Saudi Pharmaceutical Journal 25 (2017) 575–579

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Osteogenic differentiation of bone marrow mesenchymal stem cells by magnetic nanoparticle composite scaffolds under a pulsed electromagnetic field

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ARTICLE INFO

Article history:
Available online 8 May 2017

Keywords:
Magnetic nanoparticles
Bone marrow mesenchymal stem cells
Osteogenic differentiation
Pulsed electromagnetic field

ABSTRACT

This study was conducted to investigate the effect of magnetic nanoparticle composite scaffold under a pulsed electromagnetic field on bone marrow mesenchymal stem cells (BMSCs), which was achieved by examining the biological behaviors of cell adhesion, proliferation and differentiation on the surface of the scaffolds. This may provide some experimental evidence for the use of magnetic nanoparticles in medical application. The magnetic nanoparticle composite scaffolds were evaluated and characterized by the following indexes: the cell proliferation was detected by the CCK-8 method, the alkaline phosphatase (ALP) activity was examined by a detection kit, and the expression of type I collagen and osteocalcin gene were evaluated by RT-PCR. The CCK-8 test showed that there was no significant difference in Group A (BMSCs-seeded magnetic scaffolds under the electromagnetic field), B (BMSCs-seeded magnetic scaffolds) and C (BMSCs cultured alone) ($P > 0.05$). The value for the ALP activity in Group A was higher than the other two groups. In addition, the RT-PCR results showed that the expression of type I collagen gene in Group A was enhanced ($P < 0.05$), suggesting that the magnetic nanoparticles combined with the pulsed electromagnetic field had a positive effect on the osteogenic differentiation of BMSCs. However, the expression of osteocalcin was not significantly different in three groups ($P > 0.05$). To conclude, magnetic nanoparticles may induce the osteogenic differentiation with the action of the pulsed electromagnetic field.

1. Introduction

In recent years, with the development of bioengineering and biomedicine, some functional molecules of medical drugs have been introduced to improve biomedical materials (Nawaz et al., 2017). Magnetic nanoparticles have attracted much attention as a new medical drug in the biomedical field, and are widely applied in targeting delivery, bio-imaging and cancer therapy (Weizenecker et al., 2009; Wang et al., 2013; Maleki-Ghaleh et al., 2016; Hu et al., 2009; Hirsch et al., 2003). The most unique feature of magnetic nanoparticles is the response to the electromagnetic field, enabling some applications such as drug targeting and the separation of molecules and cells (Son et al., 2005). Moreover, the electromagnetic field can exert the appropriate force on the cells with magnetic particles, offering a powerful tool to control cell behaviors. For example, Bradshaw et al. (2015) found that the migration speed of fibroblasts can be enhanced with the internalized magnetic nanoparticles in the presence of a electromagnetic field. Perica et al. (2014) showed that T cell receptor antibody immobilized paramagnetic particles could bind to cell surface and formed large agglomerates under the electromagnetic field, leading to the receptors binding to each other and thus the activation of specific cell functions. Jiang et al. (2016) also showed that bone marrow mesenchymal stem cells can differentiate into osteoblasts under the action of magnetic nanoparticles and the

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2017.04.026
1319-0164/

external electromagnetic field, and promoted the expression of osteocalcin and type I collagen. It is thus believed that the force generated by the magnetic nanoparticles under a electromagnetic field will influence cell behaviors significantly. In this study, magnetic nanoparticle composite scaffolds were prepared using the magnetic nanoparticles Fe_2O_3, Nano-hydroxyapatite (n-HA) and L-polylactic acid (PLLA). *In vitro* study of BMSCs seeded onto the magnetic nanoparticle composite scaffolds under a pulsed electromagnetic field was performed, which was to explore the cell adhesion, proliferation, differentiation and other biological behaviors in this case. This may provide some experimental evidence for the use of magnetic nanoparticles in medical application (Gohar et al., 2017; Muhammad et al., 2017).

2. Material and methods

2.1. Experimental materials and instruments

Fe_2O_3, n-HA and PLLA were obtained from Shandong Medical Instruments Institute, China. α-MEM, fetal bovine serum, 500 U/mL penicillin and 500 μg/mL streptomycin were purchased from Gibco, USA. CCK-8 cell proliferation and alkaline phosphatase detection kit were obtained from Keygen, China. TRIzol®Reagent was purchased from Invitrogen, USA. A low temperature rapid prototyping instrument (Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, China) was used for the fabrication of magnetic nanoparticle composite scaffolds. SEM imaging was conducted using a scanning electron microscopy (MIRA3 TESCAN, Tsinghua University, Shenzhen Institute, China). Pulsed electromagnetic field was obtained from a CLM-B-type pulse magnetic field therapy instrument (Hebei Langfang Hammer Medical Devices Company, China).

