Semi-Automated Large-Scale Expansion of Intrahepatic Cholangiocyte Organoids

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INTRODUCTION

The liver is the second most required organ for transplantation purposes. The rising demand far exceeds the number of available donor livers, and many patients do not survive the waiting time. Therefore, we propose the use of organoids. To achieve therapeutic efficacy, billions of cells are required and so there is a need for efficient culture methods that are scalable and automated.

Here, we have investigated the use of the SCINUS bioreactor for rapid production of adult stem cell-derived, bipotential Intrahepatic Cholangiocyte Organoids (ICOs) in a semi-automated fashion.

METHODS AND MATERIALS

Process development for organoid culture in the SCINUS bioreactor was initiated by small-scale investigations evaluating settings resulting in sustained organoid phenotype, morphology, and viability from inoculation to organoid maturation. Thereafter, the first large-scale run was undertaken where critical parameters were maintained at predetermined set points (Figure 1).

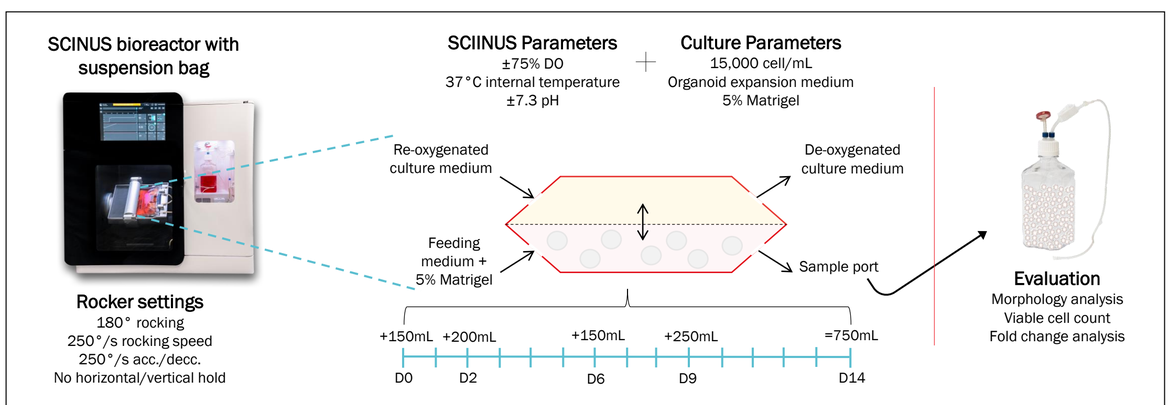

Figure 1 – Schematics of the complete culture process of ICOs in the SCINUS bioreactor.

RESULTS

ROCKING OPTIMIZATION

The influence of rocking speed on ICO growth and phenotype was first evaluated. Whole organoids were exposed to five different speeds (90°/s, 200°/s, 300°/s, 400°/s and 500°/s) for 3 consecutive days, after which cystic morphology and gene expression levels were assessed. Spinner flask cultures were used as a positive control. Early investigations demonstrated that a rocking speed between 200°/s and 300°/s with a 180° angle and no hold was most beneficial for ICO phenotype, morphology and viability (Figure 2).

