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Stimulation of Nerve Tissue Differentiation and Development Using Scaffold-Based Cell Culture in Bioreactors

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Abstract : Nerve tissue engineering is the main field of research aimed at finding an alternative to autografts as a treatment for nerve injuries. Scaffolds are used as a support to enhance nerve regeneration. In order to successfully design novel scaffolds and in vitro cell culture systems, a deep understanding of the factors affecting nerve regeneration processes is needed. Physical and biological parameters associated with the culture environment have been identified as potentially influential in nerve cell differentiation, including electrical stimulation, exposure to extracellular-matrix (ECM) proteins, dynamic medium conditions and co-culture with glial cells. The mechanisms involved in driving the cell to differentiation in the presence of these factors are poorly understood; the complexity of each of them raises the possibility that they may strongly influence each other. Some questions that arise in investigating nerve regeneration include: What are the best protein coatings to promote neural cell attachment? Is the scaffold design suitable for providing all the required factors combined? What is the influence of dynamic stimulation on cell viability and differentiation? In order to study these effects, scaffolds adaptable to bioreactor culture conditions were designed to allow electrical stimulation of cells exposed to ECM proteins, all within a dynamic medium environment. Gold coatings were used to make the surface of viscose rayon microfiber scaffolds (VRMS) conductive, and poly-L-lysine (PLL) and laminin (LN) surface coatings were used to mimic the ECM environment and allow the attachment of rat PC12 neural cells. The robustness of the coatings was analyzed by surface resistivity measurements, scanning electron microscope (SEM) observation and immunocytochemistry. Cell attachment to protein coatings of PLL, LN and PLL+LN was studied using DNA quantification with Hoechst. The double coating of PLL+LN was selected based on high levels of PC12 cell attachment and the reported advantages of laminin for neural differentiation. The underlying gold coatings were shown to be biocompatible using cell proliferation and live/dead staining assays. Coatings exhibiting stable properties over time under dynamic fluid conditions were developed; indeed, cell attachment and the conductive power of the scaffolds were maintained over 2 weeks of bioreactor operation. These scaffolds are promising research tools for understanding complex neural cell behavior. They have been used to investigate major factors in the physical culture environment that affect nerve cell viability and differentiation, including electrical stimulation, bioreactor hydrodynamic conditions, and combinations of these parameters. The cell and tissue differentiation response was evaluated using DNA quantification, immunocytochemistry, RT-qPCR and functional analyses.

Keywords : bioreactor, electrical stimulation, nerve differentiation, PC12 cells, scaffold
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