MASS SCALE LIVER ORGANOID EXPANSION IN AN AUTOMATED BIOREACTOR MOVING TOWARDS CLINICAL VIABLE NUMBERS FOR CELL THERAPY

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INTRODUCTION

The liver is the second most required organ for transplantation, with demand far exceeding available donor livers. This shortage creates a critical healthcare challenge, as many patients with end-stage liver disease tragically do not survive the waiting period. Cell therapies, including the use of adult stem cell-derived organoids, present a viable solution to donor shortages. In this context, the differentiation capacity of bipotential intrahepatic cholangiocyte organoids (ICOs) allows for the opportunity to address multiple therapeutic needs. However, to actualize this, billions of cells are needed which requires scalable and automated cell culture methods. We propose adopting larger-scale culture methods, namely, automated bioreactor technology.

METHODS AND MATERIALS

Using the Osilaris™ bioreactor system, we investigated the rapid and scalable production of organoids. ICOs were generated from 2 female and 2 male donors. Each donor was seeded at a density of 1.5x10^4 cells/ml in 100 mL expansion medium supplemented with 5% Matrigel (MG) in a volume-expandable single-use bag. Cells were similarly seeded in static droplet (100% MG) and spinner flask (10% MG) formats as controls. Single-cell suspensions developed into translucent organoid structures using a continuous rocking regime. The culture protocol included 14 days of expansion, 2 days of BMP7 priming, and 10 days of hepatic differentiation. Every 2-3 days the culture was refreshed by expanding the culture bag. Cultures were monitored by morphology, growth, and metabolite concentration at every refreshing time point. On day 14 and 26, they were characterized for stemness, proliferative and differentiation capacity.

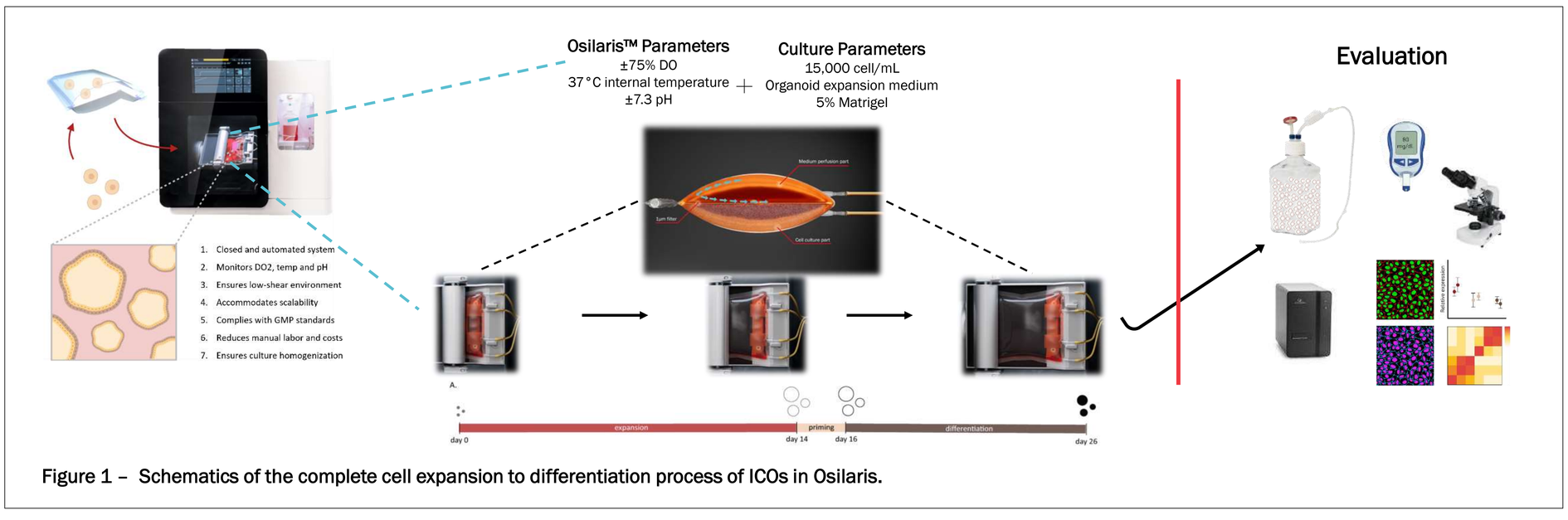

Figure 1 – Schematics of the complete cell expansion to differentiation process of ICOs in Osilaris.

