	84
SOX-9 (NM_000346)	5-AGCGAACGCACATCAAGAC-3 5-GCTGTAGTGTGGGAGGTTGAA-3	110

2.3. Adipogenic and osteogenic differentiation

For osteocyte differentiation 1.5×10^3 cells at passage 3 were seeded per well at a 4-well plate and 450 µl DMEM medium supplemented with 100 mM dexamethasone, 10 mM beta glycerol phosphate, 50 mM ascorbic acid and 500 ml FBS (all from Sigma-Aldrich, USA) was added to each well. Two wells were exposed to differentiation medium and the other two wells were exposed to DMEM supplemented with 10% FBS as control group. The medium was changed every 2 days. After 14 days the cells were fixed with Paraformaldehyde 4% for 20 min at 4 °C and then washed by PBS and stained with alizarin red (Behnogen, Iran) for 5–10 min. For adipogenic differentiation 1.5×10^3 cells at passage 3 were seeded per well at a 4-well plate and 4500 mL DMEM supplemented with 5 mL dexamethasone 1 mM, 5 mL insulin 1.7 mM, 5 mL indomethacin 200 mM, 5 mL isobutylmethylxanthine 500 mM and FBS 10% (all from Sigma-Aldrich, USA) was added to the wells. Two wells were exposed to differentiation medium and the other two wells were exposed to DMEM supplemented with 10% FBS as control group. The medium was changed every 2 days. After 14 days the cells were fixed with Paraformaldehyde 4% for 20 min at 4 °C and washed by PBS and stained with Oil red (Merck, Germany) for 5 min.

2.4. Fabrication of electrospun nanofibrous PES scaffolds

Nanofibrous PES scaffolds were fabricated and Produced by an electerospinning setup according to a protocol modified with Shabani et al. (2009).

2.5. Chondrogenic differentiation in vitro

Nanofibrous membranes (scaffolds) were placed in a 24-well culture plate. The scaffolds were floated in 70% ethanol for 2 h, and washed with PBS three times. After that, the scaffolds were pre-treated in basal medium (DMEM with 10% FBS) for 24 h to facilitate protein adsorption and cell attachment on to the scaffold surface. Finally, the scaffolds were seeded with 2×10^5 BMSC cells. In the control negative sample, the basal medium was replaced with chondrogenic medium. The chondrogenic medium contained DMEM (4.5 g/L glucose), ITS