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Physical stimulation and scaffold composition efficiently support osteogenic differentiation of mesenchymal stem cells

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ABSTRACT

Background: Despite significant achievements in the field of tissue engineering, simplification and improvement of the existing protocols are of great importance. The use of complex differentiation media, due to the presence of multiple factors, may have some undesired effects on cell health and functions. Thus, minimizing the number of involved factors, while maintaining the differentiation efficiency, provides less costly and controllable conditions. Adipose-derived Mesenchymal stem cells (ASCs), the adult stem cells present in adipose tissue, can be a suitable source of stem cells due to abundant and ease of access. The aim of this study is to optimize the osteogenic differentiation of ASCs by chemical composition of scaffold, in the first step, and then by electromagnetic treatments.

Methods: ASCs were cultured on PVA/PES scaffold and tissue culture polystyrene surfaces (TCPS) and osteogenic differentiation was performed with either osteogenic medium, or electromagnetic field or both. The impact of each treatment on ASCs growth and proliferation was measured by MTT assay. Changes in gene expression levels of osteogenic-specific markers including ALP and RUNX2 were determined by Real Time PCR. Furthermore, alkaline phosphatase activity and calcium deposition were measured.

Results: The MTT assay showed the significant effects on cell growth and respiration in scaffold-seeded ASCs treated with electromagnetic field, compared to control TCPS plate. Also, the electromagnetic treatment, increased alkaline phosphatase activity and calcium deposition. Finally, Real Time PCR showed higher expression of ALP and RUNX2 genes in electromagnetic field groups compared to control groups.

Conclusion: It can be concluded that PVA/PES scaffold used in this study improved the osteogenic capacity of ASCs. Moreover, the osteogenic potential of ASCs seeded on PVA/PES scaffold could be augmented by electromagnetic field without any chemical stimulation.

1. Introduction

Today, Bone tissue engineering is considered as a promising approach for bone defect repair (Cancedda et al., 2007; Choong et al., 2006), which needs three key components, i.e., biomaterial scaffold, osteogenic cell lineage and osteogenic stimulation factors. Different kinds of biomaterial and stimuli, weather natural or synthetic, have been used to mimic the real environment of bone tissue and osteogenic differentiation (Wang et al., 2012; Wang et al., 2017; Wang et al., 2014). The main feature of scaffold materials for bone tissue engineering is cell attachment and osteoinductive properties (Ashammakhi and Ferretti, 2004). on the other hand, the main challenge ahead for bone tissue engineering is to find suitable cell sources to optimize the differentiation efficacy of implanted cells toward osteoblasts and form neo-vasculature (Robey, 2011).

Mesenchymal stem cells (MSCs), derived from different sources like bone marrow and adipose tissue, have the ability to commit to a range of lineages including osteogenic lines (Kusuyama et al., 2014). Although bone marrow is perceived as an enriched source of MSCs, Adipose tissues have become an alternative attractive source to obtain Adipose-derived Mesenchymal stem cells (ASCs) for use in tissue engineering therapies. Both cell types can differentiate into osteoblasts and have phenotypic similarities (Zuk et al., 2002).

Different characteristics have stimulated a growing interest in ASCs to alternate MSCs in adult stem cells research. Compared with stem cells isolated from bone marrow (BMSCs), the isolation procedure of ASCs is relatively easier and is from human liposuction aspirates, which people generally want to eliminate. Furthermore, the procedure is less

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invasive for donors and patients, presents less ethical issues and led to more abundant number of MSCs. ASCs proliferate rapidly, have good attachment property and can be maintained in culture due to their self-renewing properties (Zuk et al., 2002). The application of ASCs, given their autologous cell source, minimizes the risk of immunogenicity associated with allograft sources. All these characteristics make ASCs an attractive cell source for clinical application (Zhu et al., 2012).

To date, many studies have reported the differentiation ability of ASCs, whether transplanted or injected, into osteogenic lineage, and showed improved fracture healing and bone regeneration (Shoji et al., 2010). Osteogenic differentiation of ASCs can be induced by various stimuli, including chemical and biophysical signals (Tapp et al., 2009). Biophysical signals have been actively studied for clinical applications.

Low-frequency, low-energy, electromagnetic fields (EMFs) are commonly used, with beneficial effects, to promote bone fracture healing in clinic (Fredericks et al., 2000). This effect of EMFs can be related to the activity of PEMFs on osteoblasts and osteogenesis differentiation of MSCs (Luo et al., 2012). The regulatory roles of EMFs have been observed in several in vitro, in vivo, and clinical studies and the results have shown the improved osteogenesis, decreased osteoporosis and, increased DNA synthesis (Tsai et al., 2009), extracellular matrix production, and inhibited inflammatory activities following exposure to EMF (Ongaro et al., 2014). In addition, EMFs induce the gene expression of osteogenic markers and calcified matrix production during mineralization (Fassina et al., 2006). The Frequencies used for osteogenic differentiation are in the range of 7.5–75 Hz (Tsai et al., 2009; Tsai et al., 2007; Sun et al., 2009a; Schwartz et al., 2008).