RESULTS

SUPERIOR CELL EXPANSION IN OSILARIS

Representative images of organoid morphology on days 2, 9, and 14 were selected to represent illustrate organoid integrity and growth throughout the expansion phase (Fig. 2A). After 14 days, organoid cultures were primed and subsequently differentiated, into the hepatocytic lineage. Organoids developed a collapsed, darkened morphology (day 26) as compared to during the expansion period, consistent with hepatocyte differentiation.

ICOs rapidly proliferated in dynamic cultures. Our results demonstrate expansion yields of 376-fold in Osilaris compared to 371-fold in spinner flasks, and 134-fold in the static cultures (Fig. 2B). This shows that the highest yield was achieved using the largescale bioreactor compared to conventional approaches like spinner flasks and static droplet cultures. Furthermore, LGR5 expression confirmed the stemness-like phenotype of the liver organoids during the expansion phase (Fig. 2C).

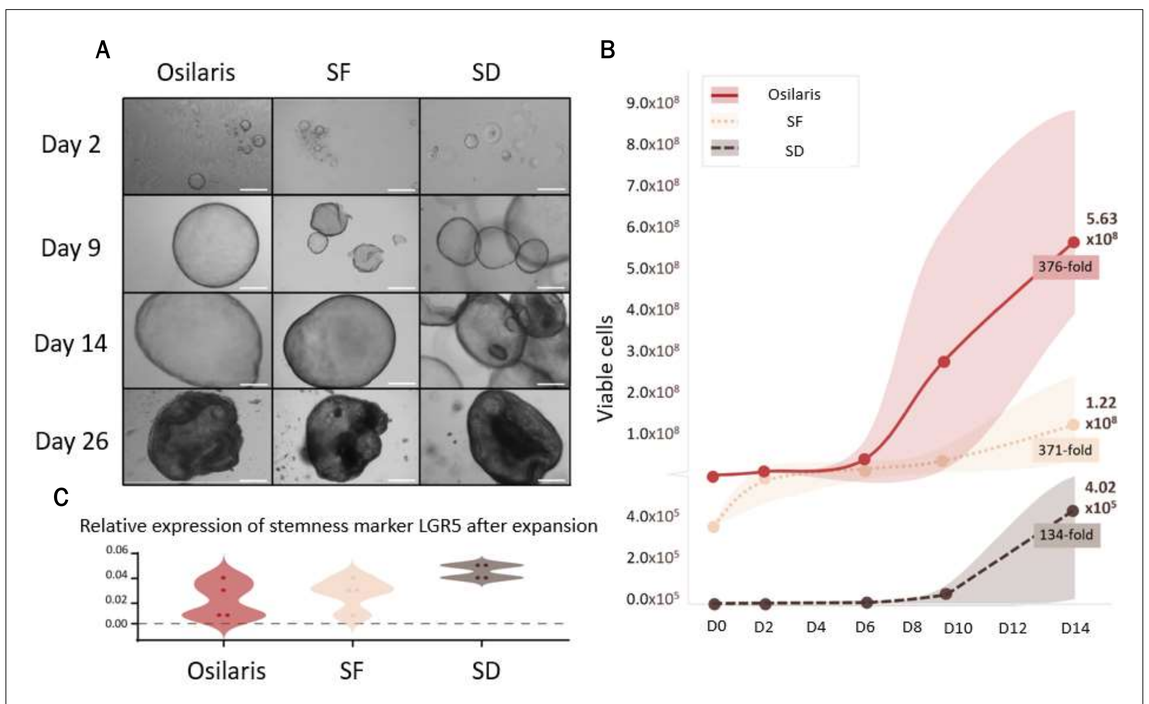

Figure 2 – (A) Representative images show organoids growth over a 14-day expansion period and shift to a darker, non-translucent morphology after hepatic differentiation. Scale bar indicates 100 µm. (B) Mean viable cell count from all four donors plotted per culture method (automated bioreactor, spinner flask, and static droplets) on days 2, 6, 9, and 14. (C) Relative expression of stemness marker LGR5 remains present after the 2-week expansion period (relative expression based on three reference genes HPRT, RPL19 and YWHAZ).

