AI Image Analysis and Other Emergent Technologies to Support Adipose Tissue Studies

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The study of adipose tissue as an endocrine organ has increased in recent years due to the development of new tools to support the scientific community to better understand the worldwide obesity epidemic. These tools will also enable scientists to study the impact of adipose tissue on diabetes, insulin resistance, and pathologies in other organs and tissues, including cardiovascular disease, cancer, and sleep disorders. 3D cell cultures and Organ-on-a-Chip technologies are helping to recapitulate human adipose features in vitro, facilitating the rapid study of this organ and its responses taking in account the native-like drug metabolism, multicellular complexity, and paracrine factors affecting other organs and systemic responses.

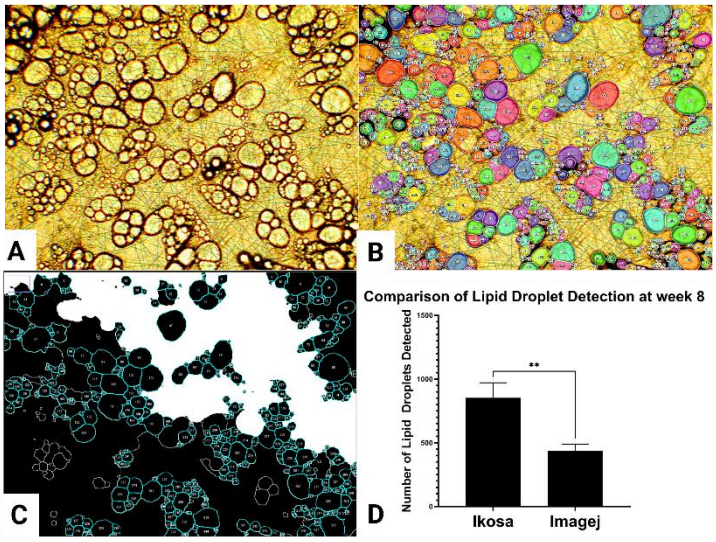

Figure 1. (A) Brightfield images of adipocytes after 8 weeks of culture on PCL fibers. (B) Visualized output of IKOSA AI image analysis algorithm trained to detect lipid droplets. (C) Output of ImageJ morphological segmentation algorithm. (D) Average +/- standard deviation of number of lipid droplets detected in a set of 8 images by IKOSA and ImageJ respectively (p<.01).

Traditionally, scientists have relied on endpoint assays to understand adipocyte biology in the existing models including metabolic responses (including lipolysis and glucose uptake), and adipokine secretion assays (Gibler et al. 2021). Other studies aim to quantify size and quantity of lipid droplets in the adipocytes, to measure hypertrophy (large volume) and hyperplasia (large number). Lipid droplets are commonly analyzed using fluorescent dyes or stains. Fluorescent staining can be time consuming and expensive, requiring specialized equipment and invasive protocols that can be cytotoxic (Helgadottir et al. 2021). Other methods, like Oil Red O, require cells to be fixed prior to staining, meaning they are only suitable for endpoint analyses (Du et al. 2023).

Adipocyte differentiation in pathophysiological conditions can be analyzed by label-free image analysis such as stimulated Raman scattering microscopy and three-dimensional sectioning (Ferrara et al., 2019). Emerging technologies, such as **AI-based image analysis** in live-cell imaging allows quantification of

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adipogenic differentiation kinetics. Obatala Sciences uses AI image processing to monitor the kinetics, size and morphology of lipid droplets using bright field images over the course of an experiment without invasive protocols or cellular manipulation. Briefly, images acquired at different time points of adipocyte differentiation were submitted to the IKOSA AI platform (KML Vision GmbH, Austria). IKOSA is a deep learning software platform that doesn't rely on a specialized instrument and can be used through a simple web browser. We trained the system on 20 images for about 1 hour to annotate the features of adipocytes for the automatic detection of adipose area, total cell area, and individual lipid droplets at different time points during differentiation. The IKOSA AI model was quickly able to accurately identify and measure all lipid droplets in each consecutive brightfield image (Figure 1B). We compared the data to ImageJ analysis using the morphological segmentation plugin. As presented in Figure 1, the comparative study confirms that ImageJ method is moderately successful. It often misses droplets in large clusters and is very sensitive to image resolution (Figure 1C). The IKOSA AI platform provides clear benefits for convenient data management, annotation and AI model training, hence was further implemented in quality control and kinetic studies during the development of the adipocyte hypertrophy model as presented in Figure 2A-B. Here, hypertrophy was defined as a minimum area of 706.85 um^2.

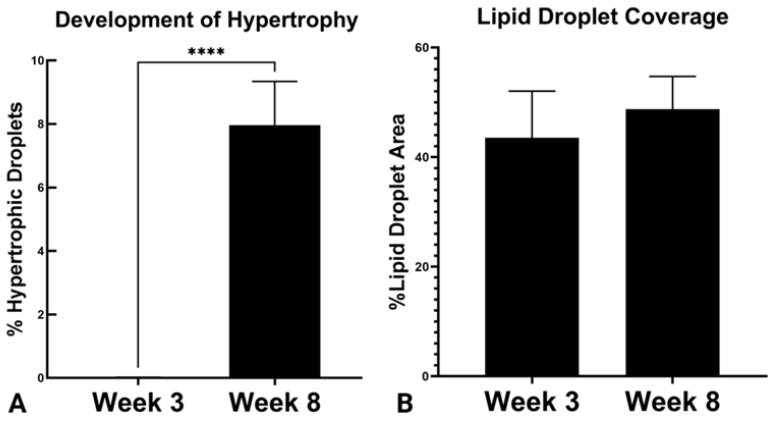

Figure 2. N=6 wells for week 3, n=8 wells for week 8. (A) The percentage of lipid droplets that are hypertrophic (>706 um^2 area) at week 3 compared to week 8, P<0.0001. (B) The percentage of images that are covered by lipid droplets at week 3 compared to week 8.

AI image analysis for adipose tissue organ-on-a-chip model is a robust and reliable method, compatible with real time GO/NO GO decisions concerning quality of micro-physiological systems or cellular responses during drug screening. The development and use of all-inclusive platforms for liquid handling, culture maintenance, and AI image analysis will help us to understand the kinetics of adipose tissue in terms of differentiation and responses during high throughput drug screenings, but also as a system to maintain quality control for in vitro tissue models.

As recently presented by Avtanski et al., other emergent technologies in adipose tissue research includes **Single cell analysis (**scProteomics, scRNA-seq, scDNA-seq, scATAC-seq and sc DNA methylome,), to evaluate the individual intrinsic differences in the adipose tissue (Avtanski D, et al., 2023). Finally, the integration of **Multiple-Omics** (proteomics, transcriptomics, genomics, metabolomics) approaches on adipocytes further support the deep understanding of molecular mechanisms of adipogenesis, remodeling, inflammation, insulin resistance, thermogenesis, and plasticity and adipose/cancer crosstalk (Hamel K et al., 2023; Holowatyjet al. 2020; Krieg et al., 2021).

Obatala Sciences supports the scientific and pharmaceutical community with the development of two 3D models for the study of adipose tissue compatible with the emergent technologies discussed above. ObaCell® Fat-on-a-Chip is a 3-D encapsulated cellular system used to evaluate immune responses and paracrine factors while the ObaCell® Obesity-on-a-Chip model, provides features of hypertrophic adipocytes, a characteristic of tissue from obese individuals.

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