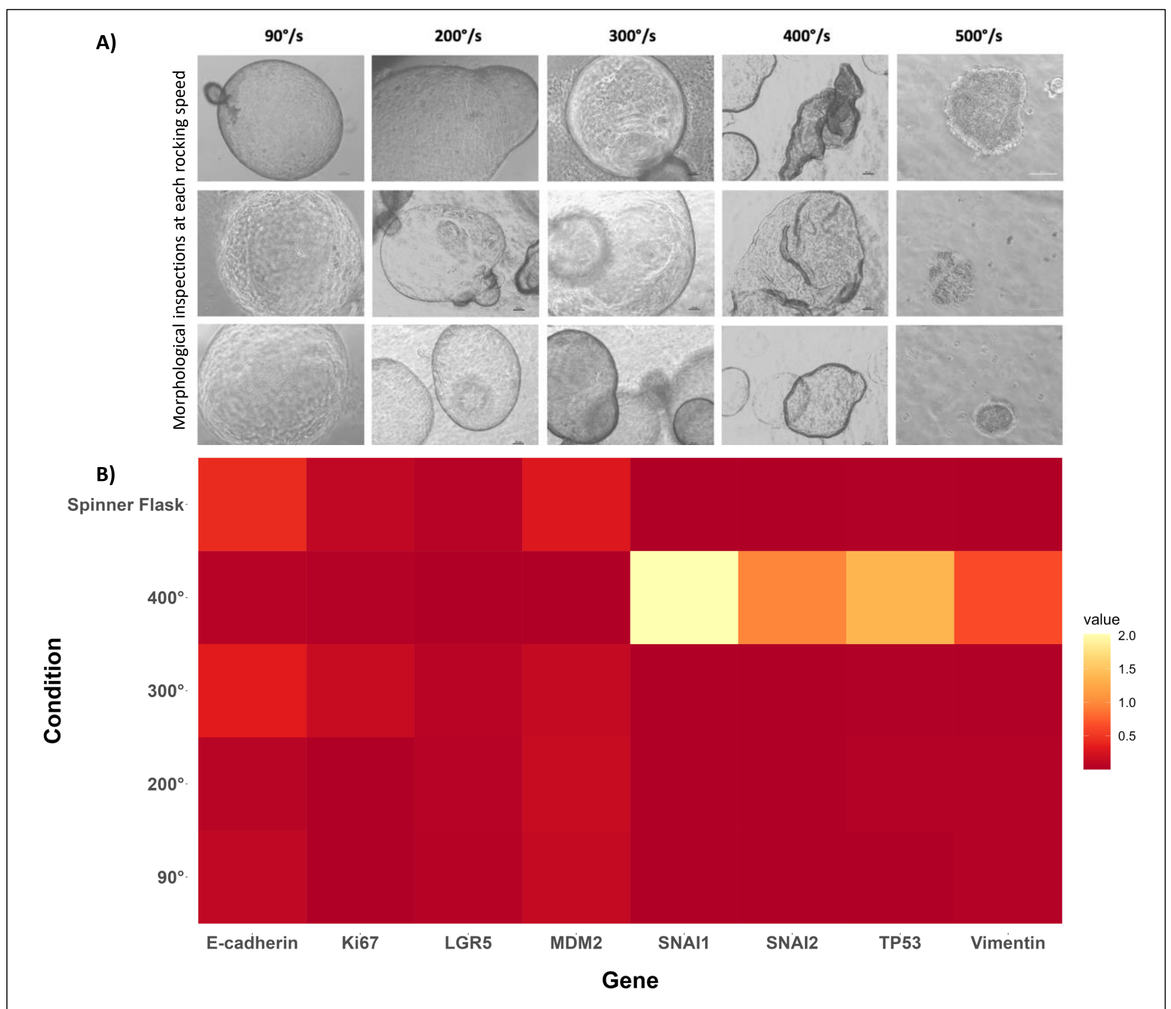

Figure 2 – (A) Organoid morphology and (B) gene expression with different rocking speeds. Organoid morphology and gene expression levels were assessed after three days in culture.

SMALL FRAGMENT SEEDING

Next, the feasibility of small fragment seeding on ICO growth and viability in the bioreactor for largescale expansion was explored. By means of mechanical and TrypLE treatments, small fragments were produced for two donors. They were cultured at a rocking speed of 250°/s and 180° angle for 2 weeks. Spinner flasks and static cultures were used as a positive control. Results show that organoids could be generated from small fragments in the bioreactor similar to standard culture methods (Figure 3).

