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Synthetic hydrogel-based 3D culture system for maintenance of human induced pluripotent stem cell

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Human induced pluripotent stem cells (hiPSCs) are generated from human somatic cells using defined transcription factors. These cells possess characteristics very similar to that of human embryonic stem cells including the ability to differentiate into cell types of all three germ layers. HiPSCs show great potential in clinical researches like drug screening and regenerative medicine, that all require large amount of cells cultured under well-defined conditions. The most common culture methods used for hiPSCs are 2D culture methods using Matrigel or vitronectin coated culture plates or flasks. 2D culture methods require large surface area to produce the same amount of cells compared to 3D methods. In addition, cells cultured in 2D culture environment are far from that *in vivo*. In this study, we developed a robust 3D culture condition based on hiPSC-qualified PGmatrix (PGmatrix-hiPSC) hydrogel. This 3D culture system provides hiPSCs with well-defined, more *in vivo*-like environment that encapsulate cells in liquid rich hydrogel with appropriate oxygen supply that resembles the hypoxia condition *in vivo*. Two hiPSC lines grown continuously in PGmatrix-hiPSC showed higher total population fold expansion and higher viability with more consistency compared to the same cell lines grown in 2D on matrigel or vitronectin-XF. After grown in 3D PGmatrix-hiPSC for over 25 passages, major pluripotency markers, such as Oct4, Sox2, Nanog, and SSEA4 are expressed in most hiPSCs examined by flow cytometry. RT-qPCR also confirmed adequate expression levels of major pluripotency related genes. In addition, karyotype analysis of hiPSC after 37 passages in 3D PGmatrix-hiPSC was found normal. The same hiPSC lines cultured continuously in parallel in 2D and 3D showed differences in gene expression and surface marker TRA-1-81 expression. These results indicated the 3D PGmatrix-hiPSC system is likely superior in maintaining hiPSC growth as well as pluripotency. The findings also suggest that it is very important to study cells in 3D culture environment to better understand the mechanism of pluripotency maintenance.

Biography

Quan Li has completed her BS and MS studies from Kansas State University and is pursuing PhD studies in Biological and Agricultural Enigeering at Kansas State University. During her Master's program, she developed a novel 3D culture protocol based on synthetic hydrogel for human induced pluripotent stem cell that allows rapid and consistent cell growth without differentiation. She is continuing to explore 3D modeling for human tissue engineering.

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