SCALABLE AND PHYSIOLOGICALLY RELEVANT MICROENVIRONMENTS FOR HUMAN PLURIPOTENT STEM CELL EXPANSION AND DIFFERENTIATION

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Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), are attractive cell sources for various biomedical applications including cell therapies, tissue biofabrication, drug screening and toxicity tests. These applications require large numbers of high quality cells. However, the scalable and cost-effective culturing of high quality hPSCs and their derivatives, especially for clinical applications, remains a challenge. *In vivo*, majority of human cells including the hESCs reside in 3D microenvironments that have plenty of cell-cell and cell-ECM (extracellular matrix) interactions, sufficient supply of nutrients, oxygen and growth factors, and no or minimal hydrodynamic stresses. The current hPSC culturing methods, however, provide culturing conditions that are very different from these physiological microenvironments, leading to low culture efficiency and difficulty to culture cells at large scales. For instance, we and others showed hPSCs typically expanded 4-fold in 4 days to yield around 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 3D suspension culture volume demonstrated to date for hPSCs is less than 10 liters.

We here report a novel technology that can overcome the limitations of current hPSC culturing methods and provide physiologically relevant cell culture microenvironments. With this technology, hPSCs are processed into and cultured in microscale alginate hydrogel tubes (or AlgTubes) that are suspended in the cell culture medium in a culture vessel (Figs. 1A and B). The hydrogel tubes create cell-friendly 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 less than 400 µm (in radial diameter) to ensure efficient mass transport during the entire culture (Figs. 1A and B). Additionally, this technology is simple, scalable, defined and compatible with the current Good Manufacturing Practices that make it commercially viable. We showed that, under optimized culture conditions, the AlgTubes offered paradigm-shifting improvements in cell viability, growth, yield, culture consistency and scalability over current hPSC culturing technologies. We demonstrated long-term culturing (>10 passages) of multiple hPSC lines without uncontrolled differentiation and chromosomal abnormalities. Cultures between batches and cell lines were very consistent. hPSCs in AlgTubes 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 3D suspension culturing. The high yield and high expansion fold have high impact on large-scale cell production since they significantly reduce the culture volume and time, numbers of passaging operations, and the production cost. hPSCs could be efficiently differentiated into various tissues cells. In addition, AlgTubes-based scalable bioreactors could be readily built. Our comparative study showed the AlgTubes did not significantly alter hPSCs' gene expression profiles, but significantly reduced cell death, resulting in high cell expansion and yield.

In summary, the AlgTube technology combines physiologically relevant culture microenvironments, high performance, high scalability, cGMP compliance and commercial viability, and has potential to address the hPSC manufacturing challenge. This research well demonstrates the using of microfluidics to create cell niche to address a significant biomedical problem.

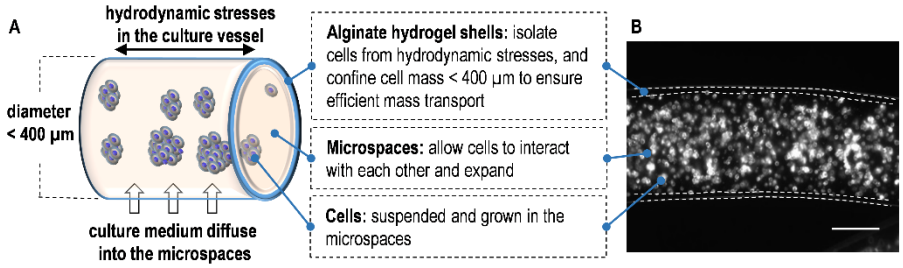

Figure 1- The AlgTubes cell culture system.