Differentiation of Mesenchymal Stem Cells within GrowDex® using SARSTEDT TC inserts

SARSTEDT AG & Co. KG

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Introduction

Mesenchymal stem cells (MSCs) are clinically relevant tripotent adult stem cells which give rise to bone (osteogenic), cartilage (chondrogenic) and fat (adipogenic) cells [1]. Since their discovery, differentiation of MSCs has been traditionally conducted on flat two-dimensional (2D) glass or polystyrene surfaces. 2D cell culture is known to result in unnatural cell polarity and morphology in addition to lacking the three-dimensional extracellular matrix. Moreover, MSCs are known to sensitively respond to topological and mechanical cues. 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]. In addition, 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 22].

Here, we describe osteogenic and adipogenic differentiation of human MSCs embedded within GrowDex® in SARSTEDT 24-well TC inserts.

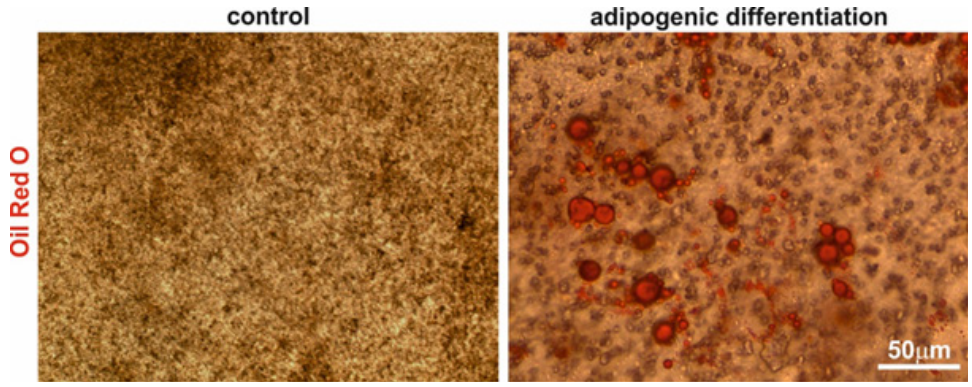

Fig. 1
Images showing MSCs stained with Oil Red O following 21 days of incubation in either control (left) or adipogenic differentiation (right) media. Deposition of intracellular lipid droplets can be seen within differentiated cells (right). Scale bar is 50µm.

Materials

- Palatal adipose tissue-derived Mesenchymal Stem Cells 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) supplemented with 1 % Pen/Strep
- 24 well tissue culture inserts: 3.0 µm 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 % NH_4OH, filtered (A5533-25G, Sigma Aldrich)
- EVOS Live imaging system (ThermoFisher)

