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Neurite infiltration and cellular response to electrospun polycaprolactone scaffolds implanted into the brain

David R. Nisbet [a,b,f,*], Andrew E. Rodda [a,b], Malcolm K. Horne [c,d,e], John S. Forsythe [a,b], David I. Finkelstein [f]

[a] Department of Materials Engineering, Division of Biological Engineering, Monash University, Victoria 3800, Australia
[b] CRC for Polymers, Notting Hill, Victoria 3168, Australia
[c] Florey Neurosciences Institute, University of Melbourne, Parkville, Victoria 3010, Australia
[d] Centre for Neurosciences, University of Melbourne, Parkville, Victoria 3010, Australia
[e] St Vincent's Hospital, Fitzroy, Victoria 3065, Australia
[f] The Mental Health Research Institute of Victoria, Parkville, Victoria 3010, Australia

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ABSTRACT

Assessment of axonal infiltration and guidance within neural tissue engineering scaffolds, along with the characterisation of the inflammatory response, is critical in determining these scaffolds' potential for facilitating neural repair. In this study, the extent of microglial and astrocytic response was measured following implantation of electrospun poly(ε-caprolactone) (PCL) scaffolds into the caudate putamen of the adult rat brain. The inflammation peaked at around 4 days (microglia) and 7 days (astrocytes) and subsided to homeostatic levels by 60 days. There was no evidence of microglial encapsulation and indeed neurites had infiltrated the implants, evidence of scaffold-neural integration. Whilst the inflammatory response was uninfluenced by the degree of PCL fibre alignment, the extent of neurite entry was. Large porosity, as was the case with the randomly orientated polymer fibres, enabled neurite infiltration and growth within the scaffold. However, neuronal processes could not penetrate scaffolds when fibres were partially aligned and instead, preferentially grew perpendicular to the direction of PCL fibre alignment at the implant-tissue interface i.e. perpendicular, not parallel, contact guidance was provided. This investigation shows that electrospun PCL fibres are compatible with brain tissue and provide preliminary insights regarding the influence of microglia and astrocytes in neural integration within such scaffolds.

1. Introduction

The central nervous system (CNS) is the primary computing area within the body and consisting of the brain and spinal cord. During development, its architecture is established by a highly complex set of guidance cues including cell–cell and cell–extracellular matrix (ECM) interactions and cytotrophic molecules such as growth factors and guidance molecules [1–3]. When the CNS is damaged by injury or disease, these same factors are induced to re-establish connectivity but their efficiency is severely attenuated and hence there is highly limited functional recovery of this complex cellular network [4–7]. As a consequence, people with brain and spinal cord injury are left with major disabilities. There are no treatments to facilitate neural repair within the adult mammalian CNS [2,8,9], and regeneration or repair of the neural circuitry is actively inhibited by the microenvironment surrounding damaged neurones and axons [6,10]. Consequently there has been interest in developing neural tissue engineering materials and methods to provide microenvironments that permit CNS regeneration.

Whist there is currently an array of scaffold materials and designs available, PCL nanofibres were selected for this study on the basis of *in vitro* evidence that they support, maintain and control the differentiation of neural stem cells (NSC) [9]. Furthermore, the size and alignment of the fibres can be manipulated to simulate the native ECM. *In vitro* evidence indicates that aligned nanofibrous scaffolds promote NSC to differentiate towards the neuronal lineage, and provide contact guidance cues and direct axonal outgrowth [11]. The neurites of dorsal root ganglion (DRG) explants cultured with aligned polymer nanofibres initially grew radially outwards to contact the aligned nanofibres and then turned to follow the fibres [12,13]. In addition, contact guidance is not exclusively controlled by fibre alignment; the diameter of the electrospun fibres may also impart an influence [11].

* Corresponding author. Department of Materials Engineering, Division of Biological Engineering, Monash University, Victoria 3800, Australia. Tel.: +61 3 99055345.
E-mail address: david.nisbet@eng.monash.edu.au (D.R. Nisbet).

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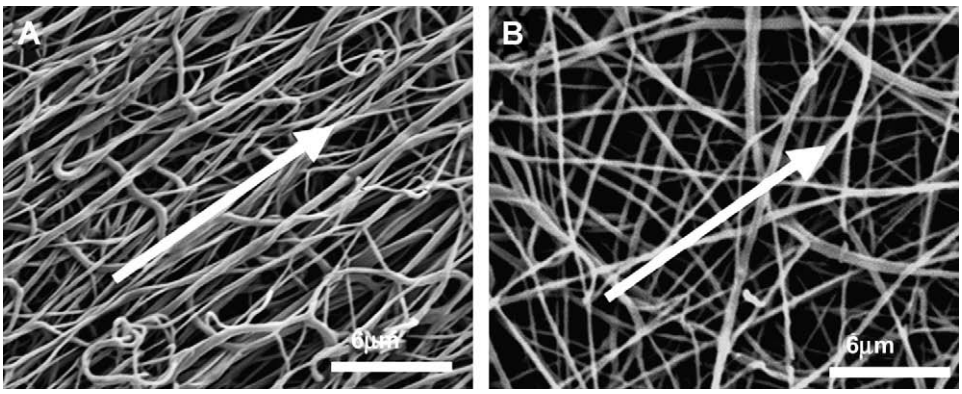

Fig. 1. Partially aligned (A) and random (B) electrospun PCL nanofibrous scaffolds. Arrow shows rotation direction of the mandrel.

