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Functional improvement following implantation of a microstructured, type-I collagen scaffold into experimental injuries of the adult rat spinal cord

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ABSTRACT

The formation of cystic cavitation following severe spinal cord injury (SCI) constitutes one of the major barriers to successful axonal regeneration and tissue repair. The development of bioengineered scaffolds that assist in the bridging of such lesion-induced gaps may contribute to the formulation of combination strategies aimed at promoting functional tissue repair. Our previous in vitro investigations have demonstrated the directed axon regeneration and glial migration supporting properties of microstructured collagen scaffold that had been engineered to possess mechanical properties similar to those of spinal cord tissues. Here, the effect of implanting the longitudinally orientated scaffold into unilateral resection injuries (2 mm long) of the mid-cervical lateral funiculus of adult rats has been investigated using behavioural and correlative morphological techniques. The resection injuries caused an immediate and long lasting (up to 12 weeks post injury) deficit of food pellet retrieval by the ipsilateral forepaw. Implantation of the orientated collagen scaffold promoted a significant improvement in pellet retrieval by the ipsilateral forepaw at 6 weeks which continued to improve up to 12 weeks post injury. In contrast, implantation of a non-orientated gelatine scaffold did not result in significant functional improvement. Surprisingly, the improved motor performance was not correlated with the regeneration of

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lesioned axons through the implanted scaffold. This observation supports the notion that biomaterials may support functional recovery by mechanisms other than simple bridging of the lesion site, such as the local sprouting of injured, or even non-injured fibres.

1. Introduction

Severe SCI results in the loss of motor, sensory and autonomic function, largely due to the disconnection of projection neurons from their target neurons (Schwab and Bartholdi, 1996). The dramatic changes at the lesion site following acute SCI include the development of molecular and physical barriers (scarring), as well as the formation of cystic cavitations, resulting in abortive sprouting by long distance projection neurons (Eng, 1985; Fawcett and Asher, 1999; Hagg and Oudega, 2006; Hall and Springer, 2004; Morgenstern et al., 2002; Profyris et al., 2004). The presence of large cystic cavities, combined with the loss of the normal orientated geometry of spinal cord white matter tracts at the lesion site have been reported to be detrimental to axonal regeneration (Pettigrew and Crutcher, 1999; Pettigrew et al., 2001).

Although a large number of experimental strategies have been developed that are capable of inducing some degree of functional recovery (Bauchet et al., 2009; Deumens et al., 2005; Schwab et al., 2006; Thuret et al., 2006), it is likely that the most effective treatment will involve a combination of approaches (Lu and Tuszynski, 2008), including the implantation of a scaffold that is capable of restoring the normal orientated geometry of the damaged fibre tracts. A vast array of engineered materials (based on either natural or synthetic polymers) has been developed to act as scaffolds that are capable of supporting axon regeneration (Führmann, 2011; Schmidt and Leach, 2003), however, the ideal bridging material has yet to be identified.

Collagens have proved to be popular natural polymers in bioengineering due to their biocompatibility, non-toxic degradation products, and the relative ease of controlling their shape and structure (Burke et al., 1981; Pachence, 1996; Schoof et al., 2001; Yannas et al., 1982). Over recent years, our in vitro studies have demonstrated the cytocompatibility and axon growth promoting properties of an orientated microstructured porcine type-I collagen scaffold. The scaffold has been demonstrated to support cell attachment, proliferation and orientated migration of a range of central- and peripheral nervous system-related glia, including astrocytes, olfactory ensheathing cells, Schwann cells as well as microglia and macrophages. Furthermore, orientated axonal growth has been demonstrated by sensory neurons from dorsal root ganglia (DRG), by spinal cord ventral horn motoneurons and by differentiated human neuroblastoma cell line (SH-SY5Y) (Bozkurt et al., 2007, 2009; Gerardo-Nava et al., 2014; Mollers et al., 2009). The framework of longitudinally orientated channels of the hydrophilic scaffold with visco-elastic properties similar to those of mammalian spinal cord (Ozawa et al., 2001; Tunturi, 1978) are widely regarded as ideal physical characteristics for the promotion of graft-host integration (e.g. (Saglam et al., 2013)) and tissue repair following traumatic injury.

The use of such collagen in bridging materials in experimental models of SCI (as well as peripheral nerve injury) has taken various forms, including hollow conduits, sponge-filled conduits, gels, extruded filaments and orientated microporous scaffolds (Bozkurt et al., 2012; Cholas et al., 2012a, 2012b; Deumens et al., 2010; Joosten et al., 1995; Marchand et al., 1993; Yoshii et al., 2003a, 2003b). The hollow collagen conduits and the orientated microporous scaffolds have proven successful in supporting axon regeneration across 10–20 mm gaps of the lesioned PNS (Bozkurt et al., 2012; Chamberlain et al., 1998). Similarly structured scaffolds composed of a mixture of collagen (either bovine or porcine type-I collagen) and glycosaminoglycan (chondroitin sulphate proteoglycan) have been used in attempts to promote functional tissue repair following implantation into 5 mm full resection injuries of the adult rat thoracic spinal cord (Cholas et al., 2012a, 2012b), or into hemi-resection injuries of the thoracic spinal cord (Cholas et al., 2012a). Using a subjective (modified Tarlov) behavioural test, a small but statistically significant improvement of function was noted for the implantation of the naïve scaffolds, as well as those associated with chondroitinase ABC or with mesenchymal stromal cells in the hemi-resection model of SCI, but not in the complete spinal cord transection model (Cholas et al., 2012a, 2012b; Joosten et al., 1995). Our own attempts to implant cell seeded- or naïve orientated collagen scaffolds into low thoracic hemi-resection injuries of the adult rat spinal cord had demonstrated no improvement (Deumens et al., 2013). However, our earlier observations and those of others have suggested that the hemi-resection model at low thoracic spinal cord levels may not be the ideal experimental model for biomaterial-based bridging strategies: local inflammation and distortion of the implanted scaffold may lead to reduced graft-host integration (Deumens et al., 2013), also the natural tendency for plastic reorganisation of spared or uninjured populations of descending axons (observed after partial thoracic SCI) may confound the search for axon regeneration-mediated functional recovery (Ballermann and Fouad, 2006; Fouad and Tse, 2008).

The present investigation focuses on the implantation of a longitudinally orientated microstructured collagen scaffold into a unilateral 2 mm resection injury of the lateral funiculus at the mid-cervical level of the adult rat spinal cord. An objective, quantitative approach to assess behavioural recovery was performed by determining the lesion-induced deficits in food pellet retrieval of the ipsilateral forepaw, revealing a progressive, implant-mediated improvement in performance over a period of 3 month. No such recovery was noted in the control groups that were subjected to a lesion without implantation, or were implanted with a non-orientated gelatine sponge.