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Application Note 24

UPMBIOFORE BEYOND FOSSILS

Osteogenic and adipogenic differentiation of human mesenchymal stem cells

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INTRODUCTION

Mesenchymal stem cells (MSCs) are adult stem cells that are able to give rise to bone, cartilage and fat cells [1]. Since their discovery, differentiation of MSCs has been traditionally conducted on a flat two-dimensional (2D) glass or polystyrene surface. In addition to lacking the three-dimensional extracellular matrix, 2D cell culture is known to result in unnatural cell polarity and morphology. Moreover, MSCs are known to sensitively respond to topological and mechanical cues, and thus differentiation in 3D can be considered more physiological than under 2D conditions. Indeed, modulated osteogenic and adipogenic differentiation properties of MSCs have been reported [3, 4, 5]. Delivery of bone forming cells within a known and controlled 3D scaffold may help towards regenerating bone fractures or critical size defects [6, 7].

Recently, we reported successful culture expansion of MSCs within three dimensional nanofibrillar cellulose, GrowDex® [8, Application note AN 022]. Here, we describe osteogenic and adipogenic differentiation of human MSCs embedded within GrowDex in 24-well tissue culture inserts.

MATERIALS

- Palatal adipose tissue derived Mesenchymal Stem Cells were obtained from adult donors with written informed consent with an approval from the Ethics Committee of Dental Faculty of Selcuk University (approval number 2012-08)
- Complete Media: DMEM (High Glucose) supplemented with 10% FBS, 1% Pen/Strep and 1% L-Glutamine (Sigma Aldrich)
- Differentiation Media: StemPro™ Adipogenesis or Osteogenesis Differentiation Kit (Cat# A1007001 and Cat# A1007201, ThermoFisher) supplimented with 1% Pen/Strep
- 24 well tissue culture inserts: 3.0µm pore PET membrane (Cat# 83.3932.300, Sarstedt)
- Low adhesion 24-well cell culture plate (Cat# 83.3922.500, Sarstedt)
- GrowDex, 1.5% (Cat# 100 103 005, UPM)
- 4% Paraformaldehyde (PFA)
- Oil Red O: 300 mg Oil Red O in 100 ml 99% isopropanol (Cat# O0625-25G, Sigma Aldrich)
- Alizarin Red S: 2 g Alizarin Red S in 100 ml distilled water, pH 4.1 with 0.1% NH4OH, filtered (A5533-25G, Sigma Aldrich)
- EVOS Live imaging system (ThermoFisher)

METHODS

1. Adipose derived and palatal derived MSCs were cultured in complete media and incubated at 37°C with 10% CO2.
2. Following trypsinization, cells were resuspended in complete media at a concentration of 1 x 106 cells/ml.
3. Subsequently, cells were mixed with the appropriate volume of complete media and GrowDex to provide a final concentration of 0.2% GrowDex seeded with 2x105 cells (2000cells/µl).
4. Embedded cells were then transferred to 24-well tissue culture inserts and fed with 500 µl complete media within the outer well.
5. Medium was changed after 3 days to either complete media (control), or Stem Pro Adipogenic/Osteogenic media and incubated at 37°C with 10% CO2.
6. Control and differentiation media was replaced every 2 to 3 days and cells were maintained for 21 days.
7. Following the differentiation period, media was removed from the wells and inserts and cells were washed with PBS and then fixed with 4% PFA for 30 mins.
8. For Oil Red O staining: 3 parts of the stock solution was mixed with 2 parts ddH2O and filtered. Following fixation, cells were washed with sterile ddH2O. Staining solution was added to the cells for 5 mins. For Alizarin red staining: following fixation, Alizarin red staining solution was added to cells and incubated for 45 mins at RT in the dark.
9. The staining solutions were removed and unbound dye was washed off by 3 to 5 washing steps with ddH2O.
10. Images were taken using the EVOS imaging system.

RESULTS

Following 21 days of differentiation treatments, MSCs stained positive for lipid accumulation (Fig.1) and calcium deposition (Fig.2).

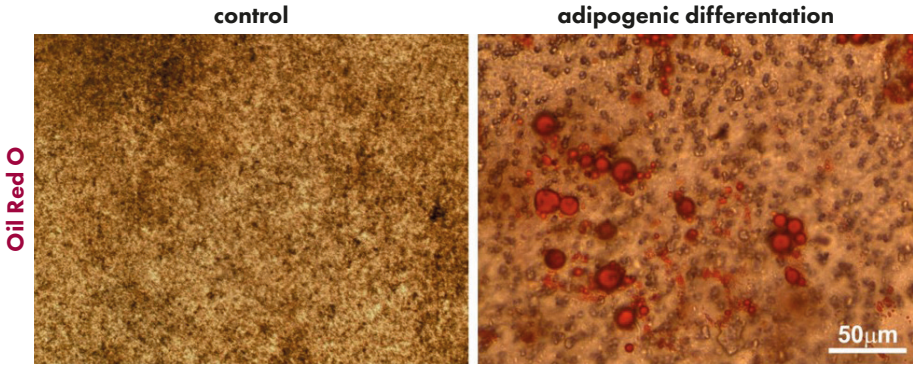

Figure 1. Images showing MSCs stained with Oil Red O following 21 days of incubation in either control (left) or adipogenic differentiation (right) media. Deposition of intracellular lipid droplets can be seen within differentiated cells (right). Scale bar is 50µm.

