DIFFERENTIATION OF 3T3-L1 CELLS INTO ADIPOCYTE-LIKE CELLS IN 3DPROSEED™ HYDROGEL WELL PLATE

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Summary

This note describes the seeding of 3T3-L1 cells in the 3DProSeed™ hydrogel well plate and the chemically-induced differentiation into adipocyte-like cells. Differentiation was induced 3 days after 3T3-L1 cell seeding and was monitored until day 21 with bright-field microscopy. Finally, the cultures were fixed and stained with Bodipy (green, lipids droplets), Hoechst (blue, nuclei) and Phalloidin (red, actin), and analysed with laser scanning confocal microscopy. These 3D cultures can be established by the simple and automation-compatible seeding of 3T3-L1 cell line on the pre-assembled 3DProSeed™ hydrogels and can be used for in-vitro assays in biological research on adipose tissue.

Introduction

The 3T3-L1 cell line is an established pre-adipose cell line derived from disaggregated 17- to 19-day-old Swiss 3T3 mouse embryo. These cells display a fibroblast-like morphology. Under appropriate conditions 3T3-L1 cells acquire an adipocyte-like phenotype and are therefore cwidely used for studies of adipogenesis and obesity-related mechanisms [1].

Materials and methods

3DProSeed™ hydrogel well plate. The 3DProSeed™ hydrogel plate consists of a 96-well black microtiter plate with 180 micron glass bottom, containing a fully synthetic pre-assembled and hydrated hydrogel for ready-to-use and automation-compatible cell-based assays. The key innovation is the hydrogel surface featuring a so-called "in depth-density gradient" which enables the penetration into the hydrogel bulk of cells deposited on it. The hydrogel is a poly (ethylene glycol)-based formulation containing cell adhesion (RGD sequences) and degradation (MMP-cleavable sequences) motives [2].

Cell seeding procedure and differentiation. The storage buffer was removed from 3DProSeed wells and 200µL of cell suspension containing 1.0×10^4 3T3-L1 cells (ATCC® CL-173™) in pre-adipocyte expansion medium (EM, 90% Dulbecco's Modified Eagle's Medium (DMEM), 10% Bovine Calf Serum) were added to each well of a 3DProSeed plate. The adipogenic differentiation was carried out following the ATCC standard procedure [3]. Cells were fed with expansion medium until they reached confluent culture. The growth medium was then removed and cells were incubated in differentiation medium for 48 hours (90% DMEM, 10% Fetal Bovine Serum (FBS), 1.0 µM Dexamethasone and 0.5 mM Methylisobutylxanthine). After the induction, cells were switched to an adipocytes maintenance medium (90% DDMEM, 10% FBS, 1.0 µg/mL Insulin) till the end of the culture.

Immunostaining. Samples were fixed with paraformaldehyde 4% (30 min at room temperature (RT)) and stained for fatty acids (BODIPY® lipid probe 493/503, 1 : 500 in PBS and 0.20 µm filtered, Thermo Fischer , cat. no. D3922), actin (Phalloidin, 1:500 in PBS Thermo Fischer , cat. no. R415) and dsDNA (Heochst 1:100 in PBS Sigma-Aldrich , cat. no. 94403-1) (1h, RT). Samples were rinsed three times in PBS within one hour. Inspection of the cell cultures was performed with a Leica SP5 confocal microscope equipped with long distance 10x objective. Image treatment and 3D reconstructions was performed with Imaris.

Results

3T3-L1 cells were seeded on the hydrogel and started to infiltrate the hydrogel displaying a spindle-like morphology typical of fibroblast-like cells. Cells were chemically differentiated into adipocyte-like cells according to ATCC standard procedure [3]. We could observe the formation of grape-like 3D structures which contained lipid vacuoles and spanned in depth throughout the gel (Figure 1). We observed the growth of these structures over the 21 days of culture.

Conclusion and outlook

We described the protocol for the seeding and differentiation of 3T3-L1 cells into adipocyte-like cells in the 3DProSeed™ hydrogel plate. In future application notes, we aim to further describe the 3D culture phenotype especially in comparison to a regular 2D set up. It has been shown that adipocytes culture could benefit in terms of differentiation potential from the three-dimensionality of their culture environment [4-5]. Co-culture of adipocyte-like cells with other cells can also be established (e.g. endothelial cells or macrophages) to create *in vitro* models for the study of angiogenesis and inflammation mechanisms in the adipose tissue.

Literature

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Figure 1: 3T3-L1 cells after 21 days from adipogenic differentiation. In left panel a control culture maintained in expansion medium (no differentiation). In the central panel a culture treated with the differentiation medium. and on the right panel a zoom-in on the same culture showing the 3D grape-like structure containing lipid vacuoles , (red: actin; green: lipids and blue; nuclei).