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Growth of human stem cell-derived neurons on solid three-dimensional polymers

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Abstract

Understanding neural differentiation and the development of complex neurite networks in three-dimensional matrices is critical for neural tissue engineering in vitro. In this study we describe for the first time the growth of human stem cell-derived neurons on solid polystyrene matrices coated with bioactive molecules. Highly porous foams were prepared from poly(styrene/divinylbenzene) using a high internal phase emulsion (HIPE) as a template to create the porous structure. The resulting polyHIPE matrices were readily coated with aqueous-based solutions including poly-D-lysine and laminin. Human neurons adhered well to poly-D-lysine coated surfaces and extended neural processes, however, neurite outgrowth was particularly enhanced when polymers also received a coating of laminin. These data clearly demonstrate the potential use of solid polystyrene scaffolds to create three-dimensional environments for cell growth and differentiation. We propose that these robust and stable matrices can be conveniently and routinely used in the tissue culture laboratory to study the behaviour of cells grown in three-dimensions.

Keywords: Polymer; Three-dimensional-culture; Human; Neurogenesis; Differentiation; Cell culture

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1. Introduction

Culturing adherent cells in the laboratory has traditionally been carried out in petri dishes and tissue culture flasks in which cells grow and proliferate over the flat bottom surface of the vessel in two-dimensions (2-D). More recently however scientists are beginning to explore the advantages of culturing cells in three-dimensions (3-D) and have been surprised by the changes in cell structure and function. This is particularly relevant to the growth and differentiation of cells where interactions between neighbouring cells play an important role in cell proliferation, survival, migration and the determination of cell fate. For example, Cukierman and co-workers [1] have shown that fibroblasts grown in 3-D migrated and proliferated more rapidly compared to their counterparts grown in 2-D cultures. These such changes appear to relate more closely to cell behaviour in vivo, which after all is a complex 3-D environment, and allowed fibroblasts grown in 3-D cultures to adopt the characteristic asymmetrical shape that such cells possess in living tissues [1].

Encouraging cultured cells to adopt a phenotype typical of their counterparts in vivo is difficult and given the enormous complexity of the nervous system it is especially challenging to model and differentiate neural tissues in vitro. Using a 2-D culture system, we have shown that the development of neural cell types from human pluripotent stem cells in vitro closely resembles that in the embryo [2,3]. However, such development could be considered incomplete since the formation of networks by these neurons is limited to 2-D and this obviously differs significantly from the complexity of connections formed by neural cells in vivo. Accordingly, in this study we investigated the ability of human neurons to form neurites in 3-D cultures as a first step in developing a culture system that will enhance cell differentiation towards tissues that compare more closely with living cells in the brain.

Recent evidence demonstrates that 3-D culture systems are likely to play an important role in neuroscience research and have potential therapeutic applications. Gel-based materials that have often been coupled with bioactive substances have proven useful in promoting neurite outgrowth in vitro [4–7] and have also encouraged regeneration of damaged nerves in the invertebrate nervous system [8]. The routine everyday use of 3-D gel based systems is, however, limited by various practical issues including gel preparation, storage and variability.

Solid but highly porous supports may offer an attractive alternative for routinely growing cells in 3-D within the tissue culture laboratory since they are convenient to use, they can be manufactured in a controlled and reproducible fashion, they can be moulded and shaped as appropriate, and their inert structure remains stable over time. Porous 3-D matrices can be produced by polymerisation in high internal phase emulsions (HIPEs), a process that is conducted routinely in our laboratories [9–11]. The porosity of these structures can be controlled during the production process and therefore can be tailored towards the types of cells to be cultured. Moreover, we have successfully tested the ability of these materials to support the growth of primary cells [12,13]. Here we describe the culture of human stem cell-derived neurons on 3-D polystyrene matrices. In addition, we show that these materials can be readily coated with bioactive molecules that support and promote the outgrowth of neural processes.