INCREASED EXPRESSION OF DIFFERENTIATION GENES

INTRODUCTION

The morphological changes in cells during differentiation are accompanied by a profound change at the molecular level. In vivo, the adipocyte differentiation process includes early and late events due to differential gene regulation. The appearance of these markers is a sign of differentiation of pre-adipocytes and hence the development of adipose tissue.
Genetic markers such as C/EBPα (1) and PPARγ2 (2) are used to assess the complete differentiation of pre-adipocytes.

Materials required

- Human White Pre-adipocytes (HWP, PromoCell) grown in BIOMIMESYS®*Adipose tissue*
- RNA extraction by Trizol (Thermo Fisher)
- RT-PCR devices and reagents

Hydroscaffold properties

Porous and Translucent

Method

- RNA Extraction: follow the usual protocol
- RT-PCR: follow the usual protocol

RESULTS

The expression of early differentiation genes in HWPs (until 28 days of nutrition) was analysed by RT-PCR on the PPARγ2, C/EBPα and FABP-4 genes in 2D vs 3D.

- C/EBPα, a second transcription factor induced during adipocyte differentiation, can cooperate with PPAR γ2 to stimulate the adipocyte differentiation (3)
- PPARγ2 is an adipocyte specific nuclear hormone receptor stimulating adipose differentiation
- FABP-4 is a transporter allowing the absorption and transport of fatty acids,
- RNA polymerase II (RPII) housekeeping gene was used (4)

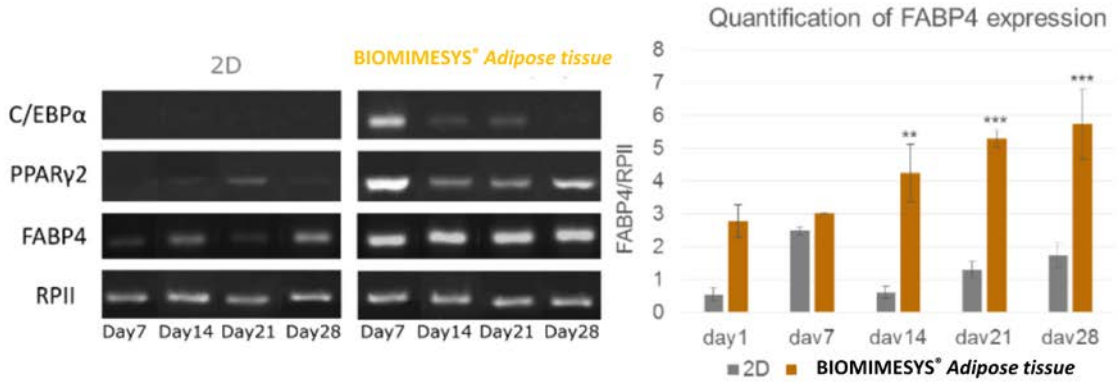

Figure 1: Semi-quantitative RT-PCR of adipogenesis early genes in 2D and 3D cultures in BIOMIMESYS® *Adipose tissue and an example of a quantitative analysis of gene expression from 3 independent experiments: FABP-4. C/EBPα, a second transcription factor induced during adipocyte differentiation, can cooperate with PPAR γ2 to stimulate the adipocyte differentiation (1). PPARγ2 is an adipocyte specific nuclear hormone receptor that stimulates adipose differentiation. FABP-4 is a transporter that allows the absorption and transport of fatty acids. RNA polymerase II (RPII) housekeeping gene was used as a control (2).*

As described in the literature (3-6), during adipogenesis, the expression of C/EBPα, PPAR γ2 and FABP4 genes is induced and is more pronounced in 3D than in 2D.

CONCLUSION

Adipocytes grown in BIOMIMESYS®*Adipose tissue* display earlier and increased expression of differentiation gene compared to 2D condition.

References

1. The role of C/EBP genes in adipocyte differentiation, Darlington J., Ross E. et al. The Journal of Biology Chemistry 273,46:30057-30060, 1998

2. PPARγ in adipocyte differentiation and metabolism – Novel insights from genome-wide studies, Siersbæk R. et al., FEBS Letters , 584,15:3242 – 3249, 2010

3. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. Tontonoz P. et al. Cell, 79,7 :1147-1156, 1994

4. Validation of reference genes for the relative quantification of gene expression in human epicardial adipose tissue. Chechi K. et al. Plos One, 7, 4, 2012.

5. The roles of PPARs in adipocyte differentiation. Grimaldi P. Progress in lipid research,40(4) : 269- 281, 2001

6. Modulation of Adipogenic Conditions for Prospective Use of hADSCs in Adipose Tissue Engineering, Galateanu et al., Int. J. Mol. Sci. 13, 15881-15900, 2012

Contact Information

HCS Pharma

hello@biomimesys.com

www.biomimesys.com