

In Vivo Monitoring of Liver Organoids Differentiation Using Live-Screening-Optical Technologies

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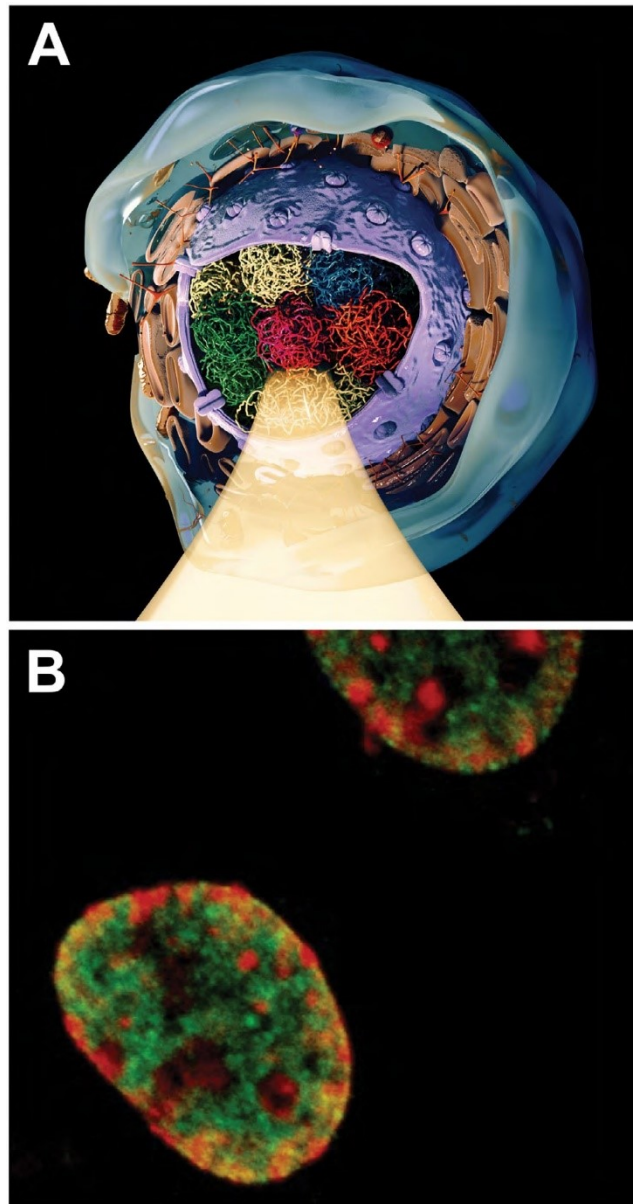
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Introduction: Tissue engineering is a promising technology for the cure of liver diseases¹⁻². Engineered liver organoids have been demonstrated to be a potential alternative to isolated liver primary hepatocytes³⁻⁵. Monitoring the progression of the differentiation process in live cells has great implications for the obtainment of functionally differentiated liver organoids. In this study, we have applied live-screening-optical technologies to monitor the progression of our differentiated human induced pluripotent stem cells (hiPSCs) in order to be able to improve the maturation and function of our liver organoids by looking at live chromatin remodeling and various other liver markers⁶⁻⁷.

Methods: The generation of human embryoid bodies (hEBs) was achieved utilizing HLA-Class II knockout hiPSCs mixed with endothelial cells, employing our ROCKi/Spin-free technology⁸. Using confocal light scattering spectroscopic microscopy technology we monitored the chromatin remodeling of our hEBs during the whole differentiation process. This analysis allowed us to characterize our four-stage hepatocyte differentiation protocol according to the whole genome modifications observed. We also carried out classical biochemical analyses to confirm results obtained with our live-screening-optical technologies.

Results: Differentiating liver organoids were followed by performing real-time chromatin remodeling to show changes in chromatin structure (euchromatin vs heterochromatin) over the course of the differentiation process (Fig. 1 A and B). Gene expression of several hepatic markers, as well as coagulation factors, were detected by real-time PCR showing a progression in the maturation of our liver organoids.

Conclusions: Real-time monitoring of our differentiating liver organoids represents a great tool to evaluate the differentiation advancement in live cells. Our data demonstrated the ability of confocal light scattering spectroscopic microscopy to provide critical information of the differentiation status *in vivo* during liver differentiation, providing a new tool necessary for the progression to clinical translation.



References:

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