Title: Modelling liver zonation: Enhancing iPSC-derived hepatocyte maturation via controlled oxygen microenvironment

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Abstract
Establishing zonation in liver cell cultures is essential for creating accurate *in vitro* liver models, which are vital for drug testing and disease studies. Since atmospheric oxygen levels differ significantly from the physioxia found in tissues like the liver, this study utilizes varying oxygen levels to guide the differentiation of induced pluripotent stem cells (iPSCs) into hepatocytes.

In the liver, hepatocytes are arranged in hexagonal units called hepatic lobules, each further divided into functionally distinct zones. The periportal zone, high in oxygen, favors the secretion of plasma proteins such as albumin, while the pericentral zone, lower in oxygen, focuses on drug metabolism and detoxification processes.

To mimic the zones, we expose iPSC-hepatocytes to varying oxygen levels during their differentiation process. We utilize 1-well culture chambers (BioGenium Microsystems) designed specifically for physiological oxygen studies. These chambers are connected to a gas supply and coated with an oxygen-sensing film, which allows for real-time monitoring of the oxygen microenvironment. We then assess how different oxygen levels impact cell functionality, including enzyme activity, protein production, and gene expression.

Our results show that by controlling the oxygen environment, the functional maturation of iPSC-hepatocytes is enhanced, even in the absence of media perfusion or 3D culture conditions. Ratiometric oxygen imaging reveals that the oxygen concentration experienced by the cells differs from the set gas level, highlighting the importance of O_2 monitoring during the culture period. Our approach contributes to the development of more accurate *in vitro* liver models that better reflect human liver physiology.