A 3D Culture System for Pluripotent Stem Cells by the Cell Fiber Technology

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ABSTRACT

Human pluripotent stem (PS) cells including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are expected to be an ideal cell source for drug screening and regenerative medicine. These applications such as transplantation therapy require large numbers of PS cells and PS cell-derived cells. For the large-scale culture of PS cells, scalable three-dimensional (3D) culture systems are attractive ways and widely studied. An important issue in previous 3D culture systems is controllability of the cell aggregation size and thickness because cell death and differentiation are induced within the large cell aggregations. Alginate microencapsulation culture system ensures avoiding excessive cell aggregations. However, efficient PS cell expansion hasn't been achieved in alginate microencapsulation culture system because of the growth inhibition due to the lack of expansion space within alginate microcapsules. Furthermore continuous PS cell expansion by serial passages hasn't been investigated with alginate microencapsulation culture system. In this study, I developed a cell-fiber based culture system called core-shell microfiber culture system to achieve efficient cell expansion in alginate gel structure by using the cell fiber technology. The cell fiber technology enables encapsulation of cells with extra cellular matrix (ECM) proteins into core-shell

microfibers by utilizing double co-axial microfluidic device. Human and mouse iPS cells were encapsulated into alginate gel's shell with extracellular matrix (ECM) proteins or medium. The cells were proliferated and formed rod-shaped or fiber-shaped aggregations with uniformed diameter. To retrieve cells from the microfibers for evaluation of the cell properties and serial passaging, I used an enzymatic treatment method for iPS cell-laden core-shell microfibers and it enabled high efficiency cell retrieval with preservation of viability. Under an optimized protocol using the core-shell microfibers with encapsulation of iPS cell suspensions in collagen, I demonstrated the long-term culture of human iPS cells by serial passaging with a high expansion ratio and maintenance of pluripotency. Thus, my work may be a key step toward the large-scale expansion of human pluripotent stem cells for use in regenerative medicine.