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PHBV microspheres as neural tissue engineering scaffold support neuronal cell growth and axon–dendrite polarization

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ABSTRACT

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) microspheres, with properties such as slower degradation and more efficient drug delivery properties, have important benefits for neural tissue engineering. Our previous studies have shown PHBV microspheres to improve cell growth and differentiation. This study aimed to investigate if PHBV microspheres would support neurons to extend these benefits to neural tissue engineering. PHBV microspheres' suitability as neural tissue engineering scaffolds was investigated using PC12 cells, cortical neurons (CNs), and neural progenitor cells (NPCs) to cover a variety of neuronal types for different applications. Microspheres were fabricated using an emulsion-solvent-evaporation technique. DNA quantification, cell viability assays, and immunofluorescent staining were carried out. PC12 cultures on PHBV microspheres showed growth trends comparable to two-dimensional controls. This was further verified by staining for cell spreading. Also, CNs expressed components of the signaling pathway on PHBV microspheres, and had greater axon–dendrite segregation (4.1 times for axon stains and 2.3 times for dendrite stains) than on coverslips. NPCs were also found to differentiate into neurons on the microspheres. Overall, the results indicate that PHBV microspheres, as scaffolds for neural tissue engineering, supported a variety of neuronal cell types and promoted greater axon–dendrite segregation.

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1. Introduction

Scaffolds are important, if not essential, to the viability of cells used in regeneration therapies [1]. Physical and biochemical cues provided by scaffolds are crucial to keeping implanted cells viable and to improve therapeutic effects. Furthermore, scaffolds can localize cells at therapeutic sites. Cell-replacement, drug-delivery, and cell-delivery therapies are being developed for neurodegenerative diseases and traumatic injuries to the nervous systems. Some challenges for effective therapy include avoiding further inflammation, providing neuroprotection, stimulating tissue regeneration, overcoming inhibitory cues, and promoting functional recovery [2–5].

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) microspheres offer benefits as tissue engineering scaffold for neural therapy. Firstly, it has well-established drug delivery properties and controllable drug release profiles, providing sustained and controlled release of neurotrophins for neuroprotection, as well as various biocompounds for stimulating tissue regeneration, overcoming inhibitory cues and promoting functional recovery, which was demonstrated in our previous studies [6–12]. Secondly, its biodegradability enables it to disappear from implant sites, making space for regenerated tissue [13]. Thirdly, its slow degradation, as compared to PLGA [9], better matches the longer duration required for neural tissue treatment [14–17], therefore there is no accumulation of acidic degradation products in vivo which could be a problem [18]. Furthermore, PHBV could be added to slow down degradation of other polymers, as we have shown [9]. Fourthly, PHBV microspheres cause less inflammation and require less invasive surgery due to their biocompatibility, small sizes and spherical shape [19–23]. Finally, the three-dimensional (3-D) nature of PHBV microsphere scaffolds benefits cell growth and differentiation. Our previous studies showed PHBV microspheres to promote higher hepatocyte proliferation and function as compared to two-dimensional (2-D) cultures [9,12].

Despite all these, PHBV has not been widely studied for neural tissue engineering (TE), where recent literature reviews did not report on its use for neurons [24–26]. This work aims to investigate the suitability of PHBV microsphere in neural TE, so as to utilize its advantages as a suitable biomaterial. In neuronal TE research, different neuronal cell types have been used in different applications and models. For example, PC12 is a neuronal cell line that responds to nerve growth factor by differentiating to a neuronal phenotype, and thus has been used as a model for neuronal differentiation and biomolecule secretion [27–30]. It has also been used in the development of therapies for neural diseases as a highly potent cell

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