Journal of Magnetism and Magnetic Materials 393 (2015) 526–536

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

The effect of magnetic stimulation on the osteogenic and chondrogenic differentiation of human stem cells derived from the adipose tissue (hASCs)

João Lima [a,b], Ana I. Gonçalves [a,b], Márcia T. Rodrigues [a,b], Rui L. Reis [a,b], Manuela E. Gomes [a,b,*]

[a] 3Bs Research Group–Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal
[b] ICVS/3Bs–PT Government Associate Laboratory, Braga/Guimarães, Portugal

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form
27 May 2015
Accepted 30 May 2015
Available online 6 June 2015

Keywords:
Adipose-derived stem cells
Osteogenic differentiation
Chondrogenic differentiation
Magnetic nanoparticles
Tissue engineering
Regenerative medicine

ABSTRACT

The use of magnetic nanoparticles (MNPs) towards the musculoskeletal tissues has been the focus of many studies, regarding MNPs ability to promote and direct cellular stimulation and orient tissue responses. This is thought to be mainly achieved by mechano-responsive pathways, which can induce changes in cell behavior, including the processes of proliferation and differentiation, in response to external mechanical stimuli. Thus, the application of MNP-based strategies in tissue engineering may hold potential to propose novel solutions for cell therapy on bone and cartilage strategies to accomplish tissue regeneration.

The present work aims at studying the influence of MNPs on the osteogenic and chondrogenic differentiation of human adipose derived stem cells (hASCs). MNPs were incorporated in hASCs and cultured in medium supplemented for osteogenic and chondrogenic differentiation. Cultures were maintained up to 28 days with/without an external magnetic stimulus provided by a magnetic bioreactor, to determine if the MNPs alone could affect the osteogenic or chondrogenic phenotype of the hASCs.

Results indicate that the incorporation of MNPs does not negatively affect the viability nor the proliferation of hASCs. Furthermore, Alizarin Red staining evidences an enhancement in extracellular (ECM) mineralization under the influence of an external magnetic field. Although not as evident as for osteogenic differentiation, Toluidine blue and Safranin-O stainings also suggest the presence of a cartilage-like ECM with glycosaminoglycans and proteoglycans under the magnetic stimulus provided.

Thus, MNPs incorporated in hASCs under the influence of an external magnetic field have the potential to induce differentiation towards the osteogenic and chondrogenic lineages.

1. Introduction

Bone and cartilage defects are a clinical problem that affects a multitude of people worldwide. Bone tissue has innate regeneration capacity granting this tissue the capacity to self-repair minor injuries. However, large bone defects are not fully healed without treatments, such as surgical procedures, whose outcomes have limited success in a long-term basis. Cartilage, on the contrary, exhibits a naturally limited regeneration capacity associated to the lack of vascularization hindering its healing process. Current strategies in the treatment of bone and cartilage, namely auto- and allo-grafting, and implantation of metallic prosthesis [1,2] are unable to fully restore complete tissue function, and evidence severe disadvantages that include limitation of autologous donor sites, risks of disease transmission and immunosuppression, risks of infection and extrusion of the prosthesis and lack of functionality [3].

Tissue engineering (TE) offers novel approaches to treat and regenerate bone and cartilage defects. In the past few years, magnetic nanoparticles (MNPs) have gained a prominent position in the biomedical field, and increasingly used as contrast agents for magnetic resonance imaging or drug delivery systems [4]. In the TE field, MNPs are being studied as magneto-mechanical stimulators/activators of cell arrays, in mechano-sensitive ion channels and magnetic cell sorting procedures, and in mechanisms of controlled cell proliferation and differentiation [5]. Moreover, the application of MNPs in combination with TE strategies could result in the development of innovative solutions to stimulate cells at a

* Correspondence to: at: 3Bs Research Group–Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães 4806-909, Portugal.
E-mail address: megomes@dep.uminho.pt (M.E. Gomes).

http://dx.doi.org/10.1016/j.jmmm.2015.05.087
0304-8853/

nanoscale, that is, at the cellular level, influencing cellular processes and responses such as cell migration or differentiation. Mesenchymal stem cells are often considered a promising cell source due to their intrinsic and well described properties in many studies aiming at cell-based therapies. Among them, human adipose-derived stem cells (hASCs) can be harvested with minimal invasive procedures and isolated and expanded to high numbers. Moreover, hASCs have long-term genetic stability and have demonstrated potential to undergo adipogenic, neurogenic, osteogenic and chondrogenic differentiation [6–10].

Considering the impact of MNPs in cell guidance and behavior aiming at cell based strategies, this study proposes to investigate the influence of MNPs in promoting hASCs differentiation towards osteogenic and chondrogenic lineages, under the actuation of a remote mechano-magnetic stimulus provided by an external magnetic field.

For that purpose, MNPs were incorporated in hASCs, and cultured in basic medium or media containing osteogenic or chondrogenic specific supplements. The influence of the MNPs per se or under the actuation of an external magnetic stimulus generated by a bioreactor apparatus in synergy with medium biochemical factors was also assessed in the differentiation process of hASCs.

Cell viability and proliferation levels were weekly evaluated by MTS and DNA quantification assays, respectively for up to 28 days. Bone and cartilage lineage commitment was assessed through specific histological stains to detect markers associated to native bone or cartilage extracellular matrices.