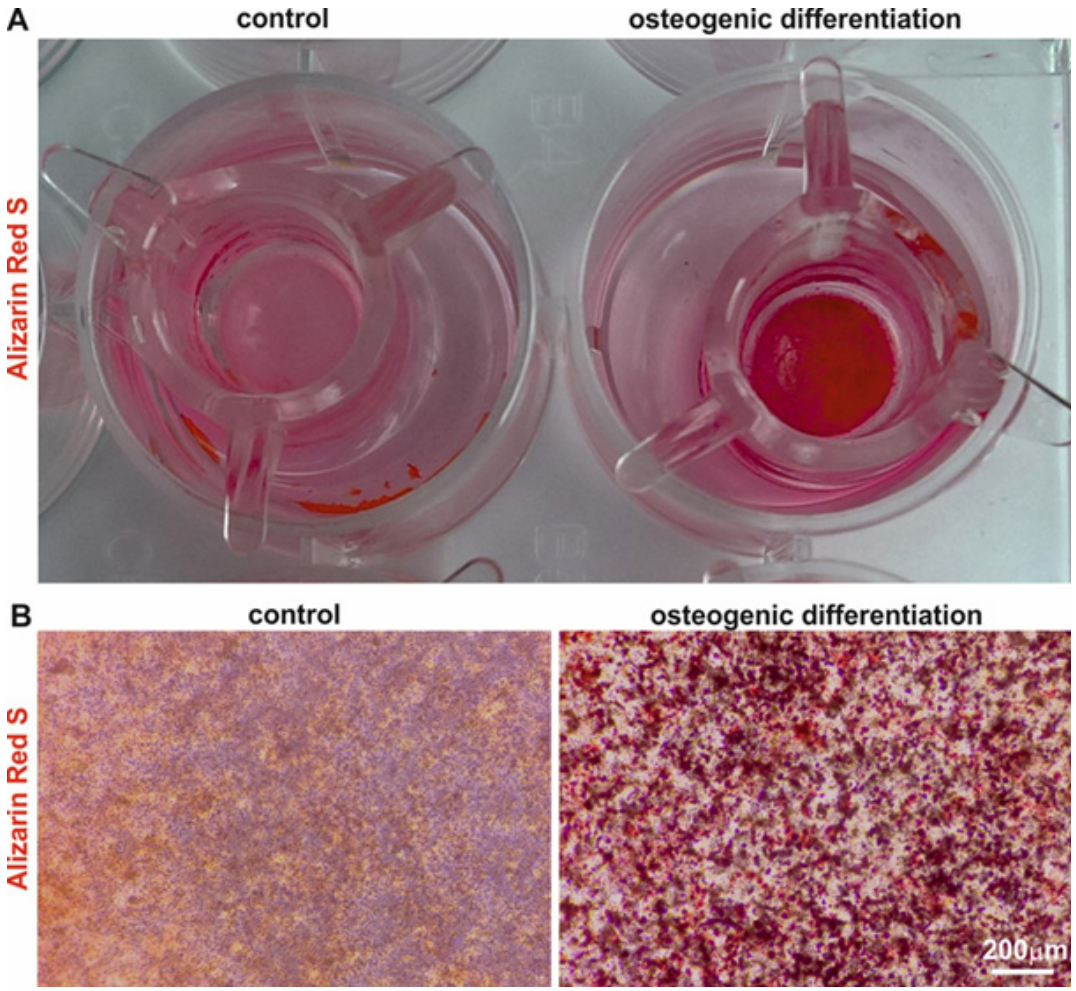

Fig. 2
Images showing MSCs stained with Alizarin Red S following 21 days of incubation in either control (left) or osteogenic differentiation (right) media. Cellular calcium deposits can be seen within differentiated cells (right). Scale bar is 200µm.

Methods

1. Adipose derived and palatal derived MSCs were cultured in complete media and incubated in a humidified incubator at 37°C and 10 % CO_2.
2. Following trypsinization, cells were resuspended in complete media at a concentration of $1x10^6$ cells/ml.
3. Cells were mixed with the appropriate volume of complete media and GrowDex® to provide a final concentration of 0.2 % GrowDex® seeded with $2x10^5$ cells (2,000 cells/µl).
4. Embedded cells were transferred into 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 StemPro™ Adipogenic/Osteogenic media and incubated at 37°C with 10 % CO_2.

6. Control and differentiation media were replaced ever 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 were mixed with 2 parts DI water and then filtered. Following fixation, cells were washed with sterile H_2O. 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 ddH_2O.
10. Images were taken using the EVOS imaging system.

Results

Following 21 days of differentiation treatments, MSCs showed lipid accumulation (Fig.1) and calcium deposition (Fig.2). Oil Red O staining of cells treated with adipogenic differentiation media revealed strong staining of intracellular lipids (Fig.1, right) in contrast to the control (Fig.1, left). Alizarin red staining of cells cultivated in osteogenic differentiation media showed high levels of calcium deposition (Fig.2, right) compared to the control (Fig.2, left).

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 nonunion [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 SARSTEDT 24-well TC inserts can be driven along both adipogenic and osteogenic differentiation lineages. The xeno-free, tuneable 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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