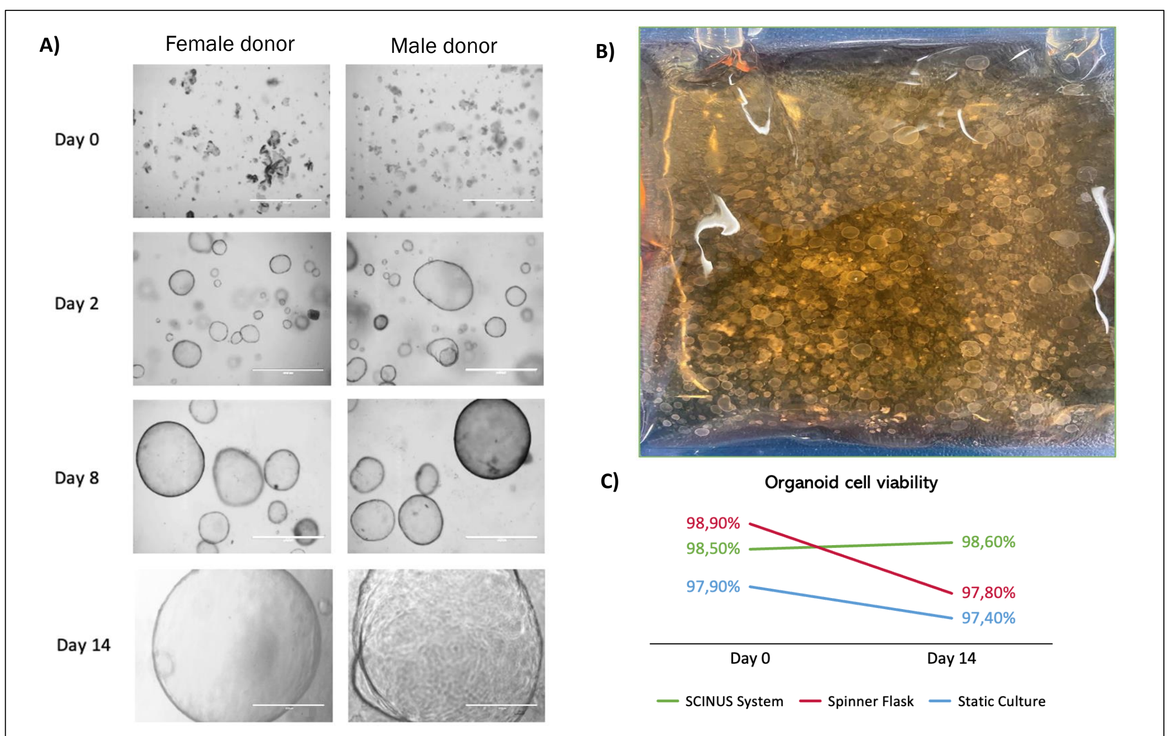

Figure 3 – Feasibility of small fragment seeding of organoids. (A) Two liver organoid donors (1 female, 1 male) were cultured with a 250°/s rocking speed and 180° angle. (B) and (C) Organoid morphology, growth and viability were assessed after two weeks in culture.

LARGE-SCALE RUN

ICOs were seeded in a 15,000 cells/mL density, and rocker speed was set to 250°/s and 180° angle with no hold. ICOs rapidly proliferated, resulting in a 383-fold expansion after two weeks, compared to a 222- and 48-fold expansion in spinner flask and static cultures, respectively (Figure 4). This shows that the highest fold change was reached using a Scinus bioreactor compared to conventional approaches like Spinner flasks and static cultures.