2.2. Fabrication of magnetic nanoparticle composite scaffolds

Fe_2O_3, n-HA and PLLA were mixed into an aqueous solution in a certain mass ratio, and the magnetic nanoparticle composite scaffolds were prepared using low-temperature rapid prototyping (Fig. 1a). The scaffold is a three-dimensional porous structure with a porosity of 80–85%. The SEM image shows that the pores are highly interconnected. At a higher magnification, the surface of the scaffold is uneven with the magnetic nanoparticles evenly distributed on the surface (Fig. 1b). Prior to the cell test, magnetic nanoparticle composite scaffolds were processed with the ultraviolet radiation for half an hour, followed by soaking in the PBS solution for cleaning and the 75% ethanol disinfection.

2.3. Isolation and culture of bone marrow mesenchymal stem cells (BMSCs)

3 months old New Zealand white rabbits were routinely anaesthetized, disinfected, and left femur trochanter. Bone marrow was aspirated with a 2 mL heparin syringe and placed in a 10 mL centrifuge tube with 5 mL PBS solution, followed by a 1200 r/min centrifugation for 10 min. High glucose DMEM medium (containing 10% FBS, 1% double antibody (penicillin/streptomycin)) was added to the cell suspension after removing the supernatant, which was then incubated with 5% CO_2 at 37 °C. At 80% confluency, BMSCs were harvested by trypsin, suspended in DMEM, centrifuged, and diluted to a concentrated cell suspension. Medium was changed every 3 days by removing 1 mL and replenishing 1 mL. BMSCs were spindle, triangular or irregular polygon under the microscope (Fig. 2).

2.4. BMSCs-seeded magnetic nanoparticle composite scaffolds

In this study, three groups were divided: In Group A and Group B, BMSCs (1×10^5 cells) were seeded on the magnetic nanoparticle composite scaffolds and were inoculated into a-MEM medium containing 10% fetal bovine serum under the aseptic condition (Fig. 3a). A pulsed electromagnetic field with the magnetic field strength of 100MT was applied in Group A (Fig. 3b). In Group C BMSCs (1×10^5 cells) were cultured alone. All the groups were placed in the 37 °C incubator supplied with 5% CO_2. The media were refreshed every 3 days.

2.5. Detection of cell proliferation activity

A Cell Counting Kit-8 (CCK-8) method was applied to the three groups. In each group, 50 μL of CCK-8 solution was added at 1, 3, 7, 14 and 21 days of cell culture (n = 5), which was incubated with the samples at 37 °C for 4 h. The light absorption value (A value) was obtained at 490 nm using a microplate reader to plot the cell proliferation curve.

2.6. Alkaline phosphatase (ALP) quantification

ALP is an early stage marker of osteogenic differentiation of BMSCs, which is elevated at relative early days and decreased at later stage. Therefore Day 7 was selected as the time point to check

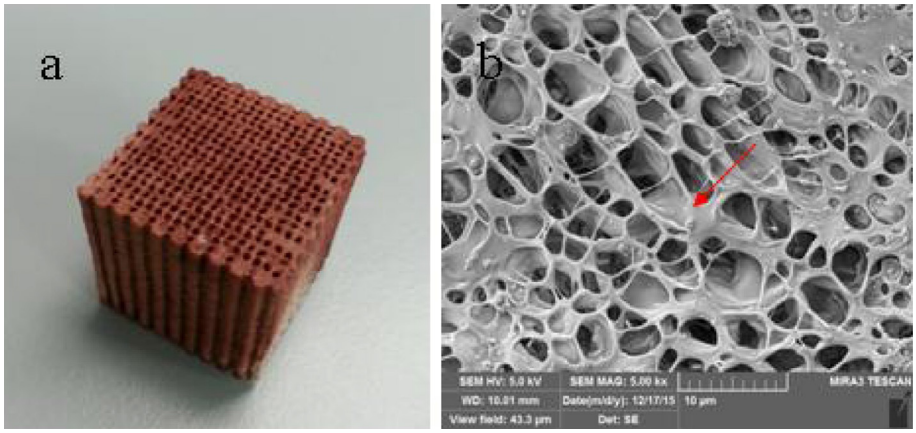

Fig. 1. (a) Magnetic nanoparticle composite scaffold (gross view) and (b) the microstructure of magnetic nanoparticle composite scaffold (SEM), the surface of the scaffold is uneven with the magnetic nanoparticles evenly distributed on the surface.