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ABSTRACT # 213

Adipocytes 3D scaffold free spheroids for toxicology and non clinical applications

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White adipose tissue (WAT) is a complex organ composed mainly of differentiated adipocytes responsible for the body energy homeostasis, the metabolism of sex steroids and glucocorticoids, and the modulation of important biological processes through adipokines acting at both local and systemic level. VitroScreen has developped human 3D scaffold free adipocyte spheroids by using the hanging drop technology. A multiple endpoint approach has been adopted to characterize the established "microadipe model" at biochemical, morphological, gene and protein expression levels compared to a 2D monolayer in term of intracellular lipid droplets (adipogenesis), amount of glycerol release in medium (lipolysis), transcriptional activity of specific markers of mature adipocytes. We have first defined protocols to model different preadipocyte differentiation stages up to 14-20 days after seeding *(Barbara de Servi et al., Adipocytes 3D scaffold free microtissues for preclinical applications, Proceedings of 76th SID Meeting 2017 – Portland, Oregon).* The biological response to reference molecule has been demonstrated (e.g. forskolin, caffein) *(Francesca Rescigno et al., New insights on the role of adipose tissue by using 3D scaffold free organoids, Proceedings of 25th IFSCC Meeting 2019 – Milan, Italy, IFSCC best poster Award).*
Given that WAT inflammation as a critical step in the pathogenesis of obesity and metabolic syndrome, an inflammatory status has been induced on the "microadipe model" dosing in the culture medium pro-inflammatory cytokines *(Francesca Rescigno et al., Scaffold-Free Human Adipe Spheroids Model: Phenotype Dependent Inflammatory Response, Proceedings of MPS World Summit Meeting 2022 – New Orleans).*

PHENOTYPE DEPENDENT INFLAMMATORY RESPONSE

CELL TYPE: Pre-adipocytes from **Male** and **Female** donors, BMI>29.