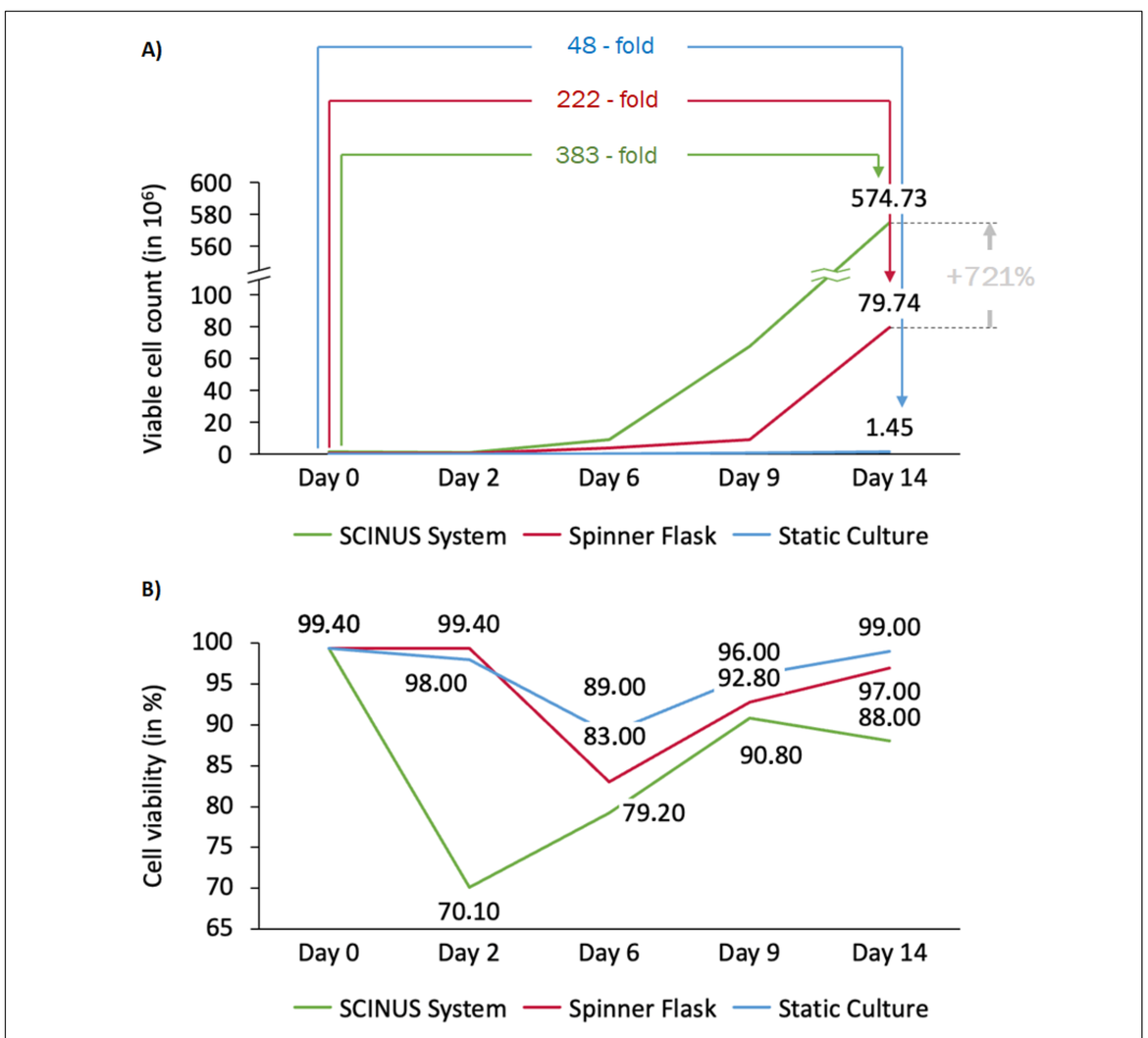

Figure 4 – Large-scale expansion of Intrahepatic Cholangiocyte organoids in the SCINUS bioreactor compared to spinner flask and static cultures. Viable cell numbers, viability and fold change were evaluated for a total period of 14 days .

CONCLUDING REMARKS

The SCINUS bioreactor allows for the large-scale expansion of ICOs in a controlled, semi-automated fashion. As well as outperforming static- and spinner flask culture in yield, the bioreactor culture revealed that ICOs retained viability and standard phenotype throughout a prolonged culture period. Therefore, the SCINUS bioreactor could be used to establish a robust, efficient, and reproducible method for upscaling organoids in a short period of time.

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