Incorporated dexamethasone influence in the PEO nanofibers on the proliferation of mesenchymal stem cells

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ABSTRACT

Tissue engineering based on the adipose derived stem cells promote a strong strategy to propagation of bone, cartilage and fat tissues in the three-dimensional scaffolds, fabricated by combining biological materials with suitable and appropriate growth factors. In this study, a PEO scaffold was used to show that mesenchymal stem cells can attach well; the aim of this study was the investigation of dexamethasone (Dex) effect on the proliferation of adipose derived mesenchymal stem cells (AT-MSCs). At first, the Mesenchymal stem cells were isolated from adipose tissue. After culturing the cells and having a good density of the population, the cells were transferred to the scaffold. MTT, DAPI and SEM assays were used for evaluation of biocompatibility of PEO scaffolds, cell survival and also cell alignment of cells cultured on the PEO scaffold in comparison with the PEO-Dex. According to the MTT results, MSCs cell viability on PEO-Dex scaffold showed the significant increase compared with PEO scaffold. SEM result also showed that mesenchymal stem cells were more proliferate and grown on the PEO-Dex scaffolds and were more confluent in comparison with PEO scaffold. Evaluation of DAPI staining indicated significantly significant cell nucleus increase in PEO-Dex compared with PEO group. The results manifested that PEO-Dex could be a suitable scaffold for growing mesenchymal stem cells and is a promising candidate for tissue engineering application.

Keywords: Polyethylene oxide; tissue engineering; dexamethasone; mesenchymal stem cells (MSCs)

INTRODUCTION

For the treatment of organ failure and damages, several solutions have been considered in the area of complications, including grafting. In cartilage allografts transplant, the tissue is transplanted from the donor to the recipient [1]. The disadvantage of this type of treatment is the repressive drug consumption which prevents rejection of transplanted tissue in the recipient [2]. Autograft treatment uses a patient's own tissue with several problems observed after autograft treatments [3]. Tissue Engineering is another suitable method for the treatment of organic lesions; it is considered as one of the best treatment options according to some points that decrease its performance [4, 5]. Tissue engineering is a process that creates three-dimensional (3D) tissues by using a 3D scaffolds combination with appropriate cells and cell growth factors [6, 7]. Stem cells derived from adipose tissue has attracted much attention in the reconstruction of soft tissues. Cartilage tissue engineering based on the adipose derived mesenchymal stem cells (AT-MSCs) is a good way to develop 3D cartilage tissues by combining biological materials with suitable and desired growth factors [8]. Dexamethasone (Dex) is a glucocorticoid and has anti-catabolic and anabolic effects on proliferation and differentiation of MSCs into the bone, cartilage and fat lineages [9, 10]. The mechanism which occurs in glucocorticoids on stromal cell differentiation is still under investigation. The use of Dex in *in vitro* cell culture has stimulated proliferation of cells, such as osteoprogenitor and its differentiation to the osteoblast cells [11, 12]. In addition to external growth factors, scaffolds are used as a substitute of extracellular matrix (ECM) with an important role in cell and tissue growth,

causing the cells express a number of factors that prevent necrosis and the uncontrolled growth of cell and tissue [13]. These scaffolds should have several properties such as hydrophilicity, biocompatibility and biodegradability [14-16]. In the present study, we incorporated Dex in the PEO nanofibers and then investigated this construction effect on the proliferation and growth of AT-MSCs in comparison with PEO and tissue culture polystyrene (TCPS) using the MTT, DAPI and SEM assays.

MATERIALS AND METHODS

Extracted mesenchymal stem cells from adipose tissue

In this vitro study, to isolate MSCs from the abdominal fat tissue content of 5 grams, 3 patients underwent abdominoplasty (mean age 30 years, Erfan Hospital, Tehran, Iran) ; the were informed and gave theier consent for such procedure according to Medical Ethics Committee guidelines, Ministry of Health I.R. Iran. Fat samples containing penicillin and streptomycin were detached transferred to the laboratory under Hospital sterile conditions in saline phosphate-buffered solution (1%) and then washed several times and were converted into small pieces with phosphate buffered saline with antibiotics. Then, the fat sample was incubated by 0.2% collagenase for an hour at a temperature C ° 37 for 1 hour. After enzyme digestion was settled, the sample was centrifuged at a speed of 1800 RPM for 10 minutes and then transferred to the flask. After cell density sticikiness of the bottom of the flask reached 80 percent, they were separated from the bottom of the flask through the identification of specific MSCs surface markers, and were isolated with monoclonal antibodies and flow cytometry.