There are many studies investigating EMFs effects on BMSCs, which showed their stimulatory effects toward osteogenesis (Sun et al., 2009b), but the research evaluating osteogenic effects of EMFs on ASCs has only been more recently performed in a limited studies (Ongaro et al., 2014; Chen et al., 2013). Specially, to our knowledge, there is no study to evaluate the osteogenic differentiation of ASCs on scaffold and under EMFs exposure.

With this background, the aim of the present study is to evaluate whether ASCs seeded on PVA/PES scaffold exposed to EMFs may differentiate toward osteogenic lineages. To this purpose, we studied the effects of EMFs on ALP activity, calcium deposition and expression of osteogenic-specific genes including ALP and RUNX2. The results showed that PVA/PES scaffold along with electromagnetic treatment can provide a good osteogenic differentiation condition for ASCs and obviate the exogeneous addition of chemical/biological factors.

2. Materials and methods

2.1. Cell isolation and characterization

Adipose tissue was supplied from Eram hospital (Tehran, Iran) which repeatedly rinsed with buffer solution under sterile conditions, then washed with phosphate buffer saline buffer (PBS) for 3 times; and digested with DMEM containing type I collagenase for 40 min, centrifuged with 1200 rpm for 10 min. After discarding the supernatant, the precipitated cells were suspended in DMEM medium into single cell and incubated at 37 °C, 5% CO_2. The medium was changed once every 2–3 days.

For evaluating the multipotency of the isolated cells, flow cytometry analysis was carried out to measure the expressions of CD34, CD44, CD44 and CD90 according to (Kabiri et al., 2015). Further, the capability of the isolated cells to differentiate to osteogenic and adipogenic lineage was studied. Cells between passages 3–9 were used for next evaluations.

2.2. Scaffold construction and characterization

Briefly, polyvinyl alcohol (PVA) solution was prepared by fully dissolving 1.2 g of polymer powder in 10 ml of distilled water. polyethersulfone solution (PES) was produced in a similar procedure by fully dissolving 3.16 g per 10 ml of Dimethylformamide (DMF). The PVA/PES hybrid scaffold was prepared in weight ratio of 2–1.

Characterization of three-dimensional scaffolds was determined through Scanning Electron Microscope (SEM) and the mechanical characteristics of elasticity Scaffolding (Tensile test). Tensile tests were performed using SANTAM (Iran) according to testing machine (Dodel et al., 2016). The test was conducted at the speed of 5 mm/min. Three samples were tested for each type of scaffold.

2.3. Osteogenic differentiation of culture-expanded human mesenchymal stem cells into scaffold

Plasma treatment was performed for improving cell biocompatibility of a biodegradable polymer scaffold (frequency 40 KHz, power 30W, gas pressure 0.4 mbar). After 2 days of cell seeding, cells were treated with standard osteogenic differentiation medium, electromagnetic waves or both, simultaneously. For electromagnetic field treatment, a function generator (STRC, Iran) was used. The device applied a 12 MHz microwave and 30 mili-tesla pulsed magnetic fields with the frequency range of 50–400 Hz. The differentiation period was 14 days and the cells were exposed to EMF 8 h/day.

Calcium deposition was measured by dissolution of the cell seeded on scaffolds in 0.6 N HCl (Merck). After centrifugation (1200 rpm, 5 min), the released calcium was determined using calcium assay kit (Pars Azmoon). The optical density of samples was measured in a microplate reader (BioTek instruments, USA). Finally, the data was normalized against total protein for all groups (Pakfar et al., 2017).

ALP activity was monitored by homogenization in Ripa buffer and subsequent sonication (maximum power, 2 min on ice). ALP activity was determined using alkaline phosphatase kit and according to the manufacture instructio (Pars Azmoon) for 30 min (5%Co2, 37 °C). Optical density of samples was evaluated by microplate reader at 450 nm (BioTek instruments, USA). Finally, the data was normalized against total protein for each group.

2.4. Cell viability by MTT assay

To evaluate viability of cells, MTT test was performed on days 3, 7, 10 and 14. 4000 cells were seeded on 1 cm scaffolds. Samples were incubated (5% CO2 at 37 °C) with MTT solution for 2 h then, they were vortexed with DMSO for 1 min. After that, the optical density was measured by microplate reader (BioTek instruments, USA).

2.5. Histological analysis

In order to estimate proliferation and differentiation of cells on scaffolds, Alizarin Red and Hematoxylin & Eosin staining were accomplished. After the 14th day, cells were fixed by paraformaldehyde, then sections were made and H&E staining was performed at Jamali cytopathology Laboratory (Iran). The sections were then deparaffinized with xylene and rehydrated in an ethanol series (100%, 95%, 70% and 50%). Alizarin red staining was done to evaluate calcium deposition during osteogenic differentiation.

2.6. Quantification of gene expression

Total RNA extracted (CinnaGen) from cultured cells using Qiazol lysis buffer was used as a template for complementary DNA synthesis (Fermentas). Real-time PCR was performed using the SYBR Green PCR Master Mix assay and the Corbett system. Data were normalized against β2 M gene. Gene expression levels of all experimental groups were expressed as fold changes relative to levels of the control group (ΔΔT). Gene expression data were analyzed using REST 2009 software.