VitroScreen ORA™
A Visionary Future of Human Health

Scaffold-Free Human Adipe Spheroids Model: Phenotype Dependent Inflammatory Response

Francesca Rescigno, Elisa Caviola and Marisa Meloni
VitroScreen srl, *In Vitro* Innovation Center, Milan (Italy)

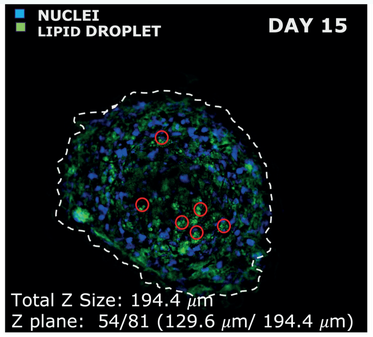
Introduction

The predisposition to obesity, the onset of diabetes and cardiovascular diseases are related to metabolic disorders, where environmental factors and phenotypical/genotypical aspects directly affect fatty acid accumulation and adipose tissue homeostasis leading to a pro-inflammatory status. VitroScreen ORA™ ADIPE represents an innovative miniaturized white adipose tissue (WAT) model, designed to mirror with high fidelity features the complexity of human adipose tissue, preserving the physiological phenotype of cellular donors. Thanks to the stable 3D configuration, primary cells are able to organize in their own micro-physiological niche and aggregate in a 3D mini-adipose unit, mimicking all the natural cell-cell and cell-stroma interactions, providing the main cellular signalling for tissue differentiation.
A miniaturized ADIPE model was obtained from overweight donors, with the aim to mirror a micro-physiological model of metabolic disorders and to study a different response to a pro-inflammatory stimulus thus modelling specific profiles of metabolic disorders and alterations in lipids accumulation.

Experimental design

Primary pre-adipocytes were obtained from two overweight donors (male and female, BMI> 29). To simulate the adipose metabolism alterations associated to a pro-inflammatory status, spheroids were stressed with increasing concentrations of LPS (10, 20 and 100 ng/mL) during 24h. Immediately after the pro-inflammatory induction, the biological responses were evaluated in terms of released IL-6 in the culture media and at gene expression level.
To investigate lipids droplets accumulation and morphology of the two ORA™ ADIPE models, the Nile Red staining was performed on whole mount cleared samples analyzed by Leica Thunder 3D imager DMi8.
Z-stacks of whole samples were acquired for all focal planes and the more representative sections were extrapolated.

MALE DONOR



FEMALE DONOR

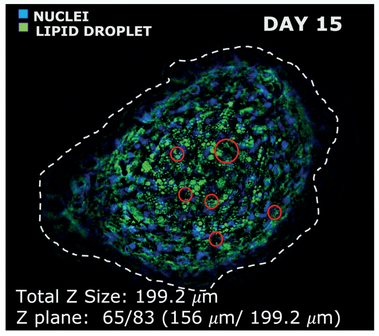
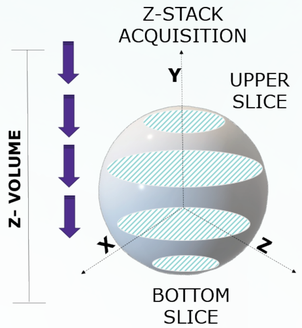


Figure 1. Differential lipids accumulation during the complete differentiation between two spheroids donors'. Mag.20X

VitroScreen ORA™ IMAGING: 3D WHOLE MOUNT ACQUISITION by LEICA THUNDER i8 IMAGER 3D



IL-6 RELEASE by ELISA

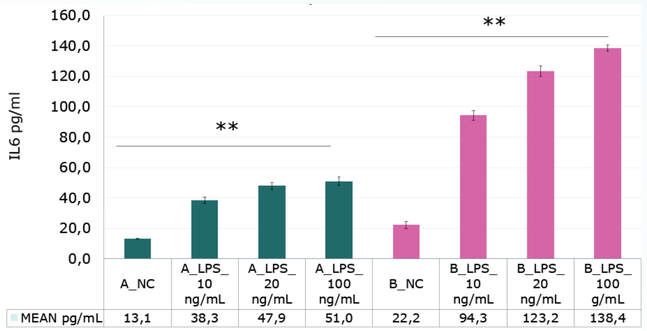


Figure 2. IL-6 release after acute exposure to LPS at different doses in ORA™ ADIPE spheroids form both donors.

LEGENDA
Male Donor: aged male donor
Female Donor: young female d onor
Both with BMI>29
LPS_A: 10 ng/mL
LPS_B: 20 ng/mL
LPS_C: 100 ng/mL

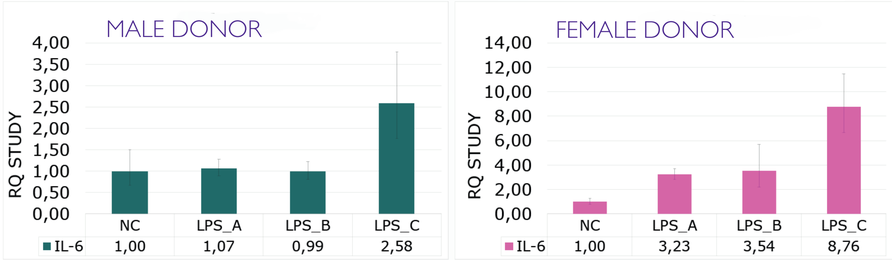


Figure 3. Differential IL-6 gene expression after acute exposure to LPS at different doses.

Results

A different 3D architecture and tissue assembly was shown by the two different donors ORA™ ADIPE spheroids (Fig.1). The exposure to higher LPS dose induced the formation of organized and diffuse mature lipids' droplets in spheroids of the female donor: furthermore, jagged boundaries related to the progressive lipid accumulation suggest a complete adipogenic differentiation (Fig.1). On the contrary, spheroids from the male donor's pre-adipocytes showed low amount of lipid droplets not fully defined. The exposure to increasing doses of LPS, triggered an increased release of IL-6 pro-inflammatory cytokine, modulated in a dose-dependent manner. According to the donor's sex the magnitude of the biological response to the LPS stimulation is higher for the female donor's spheroids, reaching values of released IL-6 higher (138 pg/mL) after 24h exposure compared to the male donor's release (51 pg/mL) in the same experimental conditions (Fig.2). Differential profiles of biological response were confirmed by gene expression: the female donor's spheroids showed a greater upregulation of IL-6 expression (RQ= 8.76) compared to the male donor's spheroids (RQ= 2.58) suggesting that the specific physiology of donors was preserved in the ORA™ ADIPE model.

Conclusion

The differential response to the acute pro-inflammatory stimulation mirrors the physiological behaviour of ORA™ ADIPE model that captures donors' phenotype preserving the natural cellular behaviour. ADIPE ORA™ is designed to mimic the complexity of human white adipose tissue and natural environment in microscale 3D culture conditions for applications in pre-clinical screening, pharmacological efficacy, personalized therapies.

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OMEICOS THERAPEUTICS