Scaffold Fabrication

For making the scaffold used in this study, 100 mM solution of polyethylene oxide was mixed with 100 nmol dexamethasone, with a 100 mM solution of poly ε-caprolactone (PCL), spun by electrospinning device. Then, the scaffolds were punched and placed in the 24 wells plate. Scaffolds were sterilized afterwards by 70% alcohol for 2 hours. Then, 4×10^4, MSCs were cultured on the surface of scaffolds.

Stem cells culture

MSCs culturing were divided in three 24 wells plates. In one of the 24 wells plates, MSCs were added on the PEO nanofibrous scaffold; in another 24 wells plate MSCs were added on the PEO nanofibrous scaffold incorporated with Dex (PEO-Dex) and finally in the last 24 wells plate, MSCs was cultured on the plate as TCPS (control group) under DMEM supplemented with 10 % FBS.

MTT assay

Checking survival was examined during 5 days of cell culture on a scaffold in the presence of DMEM supplemented with 10 % FBS. During these 5 days, 50µl MTT (3,4 -dimethyl -2 and 5- methyl thiazole -2- Yale tetrazolium bromide (5 mg / ml)) was added to the scaffolds and TCPS. After 4 hours of incubation at 37 °C, all medium was removed and 200 ml of DMSO was added as solvent and then at the wavelength of 570 nm the absorption on ELISA reader (BioTek Instruments, USA) was measured.

DAPI Staining

DAPI staining was performed to check the placement of incorporated cells on the PEO scaffold and PEP-Dex. Therefore, the paint samples were done on 1 to 4 days. Parafermo aldehyde 0.4 % (200 Ml) was used for 20 minutes at 4 ℃ for the purpose of fixation and then washed with PBS; DAPI (400 µl) was added to the sample for 20 seconds afterwards. After 2 times washing, the final sample was observed and photographed by fluorescence microscopy.

SEM

Shooting by electron microscopy was imaged in order to study the morphology of the scaffolds and the morphology of the cells on the scaffold. Fixed samples were done as cultured stem cells on the surface of PEO scaffold and PEO-Dex scaffold at day 7 after seeding. In order for fixation to happen, the scaffold was placed in 400 µl glutaraldehyde 2.5 % for 2 hours. It was then washed with PBS and dehydration process was conducted at the series of alcohol for 10 min in each (50 %, 60 %, 70 %, 80 %, 90 % and 100 %). After a day and prior to the imaging, gold sputtered in vacuum; imaging was performed by EM-3200 digital scanning electron microscope (KYKY, China).

Statistical analysis

All experiments were conducted at least for 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 with SPSS software, version 19.0.

RESULTS

As showed in Fig1, MSCs were derived and grown from tissue fat cells in spindle shape, resembling fibroblasts cells after 2 (Fig 1A) and 5 days (Fig 1B) after isolation, observed by light microscopy.

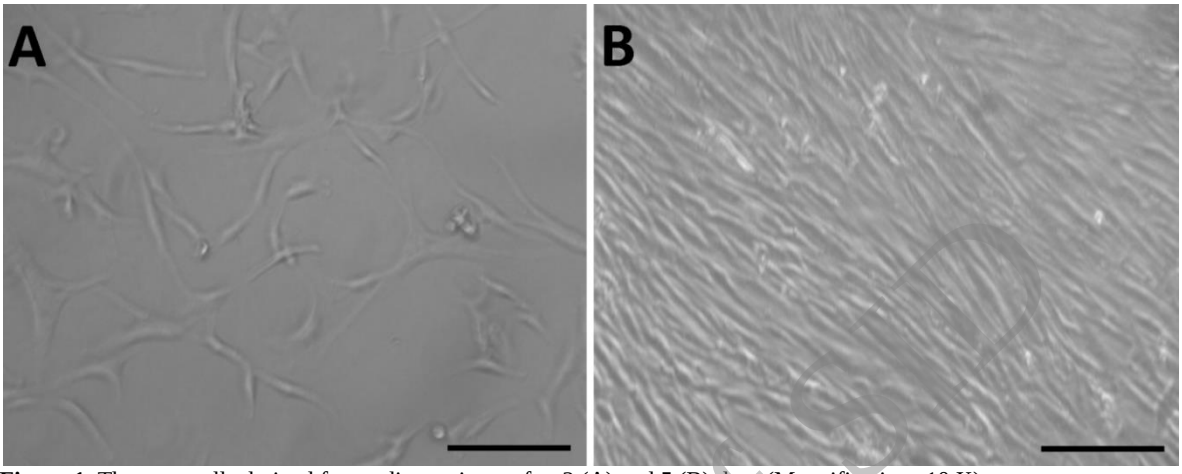

Figure 1. The stem cells derived from adipose tissue after 2 (A) and 5 (B) days (Magnifications 10 X).

