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The effect of intraluminal contact mediated guidance signals on axonal mismatch during peripheral nerve repair

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ABSTRACT

The current microsurgical gold standard for repairing long gap nerve injuries is the autograft. Autograft provides a protective environment for repair and a natural internal architecture, which is essential for regeneration. Current clinically approved hollow nerve guidance conduits allow provision of this protective environment; however they fail to provide an essential internal architecture to the regenerating nerve. In the present study both structured and unstructured intraluminal collagen fibres are investigated to assess their ability to enhance conduit mediated nerve repair. This study presents a direct comparison of both structured and unstructured fibres *in vivo*. The addition of intraluminal guidance structures was shown to significantly decrease axonal dispersion within the conduit and reduced axonal mismatch of distal nerve targets ($p < 0.05$). The intraluminal fibres were shown to be successfully incorporated into the host regenerative process, acting as a platform for Schwann cell migration and axonal regeneration. Ultimately the fibres were able to provide a platform for nerve regeneration in a long term regeneration study (16 weeks) and facilitated increased guidance of regenerating axons towards their distal nerve targets.

1. Introduction

Treatment of peripheral nerve injuries is currently limited to a small number of microsurgical treatment methods. These treatments are ineffective as these interventions often lead to painful neuropathies as a result of loss in motor control and sensory perception [1,2]. Over relatively short nerve gaps, spontaneous natural regeneration may occur. However, over larger gaps, microsurgical repair is essential for nerve regeneration [3–5]. The primary methods of repair include direct/primary repair, transplantation of autografts or allografts, and the use of hollow nerve guidance conduits [6]. Direct repair is limited to treating short nerve defects (<5 mm in length) and requires tension-free suturing of the damaged nerves [7,8].

Beyond this relatively short gap, autografting is the current gold standard for repair. Despite providing a number of advantages for repair, success in the clinic has been limited. This is partly due to a number of the intrinsic limitations of the gold standard, particularly nerve and axonal size mismatches between the donor nerve and the targeted injured nerve [6,9]. In addition there is a requirement for a second surgical site i.e. donor site that has a limited supply of nerve. Donor site complications often lead to morbidity and pain. Despite these limitations, autograft healing is characterised by some intrinsic ideal components for peripheral nerve repair. Autograft provides a natural architecture for guided nerve regeneration, as well as being readily incorporated into the regenerative process; due to the graft being derived from the host's own tissue. The intrinsic network of extracellular matrix proteins and cell adhesion molecules provides regenerating axons, and migrating and proliferating Schwann cells (from the proximal and distal nerve stumps) with appropriate topographical and biological guidance cues to achieve functional nerve regeneration [10]. To recreate this natural internal architecture for repair an extracellular matrix derived platform (i.e. one made from collagen, fibronectin or laminin) often proves useful for repair [11–13].

In efforts to reconstruct this architecture this study proposes the use of a natural extracellular matrix protein (i.e. collagen) based construct which displays topographical features and structural characteristics which are beneficial for repair. This investigation

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incorporates such a system in the form of intraluminal collagen fibres into the lumen of a hollow nerve guidance conduit. Hollow nerve guidance conduits are the commercial alternatives to grafting and have a number of advantages for repair. These advantages include limiting myofibroblast infiltration, a reduction in scar and neuroma formation, and no associated donor pain [14]. However, hollow conduits provide only limited structural support (in the form of a fibrin cable) and guidance to regenerating axons and as a result functional recovery remains poor [6,8,15]. To overcome the limitations of the use of hollow conduits, intraluminal guidance structures (e.g. fibres, films, gels) are used to supplement/replace the fibrin cable and/or to recreate the natural topographical features of autograft [12,16–20]. This study uses intraluminal biologically derived collagen fibres for such a purpose. Ultimately, the addition of these extracellular matrix (ECM) derived components aims to recreate the hierarchical organisation and biological function of the native extracellular matrix. This investigation thoroughly analyses the incorporation of these components into the host regenerative process and assesses their feasibility for improving or enhancing conduit mediated nerve repair. In particular this study investigates the influence of intraluminal fibres on the levels of axonal dispersion within the biomaterial conduit.

In addition this study incorporates a number of longitudinal grooves on the surface of the collagen fibres for additional topographical guidance (Fig. 1). The use of topographical guidance cues has shown to have beneficial effects for nerve repair. Topographical cues have been shown to introduce complex signalling responses within the growth cone of a regenerating and have resulted in profound changes in the neuronal response to injury [21]. Structural cues have been incorporated on a number of substrates *in vitro* resulting in a significant increase in aligned neurite outgrowth [21–23]. This increase in neurite alignment and growth is accompanied by a significant reduction in the number of neurites and neurite branching. Mahoney et al. demonstrated increased alignment and growth of PC12 cells on micro-structured polyimide for features in the range of 20–30 μm versus features which had a width of 40–60 μm [24]. Preliminary work by our own group demonstrated increased aligned growth of PC12 neurites on micro-structured PLGA film with features ranging from 5 to 10 μm in width [25]. Based on these results, topographical guidance features have been combined with the aforementioned intraluminal guidance structures in the form of structured collagen fibres to assess their combined ability to enhance peripheral nerve repair.

