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New insights on the role of adipose tissue by using 3D scaffold free organoids

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Introduction

The white adipose tissue (WAT) is a complex organ composed mainly of differentiated adipocytes responsible for the body energy homeostasis (storage and mobilization of energy in the form of triglycerides), the metabolism of sex steroids and glucocorticoids and the modulation of important biological processes through adipokines acting at both local and systemic level. Many evidences have supported the primary interaction of the adipose tissue with the skin and its appendages as potential target for different types of aesthetic treatments (topical, chirurgical), in particular for slimming and re-pulping efficacy.

Aim of the work

To develop and characterize 3D scaffold free adipose microtissues (adMTs) and investigate the modulatory effects derived from treatment with Forskolin, a factor involved in lipolysis regulation [1-2].

Methodology

Human 3D scaffold free adMTs have been developed by using the Hanging Drop Technology starting from a suspension of human primary preadipocytes of single/pool donors with different BMI (Tebu-Bio) or from PCi-Mesenchymal stem cells (PCi-MSC) developed from human iPSC (Phenocell). In figure 1 is reported an experimental design used for adMTs preparation.

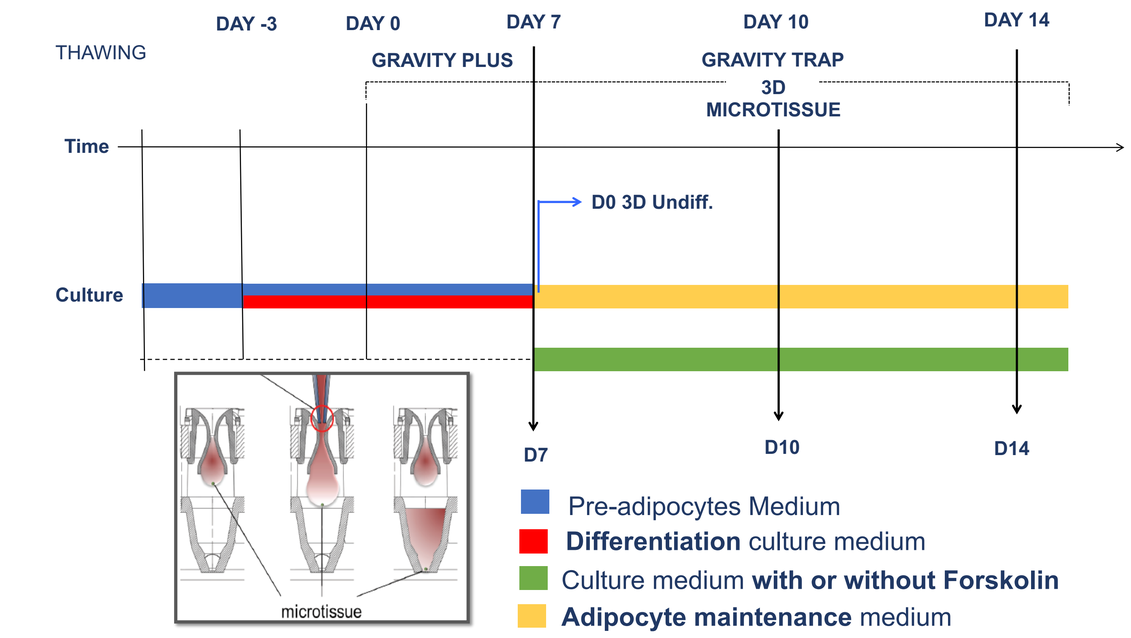

Figure 1. Example of experimental design used for adMTs preparation. After cell expansion, single cells are suspended in a hanging drop of culture medium (5000 cells/drop) to form a miniature tissue under the pull of gravity. During aggregation in the GravityPLUS™, cells are exposed to differentiation medium for 7 days, enter in close contact each other and produce their own extra cellular matrix (ECM). After cellular aggregation, the spheroids are transferred to the GravityTRAP™ plate (Day 7) in adipocyte maintenance medium with or without Forskolin. InSphero plate technology has been used for microtissue formation.

Intracellular lipid droplets content and glycerol release

Intracellular lipid droplets content and glycerol release in adipose microtissues have been quantified as reported in Figure 2 A-E. The results suggest that cell differentiation leads to the accumulation of lipids in adMTs and increase glycerol release compared to non-differentiated microtissues.