MTT assay

Over a period of 5 days from day 1 to day 5 after culturing cells on the PEO and PEO-Dex scaffolds and TCPS as control, cell survival was evaluated by MTT assay (Fig 2). Stem cells showed increasing growth pattern and no significant difference was observed until day 4; yet, on days 4 and 5, the highest significant increased proliferation or cell survival was detected on the PEO-Dex seeded stem cells.

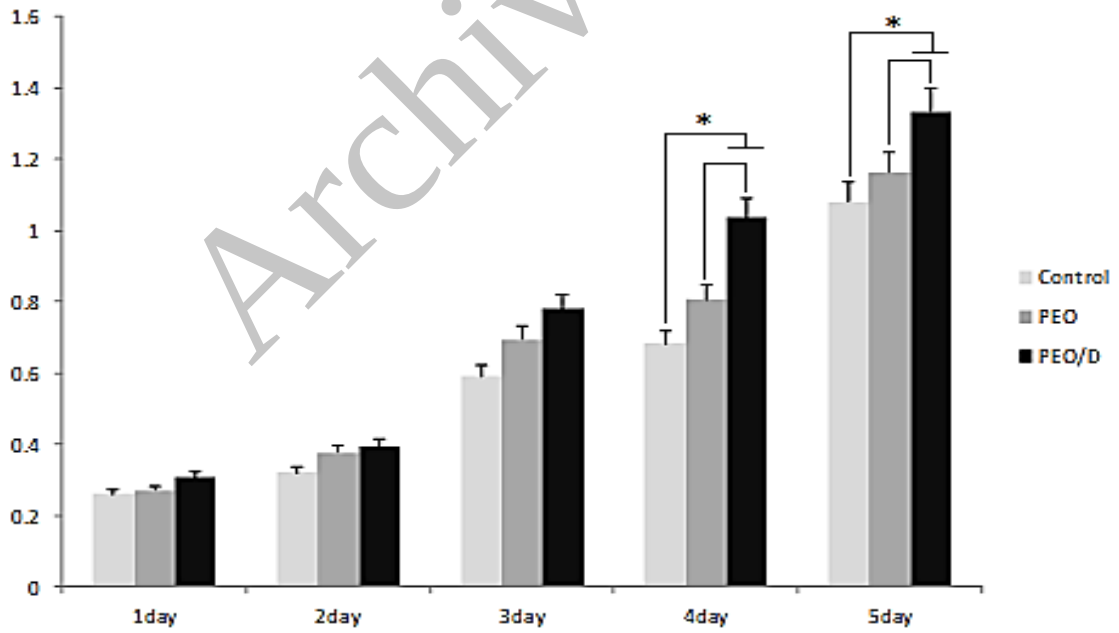

Figure 2. MTT analysis on MCs cells, cultured on the scaffold PEO/D, PEO and control groups, * indicating a statistically significant difference between the 001/0 < P groups.

DAPI staining

In this study, DAPI staining method was used for observation of stem cells cultured on the PEO scaffold and PEO-Dex during 4 days (Fig 3). Increased cell number pattern was detected on scaffolds from day 1 to day 4 after cell seeding and distribution of cells has been quite regular in the

scaffold. The larger population of cells was observed in PEO-Dex scaffold (Fig 3 B, D, F and H) in comparison with PEO scaffold (Fig 3 A, C, E and G). DAPI result also showed that the highest stem cell population was located on day 4 on the PEO-Dex scaffold (Fiig3 H).