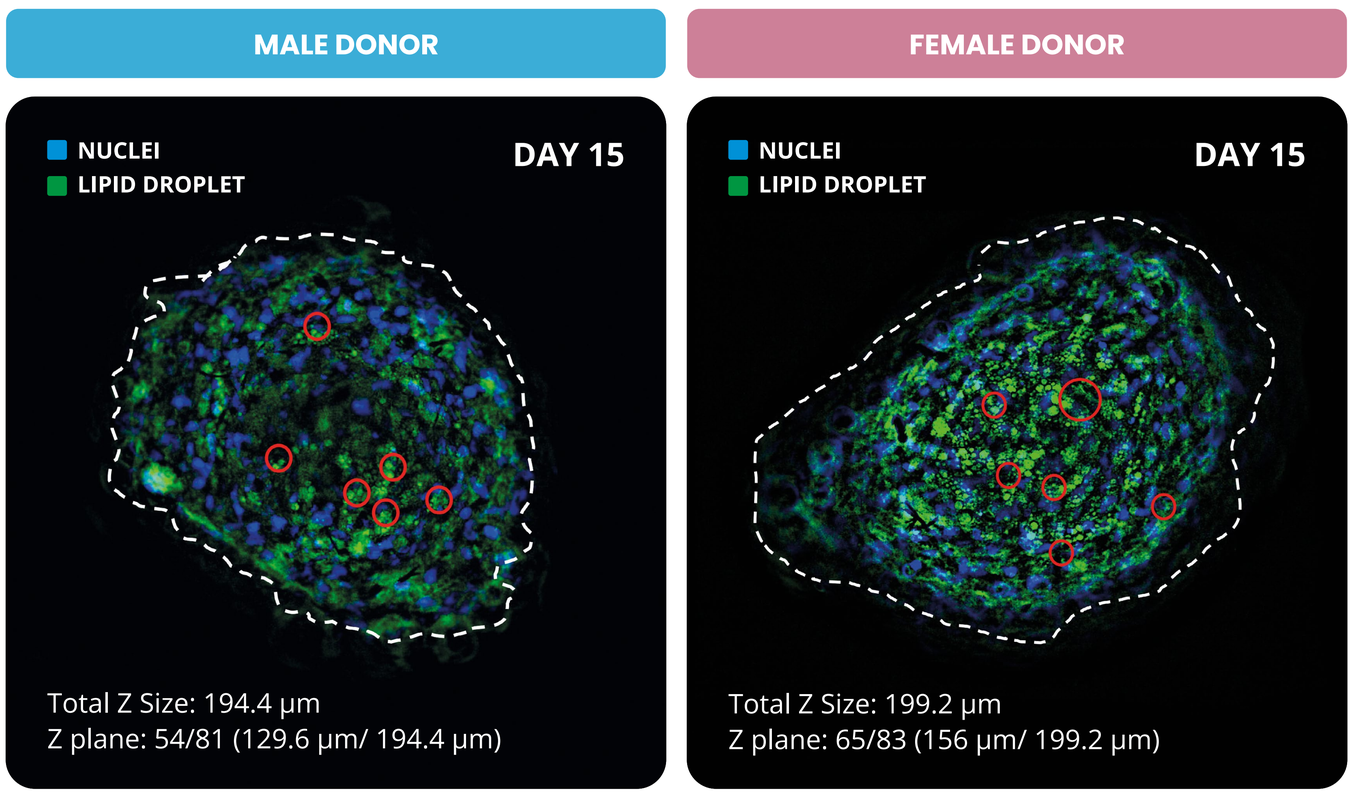


Figure 1. Differential lipids accumulation during the complete differentiation between two spheroids donors. Mag 20X.

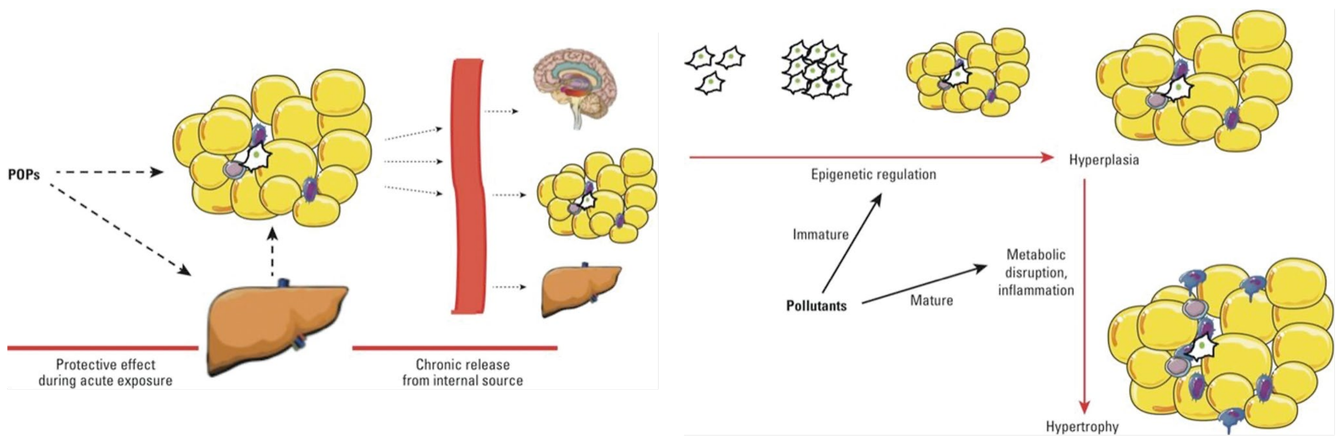
- Lipids' droplets (Fig.1): organized, diffuse and mature in spheroids of the **female donor**. Low amount of droplets not fully defined in spheroids on the **male donor's**.

TOXICOLOGICAL FUNCTION OF ADIPOSE TISSUE

Adipose tissue: modulates persistent organic pollutant (POP) toxicity through several mechanisms.

By sequestering POPs, AT can protect other organs and tissues from POPs overload. However in the long run, stored POPs are slowly released into the bloodstream and constitutes a continual source of internal exposure to POPs. AT is also a target of POPs leading to quantitative and qualitative alterations of AT. Some POPs also induce a proinflammatory state in AT and modulate the differentiation of AT precursor cells.

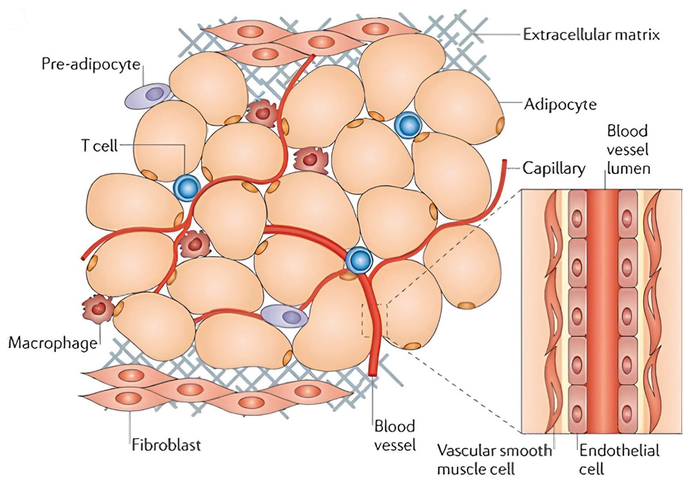
POPs are environmentally and biologically persistent, which leads to their bioaccumulation and biomagnification up the food chain. Fatty foods of animal origin (e.g., meat, fish, dairy) are important vectors of several classes of POPs, including dioxins and polychlorinated biphenyls (PCBs). Because of their hydrophobicity, POPs tend to distribute into lipophilic compartments, particularly the AT.



