

## MATURATION OF FUNCTIONAL HUMAN ADIPOCYTES

### INTRODUCTION

Pre-adipocytes differentiate into adipocytes through several phases: differentiation and maturation, where they are then completely functional (lipogenesis / lipolysis).

### Materials required

- HWP and 3T3-L1 grown in BIOMIMESYS® *Adipose tissue*
- Perilipin antibody and Phalloidin-FITC (Abcam)
- RNA extraction by TRIzol

### Hydro scaffold properties

Porous and Translucent

### Method

- The lipid quantification is accomplished by AdipoRed™ normalized by the DNA quantitation kit following manufacturer's protocol and using a Hoescht solution at 0.02 µg/ml.
- AdipoRed™ Assay Reagent, follow manufacturer's protocol
- Method for marking the perilipin / phalloidin: follow manufacturer's protocol and visualize by immunofluorescence
- RNA extraction method: follow manufacturer's protocol

### RESULTS

The storage structure of matured triglycerides can be highlighted by staining of matured adipocytes with Perilipin, also called lipid droplet-associated protein. Perilipin protein stabilizes the lipid droplet surface (1).

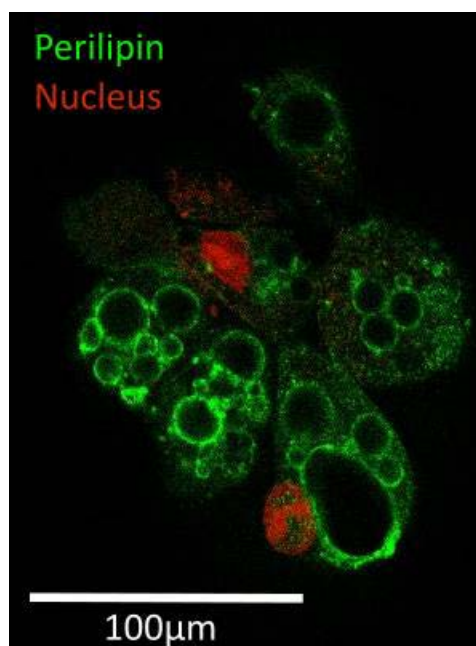


Figure 1: Confocal microscopy of a section of HWP cell aggregates in BIOMIMESYS® *Adipose tissue* at Day 21 (perilipin in green and nucleus in red) by LSCM observation of Z-stack

The adipocyte maturation can also be confirmed by the expression of a late maturation gene: GLUT-4, which is a transporter that allows glucose trafficking and its secretion from the cell.

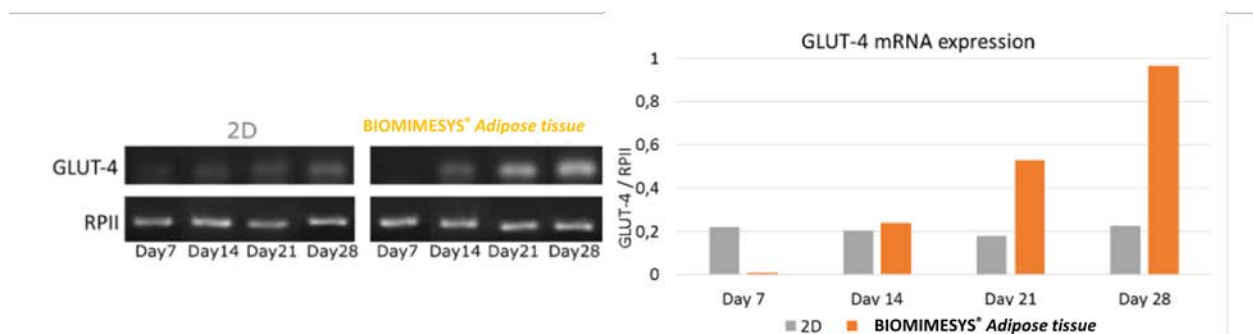


Figure 2: Semi-quantitative RT-PCR of adipogenesis late marker gene in 2D and 3D cultures in BIOMIMESYS® Adipose tissue

Induction of the late gene GLUT-4 is observed during the maturation of adipocytes, as described in the literature (2). This maturation gene is highly expressed in adipocytes grown in BIOMIMESYS® Adipose tissue, attesting the advanced stage of maturation of adipocytes in 3D culture.

## CONCLUSION

The Perilipin staining and GLUT-4 gene expression indicates a higher maturation of human adipocytes in BIOMIMESYS® Adipose tissue.

## References:

1. The Central Role of Perilipin A in Lipid Metabolism and Adipocyte Lipolysis, Tansey et al., IUBMB Life, 56(7): 379–385, July 2004.
2. Characterization of adipocyte differentiation from human mesenchymal stem cells in bone marrow, Qian SW et al., BMC Developmental Biology 10.47, 2010 ; The GLUT4 Glucose Transporter, Huang S, Cell Metabolism 5.4, 237-252, 2007.

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