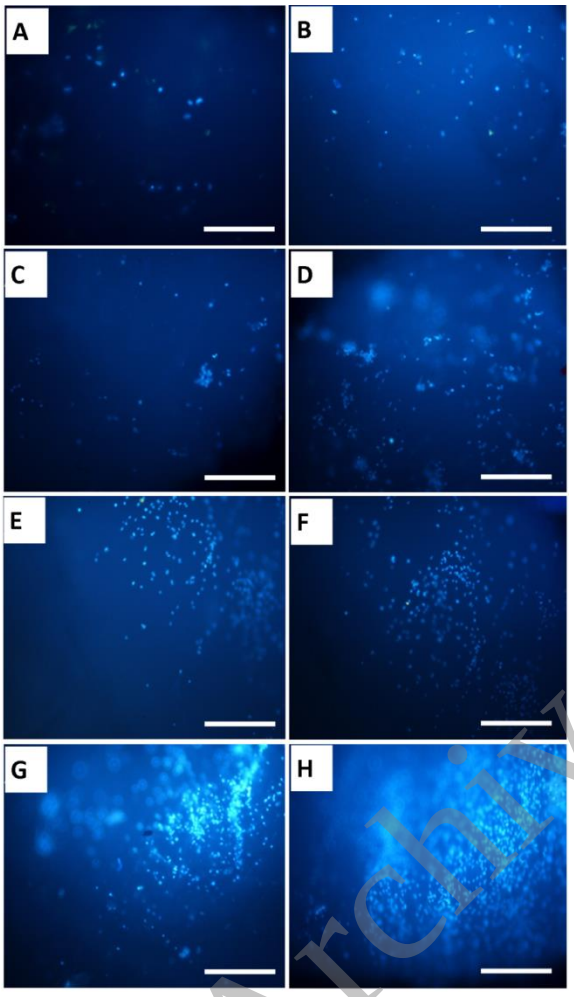

Figure 3. DAPI staining At-T-MSCs seeded scaffold during 4 days. AT-MSCs seeded PEO on days 1 (A), 2 (C), 3 (C) and 4 (G) and AT-MSCs seeded PEO-Dex on days 1 (B), 2 (D), 3 (F) and 4 (H). (Magnifications 10 X)

SEM Imaging

Photography with a SEM was done to study the morphology of the AT-MSCs seeded PEO and PEO-Dex scaffolds (Fig 4 A and B, respectively); results manifested that PEOnanofibers were linear structure without any fracture and willow trellis and contained fibers with an average of 125 ± 568 nm. MSCs at day 7 of culture was also attached and expanded on the surface of nanofibers.Increased density and regular distribution of the cells was shown on the PEO-Dex scaffold (Fig 4B) in comparison with PEO scaffold (Fig 4A) which revealed the fact that PEO-Dex is a more suitable scaffold for growing AT-MSCs.

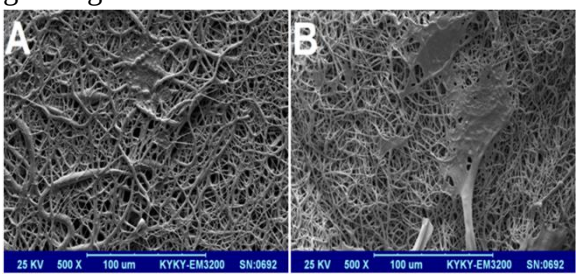

Figure 7. Imaging scanning electron microscopy, (A) in which cells were cultured on the PEO scaffold and (B) Cells were cultured on the PEO-Dex scaffold

DISCUSSION

During 3D culture process, ECM plays an important role in the cultivation and proliferation of stem cells in tissue engineering by establishing a suitable anchorage for adult stem cells and it also creates a proper environment for tissue production [17, 18]. Thus, enhancing the cell adhesion is very important in the process of tissue engineering and surface hydrophilicity and mechanical properties is important to the attachment of stem cells and differentiation into desired lineage, respectively [19].Cells needs 3D suitable matrix to proliferate because the suitable matrix allows the cells to secrete a series of critical growth factors which lead to better differentiation into considered tissue. In the stem cell culture system in hypertrophy situation that is carried out within the Falcon tube in cartilage differentiation, maximum contact is observed among the cells [19]. This method of tissue preparation has a series of restrictions, including small size and low mechanical consistency of the tissues, which limits their use in tissue transplantation. Previous studies have indicated that the fat, bone and cartilage cells on the substrate with high pore have a higher distribution and proliferation and ECM production. On hydrophilic scaffolds, these cells have better adhesion, proliferation and differentiation [20].

Over the past two decades, many degradable scaffolds have been used by scientists in tissue engineering, such as poly glycolic acid (PGA) [21], poly (lactic acid) (PLA) [12], and PCL [22], which include a large family of poly (alpha - esters hydroxyl). In this study, we used PEO scaffold on which stem cells can well connect, proliferate and differentiate. Today, numerous studies are in progress to introduce new and suitable scaffolds to be used in tissue engineering. In this study, dexamethasone

incorporated in PEO nanofibers and its impact was evaluated on the growth and proliferation of AT-MSCs. Results show that not only PEO scaffold is suitable for stem cell proliferation and viability in comparison with TCPS, but this property can be significantly increased with the dexamethasone incorporated in the PEO nanofibers. Porous scaffolds are of great importance to the tissue engineering because they cause the cells to be integrated to each other in order to produce a new tissue [23]. This type of scaffold makes up the platform for those cells to get the ability of being connected multiply and then grow up on the podium. Synthetic biocompatible materials were used as a biological scaffold. Self-assembling peptides, organic polymers, inorganic materials or compounds mixed biomass co- polymer were used for scaffold fabrication [24]. Recent reports on tissue engineering emphasize the importance of structural properties of the biological materials. Several studies were widely used on growth factors such as TGFβ and Dex to induce the proliferation and differentiation of AT-MSCs. Since PEO is hydrophilic, it can be combined with Dex and their composite can be the effective combination for growing mesenchymal cells. Dex secretes from a nanofibers continuously and slow during process and is always active and can influence cell growth in the culture medium.

"The authors declare no conflict of interest"

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