Oil Red O staining of cells treated with adipogenic differentiation media revealed positive staining of intracellular lipid accumulation (Fig.1, right panel), in contrast to the control (Fig.1, left panel). Alizarin red staining of cells treated with osteogenic differentiation media showed high levels of calcium deposition (Fig.2, right panel) compared to the control (Fig.2, left panel).

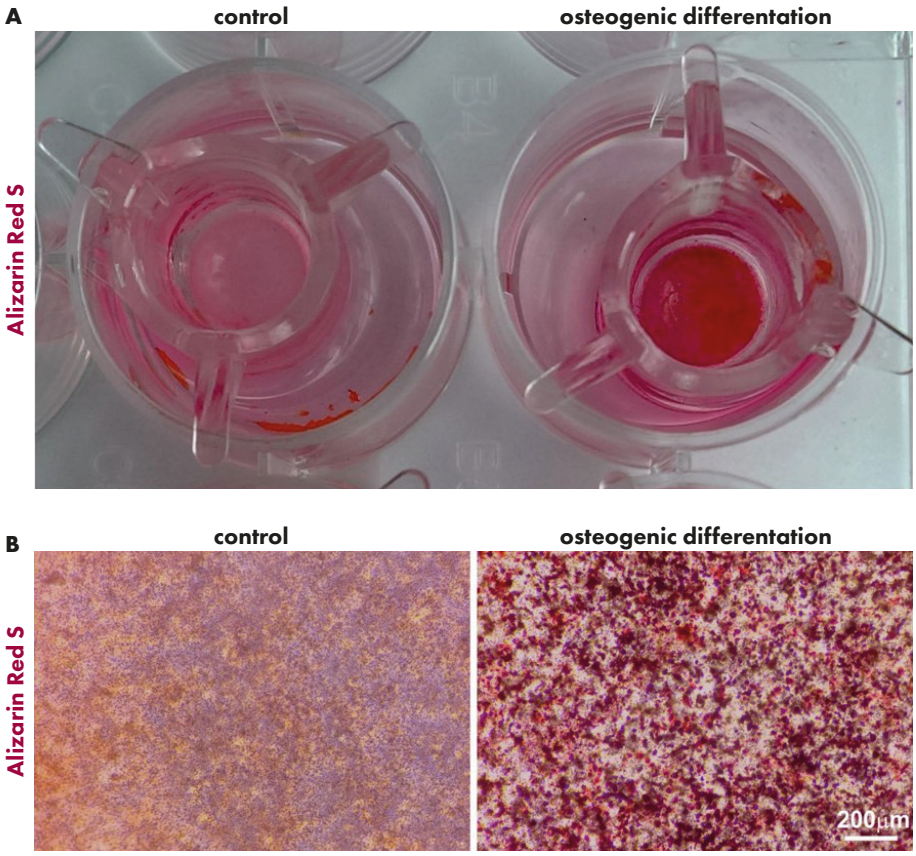

Figure 2. Images showing Alizarin Red S staining of MSCs within TC well inserts (A) following 21 days of incubation in either control (left) or osteogenic differentiation (right) media. Cellular calcium deposits (B) can be seen within differentiated cells (right) compared to the control (left). Scale bar is 200µm.

CONCLUSIONS

Adipogenic or osteogenic differentiation potential is an important identification criteria for MSCs [1,2]. Compared to 2D, differentiation of MSCs in an 3D environment represents a more physiological approach and is known to enhance the levels of adipogenesis and osteogenesis [3,4,5]. Additionally, MSCs embedded within 3D scaffolds show a greater potential for osteogenic regeneration and have previously been shown to contribute towards the treatment of fracture non-union [6]. Moreover, transplantation of a combination of pre-differentiated MSCs with the scaffold could improve the level of engraftment at the lesion side.

Here, we demonstrated that MSCs cultured within 0.2% GrowDex in 24 well cell culture inserts can be driven along both adipogenic and osteogenic differentiation lineages. The xeno-free, tunable viscosity and biocompatible nature of GrowDex in combination with 24 well TC inserts provides a promising tool in studying adipogenic and osteogenic differentiation of MSCs in 3D and could provide valuable insights in developing new strategies towards regeneration of bone injuries, non-union or potentially critical size defects.

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