2. Materials and methods

2.1. Extrusion and crosslinking of collagen fibres

The extrusion procedure and crosslinking were carried out in the same manner as reported by Zeugolis et al. [26,27]. A 5 ml syringe (BD Scientific, UK) containing 5 mg/ml bovine type I atellocollagen was extruded at a rate of 0.3 ml/min by a syringe pump (KD-Scientific 200, KD-Scientific Inc., Massachusetts, USA) through 0.03 mm inner diameter silicone tubing (Polymer Technologies Ltd, Warwickshire, UK) into a fibre formation buffer (118 mM phosphate buffer and 20% of polyethylene glycol, M_w 8000, pH 7.50 and 37 °C). Fibres were allowed to remain in the fibre formation buffer for 5 min and transferred into a fibre incubation buffer (6.0 mM phosphate buffer and 75 mM sodium chloride; pH 7.10 and 37 °C) for a further 5 min.

The extruded collagen fibres were subsequently cross-linked with N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) and N-hydroxysuccinmide (NHS), at a ratio of 30 mM:10 mM respectively, in 50 mM MES buffer (pH 5.5), for 24 h. After this period, the fibres were rinsed three times with sterile distilled water and allowed to air-dry, under the tension of their own weight. Similarly, for non-cross-linked collagen fibres, fibres were briefly dipped in distilled water for

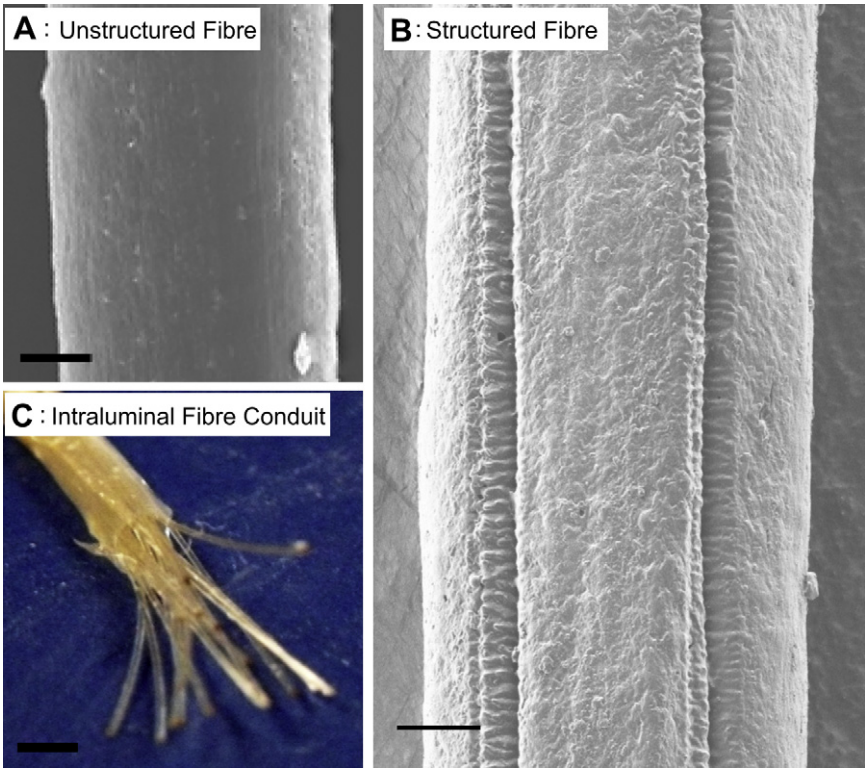

Fig. 1. Images of unstructured and structured collagen fibres and their incorporation into a hollow collagen nerve guidance conduit. (A) SEM image of an unstructured collagen fibre with a diameter of approximately 50 μm. Scale bar, 10 μm. (B) SEM image of structured collagen fibre with four channels on the surface of the fibres (channel diameter 10 μm). Scale bar, 10 μm. (C) Photo of a conduit with 18 structured collagen fibres inserted into the lumen of a hollow collagen nerve guidance conduit. Fibres are shown to be protruding from the lumen of the conduit. For implantation, fibres are trimmed to a length of 10 mm and inserted into a 12 mm long hollow nerve guidance conduit. This allows the proximal and distal nerves to be implanted into the conduit without inducing axial compression on the intraluminal fibres. Scale bar, 1.5 mm.