In this study PCL nanofibres were implanted within the caudate putamen of rats and the neurite infiltration and inflammation assessed. Injury to the CNS induces an inflammatory reaction, characterised by a chemokine and cytokine response as well as a cellular reaction by astrocytes and microglia [14–16]. In a similar manner to macrophages, microglia sense changes within their extracellular environment and are a component of active inflammation within the CNS [17], participating in phagocytosis of foreign matter [18] and cellular debris. The inflammatory response to a stab wound, which would have many similarities to the injury induced by the introduction of a tissue engineering scaffold, has two phases. The initial phase occurs in the first week to repair tissue integrity and remove damaged or foreign tissue by phagocytosis. This initial phase may also damage viable neural tissue in the vicinity [17,18]. In the second phase microglia undertake several processes that facilitate neuronal and axonal regeneration. They provide cytotrophic support through the secretion of anti-inflammatory cytokines and are also responsible for removing axonal branches from damaged tissue so as to promote regrowth of axons [19]. An understanding of the timing of microglial responses to the insertion of neural scaffolds will be important in harnessing their cytotrophic behaviour while suppressing their cytotoxic effects.

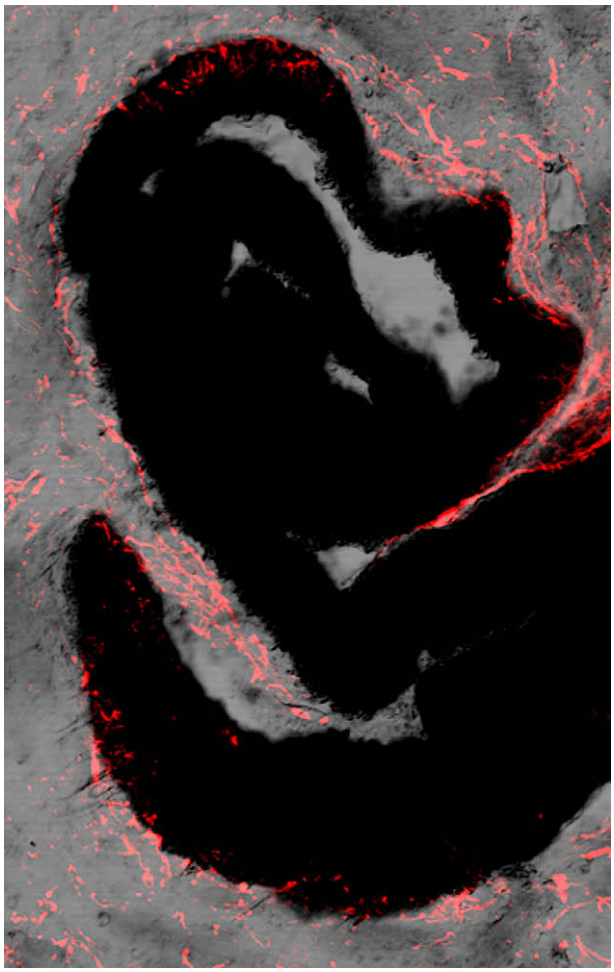

Fig. 2. Random electrospun PCL nanofibrous scaffold which was implanted within a rat brain for a 60-day duration. The scaffold has been sectioned perpendicular to the implant direction, with the red representing astrocytes that have been marked with GFAP.

Astrocytes may also be cytotoxic during certain stages in the response to injury. They proliferate and enter the site of injury to maintain the physical integrity in the region of the lesion, but in the process may inhibit neurite outgrowth and circuit repair, by releasing molecules that inhibit neurite extension [20]. However, astrocytes also contribute a cytotrophic component to repair by providing nutrients such as glucose. Astrocytes undertake many other functions *in vivo* (outlined in the following references [21–24]), however some of these processes are not fully understood or even determined. As with microglia, the extent and timing of the astrocyte response can provide an initial indication of the cytotoxic and cytotrophic contributions of astrocytes. This is an important first step in gaining an understanding of how and when to control or harness the reaction of astrocytes and encourage them to play a primarily cytotrophic role in regeneration.

Here we describe the inflammatory response that follows the implantation of electrospun sub-micron fibrous scaffolds with different architectures within the caudate putamen of adult rats over different time points. The number of astrocytes, microglia and axonal infiltration was used to assess the potential of PCL nanofibres for neural tissue engineering of the CNS.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL) was purchased from Absorbable Polymers, Inc. (Alabama, USA). Solutions containing 13% w/v polymer were prepared by dissolving PCL in a 5 mL mixture of chloroform (Merck Pty. Ltd., Australia) and methanol (Merck Pty Ltd, Australia) at a ratio of 75:25 (v/v). The solutions were placed into a 10 mL glass syringe with an 18-guage needle for electrospinning using a flow rate of 0.6 mL/h, an accelerating voltage of 15 kV and a working distance of 12 cm. The scaffolds were collected on an aluminum rotating mandrel (of diameter 5 cm) that rotated at 200 rpm to fabricate randomly orientated fibres and 4000 rpm to fabricate partially aligned fibres. The nanofibrous scaffolds were then dried in a vacuum oven overnight at 30 °C and stored in a vacuum desiccator.