La Merrill et al., Environmental Health Perspectives, vol. 121(2) 2013, 162-169

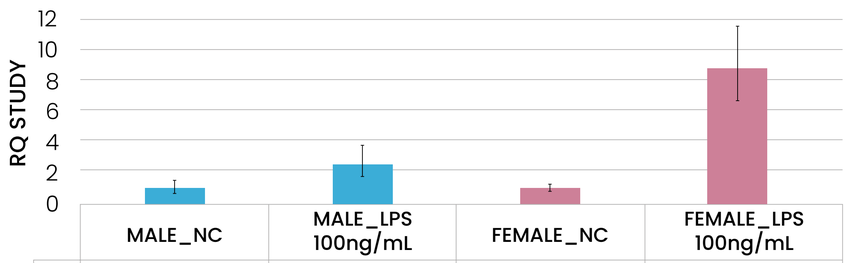
ADIPOSE TISSUE STRUCTURE

- Adipocytes are the main cellular component of adipose tissue.
- Other cell types within adipose tissue are precursor cells (including pre-adipocytes), fibroblasts, vascular cells (endothelial cells and vascular smooth muscle cells) and immune cells (macrophages and T cells).
- These cells constitute the stromal vascular fraction of adipose tissue.
- Factors that are secreted by these different cellular components are critical for maintaining homeostasis in adipose tissue and throughout the body.



Ouchi N. et al., Nature Reviews Immunology 11, 85-97

IL-6 GENE EXPRESSION



	MALE_NC	MALE_LPS 100ng/mL	FEMALE_NC	FEMALE_LPS 100ng/mL
IL-6	1,00	2,58	1,00	8,76

Figure 2. Differential IL-6 gene expression after acute exposure to LPS at different doses.

VitroScreenORA® scaffold free spheroids have shown unique features, including donor cell-dependent endogenous ECM production, metabolic competence evolution and lipids accumulation during 3 week. Their spatial geometry ensures physiological diffusion and transport of molecules through the ECM, resulting in a physiological response to detoxifying stimuli.

Scaffold free spheroids system precisely mirror the cycle of the adipose tissues as self renewining mini-organ in terms of accumulation and detoxification of xenobiotics. As suggested by the interesting data published by scientific community on the multiple role of adipose tissue, this **M**icro **P**hysiological **S**ystem has a promising future and applications in pharmaco-toxicological research.

Persistent organic pollutants (POPs)	Rolle-Kampczyk et al. *"Accumulation of distinct persistent organic pollutants is associated with adipose tissue inflammation"* Science of the Total Environment 748 (2020): 142458.
Endocrine disruptors	Cander et al. *"Effects of Endocrine-Disrupting Chemicals on Obesity and Diabetes"* Endocrinology Research & Practice 27.4 (2023). Le Magueresse-Battistoni. *"Adipose tissue and endocrine-disrupting chemicals: does sex matter?"* International journal of environmental research and public health 17.24 (2020): 9403.
Cancer Development	Bokobza et al. *"The adipose tissue at the crosstalk between EDCs and cancer development"* Frontiers in Endocrinology 12 (2021): 691658.
Pre-clinical screening	Bonet et al. *"Carotenoids and carotenoid conversion products in adipose tissue biology and obesity: Pre-clinical and human studies"* Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1865.11 (2020): 158676.
Personalized therapies	Verseijden, Femke, et al. "Comparing scaffold-free and fibrin-based adipose-derived stromal cell constructs for adipose tissue engineering: an in vitro and in vivo study." Cell transplantation 21.10 (2012): 2283-2297.
Pharmacological efficacy	Graham, Alexander D., et al. "The development of a high throughput drug-responsive model of white adipose tissue comprising adipogenic 3T3-L1 cells in a 3D matrix." Biofabrication 12.1 (2019): 015018.