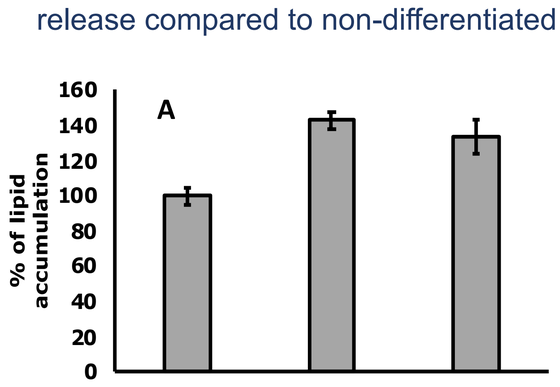

Figure 2A. Intracellular lipid accumulation in adMT quantified by Adipored™ assay at day 7 (D7) and day 14 (D14) compared to non-differentiated adMTs (ASC for adipose stromal/stem cells).

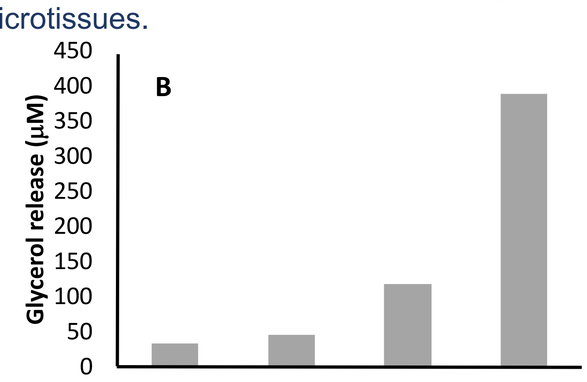

Figure 2 B. Glycerol release in non-differentiated and differentiated microtissues (D7, D10, D14) as quantified using the Glycerol Assay Kit (Sigma).

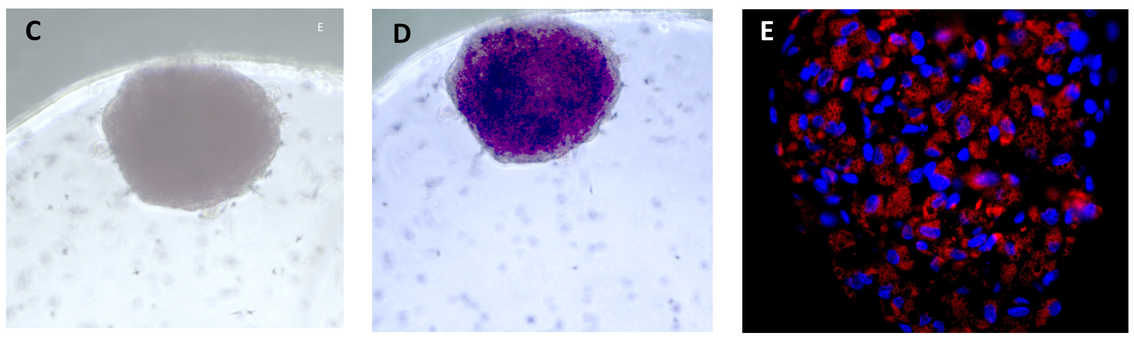

Figure 2 C-E. Microscope images representing lipid content in non-induced adMTs (C) and differentiated micro-tissues (D) stained by Adipored™. The surface of lipid droplets inside the microtissues was identified at D7 by perilipin immunostaining (red) using the rabbit polyclonal Perilipin-1 Ab (Abcam) (E).

List of biomarkers relevant for mature adipocytes characterization

GENE	FUNCTION
PPARγ2	Peroxisome proliferator-activated receptor, it is a master regulator of adipocyte differentiation
FABP4	Fatty acid binding protein 4, the gene of which is a PPARγ2 target
CEBPA	CCAAT/enhancer binding protein α expressed at high levels in adipose tissue
SLC2A4	Insulin-regulated glucose transporter (GLUT4) found primarily in adipose tissue
ADIPOQ	Adiponectin is an adipokine expressed by mature adipocyte, anti-inflammatory and improving sensitivity to insulin
LEP	Hormone (LEPTIN) produced by adipose cells that helps to regulate energy balance by inhibiting hunger
PNPL2A	The gene encodes an enzyme (ATGL) which catalyzes the first step in the hydrolysis of triglycerides in adipose tissue

Adipogenic signature in adipose microtissues

At transcriptional level it has been possible to conclude that adipogenic signature is globally stable within 14 days. The overexpression of genes involved in adipocyte metabolism (e.g. ADIPOQ, CEBPA, FABP4, SLC2A4) has been observed after differentiation as quantified by qRT-PCR at day 7 and 14 day (Figure 3). While the gene signature remains stable for almost all the genes, the decrease of ADIPOQ expression from day 7 to day 14 could be related to a higher degree of adiposity as observed in obese people [3].