EPITHELIAL PHENOTYPE AND SUCCESSFUL DIFFERENTIATION

Expanded liver organoids retained their epithelial characteristics following large-scale expansion and hepatic differentiation (Fig.3C and 3D), as confirmed by E-cadherin staining (Fig. 3A), and their proliferative capacity reflected by Ki67 expression (Fig. 3B). Importantly, there was no evidence of epithelial-to-mesenchymal transition (EMT), as gene expression analysis revealed an upregulation of E-cadherin, while Vimentin expression, though inherently low, remained stable across all culture conditions (Fig. 3C). Notably, no significant differences in these markers were observed between the various culture methods, underscoring the consistency and robustness of the epithelial phenotype.

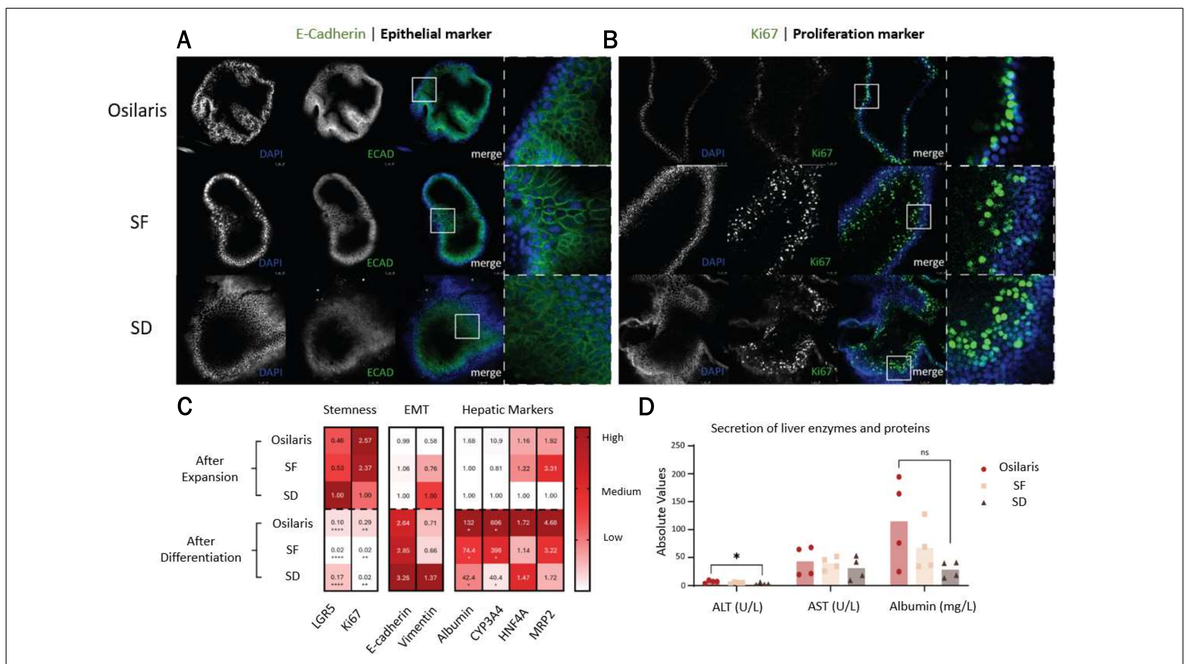

Figure 3 – (A) Immunostaining for E-cadherin indicates sustained proliferation capacity of liver organoids. (B) Immunostaining for Ki67 indicates a sustained proliferative capacity. Images are representative for all donors. (C) Gene expression profile confirms successful hepatic differentiation, as indicated by the downregulation of stemness marker LGR5, proliferation marker Ki67, and cholangiocyte marker KRT19, alongside upregulation of hepatic markers Albumin, CYP3A4, HNF4A and MRP2. (D) Hepatic-like cells produced liver enzymes ALT, AST and Albumin.

CONCLUDING REMARKS

We conclude that scalable and automated systems, such as Osilaris, offer a transformative approach to organoid expansion, bridging the gap between research and clinical application. This work represents a significant step toward providing clinically viable alternatives to donor livers.

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