# A SCALABLE AND PHYSIOLOGICALLY RELEVANT SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELL EXPANSION AND DIFFERENTIATION

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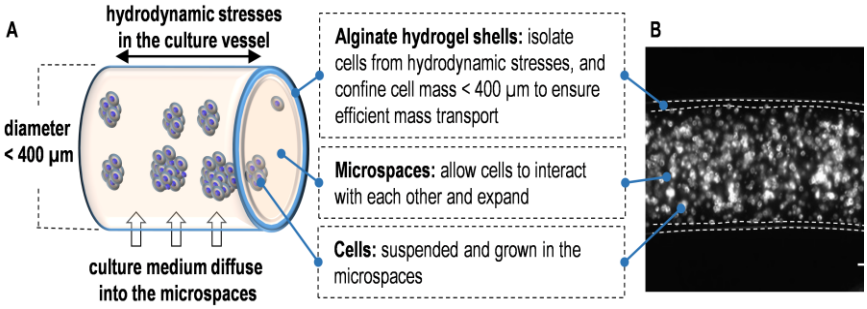
Human induced pluripotent stem cells (iPSCs) and their derivatives are needed in large numbers for various biomedical applications. However, scalable and cost-effective manufacturing of high quality iPSCs and their derivatives remains a challenge. In vivo, cells reside in a 3D microenvironment that has plenty of cell-cell and cell-ECM (extracellular matrix) interactions, sufficient supply of nutrients and oxygen, and minimal hydrodynamic stresses. The current iPSC culturing methods, however, provide highly-stressed culturing microenvironments, leading to low culture efficiency. For instance, we and others showed iPSCs typically expanded 4-fold/4 days to yield ~2.0x10^6 cells/mL with current 3D suspension culturing. These cells occupy ~0.4% of the bioreactor volume. To our best knowledge, the largest culture volume demonstrated to date for iPSCs is less than 10 liters. There is a critical need to develop new culture technologies to achieve the iPSCs' potential.

We here report a novel technology that can overcome all the limitations of current methods and provide a physiologically-relevant culture microenvironment. With this technology, iPSCs are processed into and cultured in microscale alginate hydrogel tubes (termed SFIT or stress-free intratubular cell culture) that are suspended in the cell culture medium in a culture vessel (Figs. 1A and B). The hydrogel tubes create free microspaces that allow cells to interact with each other and expand. Meanwhile, they protect cells from hydrodynamic stresses in the culture vessel and confine the cell mass <400 µm (in radial diameter) to ensure efficient mass transport during the entire culture (Figs. 1A and B). This technology is simple, scalable, defined and cGMP-compliant that make it commercially viable. We showed that, under optimized culture conditions, SFIT offered paradigm-shifting improvements in cell viability, growth, yield, culture consistency and scalability over current methods. We demonstrated long-term culturing (>10 passages) of iPSCs without uncontrolled differentiation and chromosomal abnormalities. Cultures between batches and cell lines were very consistent. iPSCs in SFIT had high viability, growth rate (1000-fold/10 days/passage in general) and yield (~5x10^8 cells/mL microspace). The expansion per passage (e.g. up to 4200-fold/passage was achieved) and volumetric yield are much higher than current methods. The high yield and high expansion fold significantly reduce the culture volume and time, numbers of passaging operations, and the production cost, making large-scale cell production technically and conically feasible.




iPSCs could be efficiently differentiated into various tissues cells in SFIT. Additionally, we have shown other human cells, such as T cells, could also be efficiently cultured in this technology. Two SFIT-based automated bioreactors for producing autologous and allogenic iPSCs and their derivatives are under developing. This technology has high potential to address the cell manufacturing challenge. Details of the method can be found in very-recent publications: Biofabrication. doi: 10.1088/1758-5090/aaa6b5; Sci Rep. doi: 10.1038/s41598-018-21927-4; ACS Appl Mater Interfaces. doi: 10.1021/acsami.8b05780; Adv Healthc Mater. doi: 10.1002/adhm.201701297.