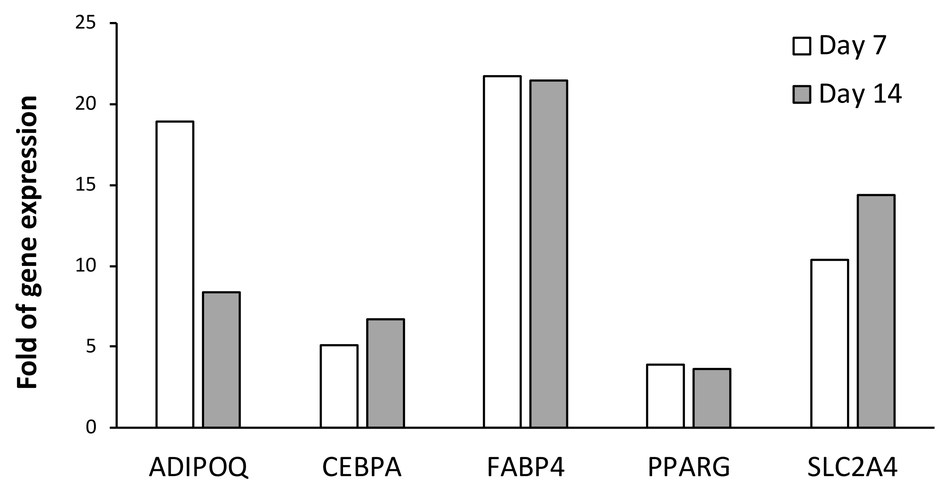

Figure 3. Adipogenic differentiation leads to the induction of genes involved in lipid production and cellular crosstalk specific of adipose tissue. Gene expression is quantified by RT-PCR, in fold of non-induced tissues (RQ= 1). Experiments were conducted on a pull of 10 microtissues.

Effects of FORSKOLIN treatment

Treatment with Forskolin induces a reduction of lipid accumulation and down regulation of adipocyte lineage genes (Figure 4).

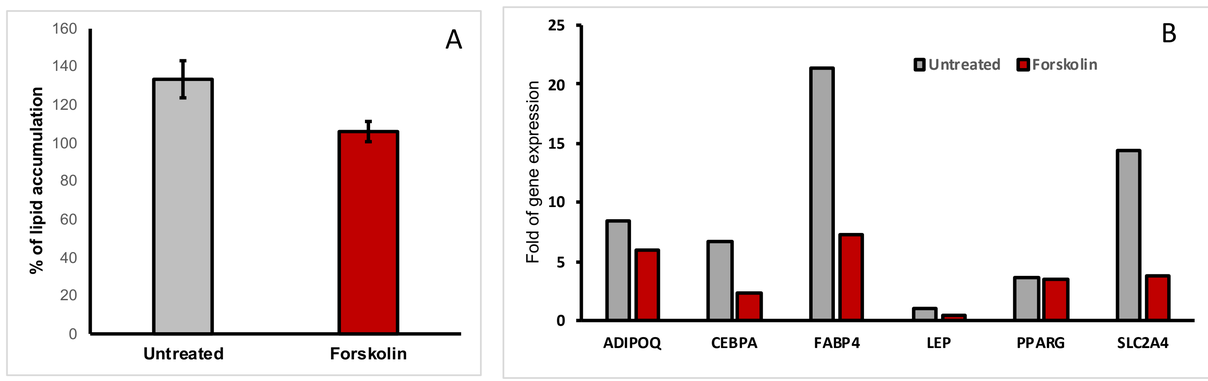

Figure 4. A significant decrease of lipid content is observed after Forskolin treatment (A) which is associated with a reduction of lipid production related genes at day 14 (B).

Conclusions

The established adMTs seem to better recapitulate the adipose tissue dynamic evolution compared to 2D monolayers offering the following advantages:

- Higher biological relevance
- Efficient ECM production and retention of cell-cell and cell-ECM contacts
- Early and efficient differentiation and polarization
- Organ-like functionality
- Low cell number compared to 2D
- Viability for 10-14 days
- Efficient (96 plate) and robust screening by multiple endpoints approach
- Histology and immunofluorescent staining of the whole tissue
- Higher doses of actives compared to 2D models
- Repeated exposure possible

AdMT can be used to screen active compounds in a 3D environment and identify their mechanism of action on adipolysis, adipogenesis, viability, inflammation and oxidative stress.

References:
1) Duncan RE et al. Regulation of Lipolysis in Adipocytes, Annu Rev Nutr. 27 (2007) 79–101
2) Litosch I et al. Forskolin as an activator of cyclic AMP accumulation and lipolysis in rat adipocytes. Mol. Pharmacol. 22(1)(1982) Jul, 109-15
3) Bullò M, et al. Adiponectin expression and adipose tissue lipolytic activity in lean and obese women Obes Surg.15(3)(2005) Mar, 382-6