2. Materials and methods

2.1. Materials

Magnetic nanoparticles (MNPs) selected for this study were commercially available at Micromod (Germany) under a product name *nanomag®-CLD-redF* (23-00-102). These MNPs with approximately 100 nm in diameter are cross-linked dextran iron oxide composite particles with an iron oxide core [CAS: 1317-61-9] of about 75–80 wt%, and with a polydispersity index < 0.2. These particles also include optical properties as being red fluorescent. Based on previous studies performed (data not shown) MNPs were re-suspended in basic culture medium at 370 μg/ml. MNPs were handled in controlled conditions and operated under aseptic conditions.

2.2. Assessment of MNPs stability in the culture medium

In order to test the stability of these particles in suspension in the culture medium, a turbidity assay was monitored over a period up to 24 h, namely 0, 1.5 h, 16 h and 24 h, with a MNP concentration of 370 μg/ml. MNPs suspended in culture medium or in PBS, as in the original suspension provided by Micromod, was analyzed in quadruplicates.

The turbidity of MNPs suspension was measured at 450 nm [11,12] using a microplate reader (Bio-tek, synergie HT).

2.3. Human adipose derived stem cells isolation and expansion

Human adipose derived stem cells (hASCs) were obtained from lipoaspirate samples following a protocol previously established with the Department of Plastic Surgery of Hospital da Prelada, Porto, Portugal. Samples were collected following informed consent and the protocol of ethics. Cells were isolated as described elsewhere [13]. Briefly, samples were digested using 0.2% collagenase type II (Sigma) in phosphate buffered saline (PBS), for 45 min, at 37 °C under stirring. A lysis buffer was used to remove the erythrocytes present. Adherent cells were then cultured and expanded in a basic medium composed of α-minimal essential medium (α-MEM; Gibco) with 10% Fetal Bovine Serum (FBS) (Gibco; heat-inactivated), 1% Antibiotic–Antimycotic solution (A/B, Invitrogen) and Sodium Bicarbonate (Sigma). Medium was changed three times per week. Then, cells were cryopreserved in a solution containing 90% FBS and 10% dimethyl sulphoxide (DMSO CryoSure, Wak-Chemie Medical GMBH) until further use. Cells were used in passage 2.

2.4. Determination of MNPs incorporation in hASCs

2.4.1. Microscopy analysis

In a previous study, we observed that hASCs were able to incorporate the particles even in the absence of a magnetic force stimulus provided by the bioreactor (Magnefect Nano II, Nanotherics) (unpublished data). Thus, to verify these outcomes for the differentiation study, hASCs were cultured in adherent 24-well plates at a density of 10,000 cells/well, and MNPs ressuspended in basal medium. After an overnight incubation, hASCs were washed in PBS and stained with Phalloidin–Tetramethylrhodamine B isothiocyanate (Phalloidin) solution, which was prepared accordingly to manufacturer's instructions (P1951, Sigma; dilution 1:200), followed by buffered formalin solution (43.05-k01009, INOPAT) fixation for 30 min, and by 4,6-Diamidino-2-phenyindole, dilactate (DAPI, 5 μg/μl, D9564, Sigma) stain for 10 min.

Moreover, cells were stained with a solution of 20% hydrochloric acid (VWR) and 10% potassium ferrocyanide (P3289, Sigma) to obtain a bright blue pigment, called prussian blue or ferric ferrocyanide, used for iron detection. Briefly, cells incorporating MNPs were immersed in a solution of equal parts of hydrochloric acid and potassium ferrocyanide for 20 min, followed by washing in distilled water and counterstained with nuclear fast red (Sigma) for 7 min. Samples were then rinsed twice in distilled water and kept in PBS until visualization. hASCs were then analyzed under a transmitted and reflected light microscope (Zeiss, Imager Z1M) for the detection and co-location of MNPs and the cells.

The MNPs within the cells were also screened by scanning electron microscopy (SEM). After MNPs incubation, hASCs were rinsed in PBS, fixed in formalin and dehydrated in a series of ethanol concentrations (30%, 50%, 70%, 80%, 90% and 100% ethanol) under mild agitation. Afterwards, the specimens were let to air-dry in a hood over night before sputter coated with gold. SEM observation was performed with a JSM-6010LV equipment (JEOL, Japan) equipped with an energy dispersive spectroscope (INCAx-Act, PentaFET Precision, Oxford Instruments, UK).

2.4.2. ICP spectrometry

The efficiency of MNPs incorporation in hASCs was further verified by measuring the levels of iron released into the culture medium after ASCs incubation with the MNPs. Briefly, the iron released was evaluated by an ICP spectrometer (JY2000-2, Jobin Yvon, Horiba, Japan) in culture medium samples collected after 24 h of incubation of the cells with MNPs, and filtered (0.22 μm, VWR) prior to analysis. Due to the presence of iron in α-MEM composition, a sample of α-MEM medium was used as blank solution. An iron (Fe, 1000 μg/mL) standard solution (13830, Specpure®) was used to prepare the standard concentration solutions.

2.5. Assessment of cell viability (MTS assay) and cell content (double strand DNA quantification assay)

After each of the selected time-point (7, 14, 21 and/or 28 days), culture medium was removed, samples rinsed with PBS and their metabolic activity assessed using a MTS assay performed following manufacturer instructions. Concisely a solution of MTS reagent (3-