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Direct Current Electric Field Stimulation against PC12 Cells in 3D Bio-Reactor to Enhance Axonal Extension

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Abstract : In this study, we developed a three-dimensional (3D) direct current electric field (DCEF) stimulation bio-reactor for axonal outgrowth enhancement to generate the neural network of the central nervous system (CNS). By using our newly developed 3D DCEF stimulation bio-reactor, we cultured the rat pheochromocytoma cells (PC12) and investigated the effects on the axonal extension enhancement and network generation. Firstly, we designed and fabricated a 3D bio-reactor, which can load DCEF stimulation on PC12 cells embedded in the collagen gel as extracellular environment. The connection between the electrolyte and the medium using salt bridges for DCEF stimulation was introduced to avoid the cell death by the toxicity of metal ion. The distance between the salt bridges was adopted as the design variable to optimize a structure for uniform DCEF stimulation, where the finite element (FE) analyses results were used. Uniform DCEF strength and electric flux vector direction in the PC12 cells embedded in collagen gel were examined through measurements of the fabricated 3D bio-reactor chamber. Measurement results of DCEF strength in the bio-reactor showed a good agreement with FE results. In addition, the perfusion system was attached to maintain pH 7.2 ~ 7.6 of the medium because pH change was caused by DCEF stimulation loading. Secondly, we disseminated PC12 cells in collagen gel and carried out 3D culture. Finally, we measured the morphology of PC12 cell bodies and neurites by the multiphoton excitation fluorescence microscope (MPM). The effectiveness of DCEF stimulation to enhance the axonal outgrowth and the neural network generation was investigated. We confirmed that both an increase of mean axonal length and axogenesis rate of PC12, which have been exposed 5 mV/mm for 6 hours a day for 4 days in the bioreactor. We found following conclusions in our study. 1) Design and fabrication of DCEF stimulation bio-reactor capable of 3D culture nerve cell were completed. A uniform electric field strength of average value of 17 mV/mm within the 1.2% error range was confirmed by using FE analyses, after the structure determination through the optimization process. In addition, we attached a perfusion system capable of suppressing the pH change of the culture solution due to DCEF stimulation loading. 2) Evaluation of DCEF stimulation effects on PC12 cell activity was executed. The 3D culture of PC 12 was carried out adopting the embedding culture method using collagen gel as a scaffold for four days under the condition of 5.0 mV/mm and 10mV/mm. There was a significant effect on the enhancement of axonal extension, as 11.3% increase in an average length, and the increase of axogenesis rate. On the other hand, no effects on the orientation of axon against the DCEF flux direction was observed. Further, the network generation was enhanced to connect longer distance between the target neighbor cells by DCEF stimulation.

Keywords : PC12, DCEF stimulation, 3D bio-reactor, axonal extension, neural network generation
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