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Research Paper

Decellularized grafts with axially aligned channels for peripheral nerve regeneration

Rukmani Sridharan[a,b,c], *Richard B. Reilly*[a,b,d], *Conor T. Buckley*[a,b,*]

[a]*Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland*
[b]*School of Engineering, Trinity College Dublin, Dublin, Ireland*
[c]*Tissue Engineering Research Group, Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland*
[d]*School of Medicine, Trinity College Dublin, Dublin, Ireland*

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ABSTRACT

At least 2 million people worldwide suffer annually from peripheral nerve injuries (PNI), with estimated costs of $7 billion incurred due to paralysis alone. The current "gold" standard for treatment of PNI is the autograft, which poses disadvantages such as high fiscal cost, possible loss of sensation at donor site and the requirement of two surgeries. Allografts are viable alternatives; however, intensive immunosuppressive treatments are often necessary to prevent host rejection. For this reason, significant efforts have been made to remove cellular material from allografts. These decellularized nerve grafts perform better than other clinically available grafts but not as well as autografts; therefore, current research on these grafts includes the incorporation of additional components such as growth factors and cells to provide chemical guidance to regenerating axons. However, effective cellular and axonal penetration is not achieved due to the small pore size (5–10 μm) of the decellularized grafts. The overall objective of this study was to induce axially aligned channels in decellularized nerve grafts to facilitate enhanced cell penetration. The specific aims of this study were to optimize a decellularization method to enhance cellular removal, to induce axially aligned pore formation in decellularized grafts through a novel unidirectional freeze drying method, to study the bulk mechanical properties of these modified decellularized grafts and to assess cell penetration into these grafts. To this end we modified an existing decellularization protocol to improve cellular removal while preserving matrix structure in rat sciatic nerve sections. Standard freeze drying and unidirectional freeze drying were employed to impart the necessary pore architecture, and our results suggest that unidirectional freezing is a pertinent modification to the freeze drying process to obtain axially aligned channels. These highly porous scaffolds obtained using unidirectional freeze-drying possessed similar tensile properties to native nerve tissue and exhibited enhanced cellular penetration after 14 days of culture when compared

Abbreviations: CPD, critical point drying; ECM, extracellular matrix; FBS, fetal bovine serum; H&E, hematoxylin and eosin; HYP, hydroxyproline; MSC, mesenchymal stem cell; NGC, nerve guidance conduit; NGF, nerve growth factor; PBS, phosphate buffered saline; PCL, poly caprolactone; PDMS, poly(dimethyl siloxane); PFA, para-formaldehyde; PGA, poly glycolic acid; PNI, peripheral nerve regeneration; PSR, picrosirius red; SEM, scanning electron micrography; sGAG, sulfated glycosaminoglycans

*Corresponding author at: Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland. Tel.: +353 1 896 2061; fax: +353 1 679 5554.
E-mail address: conor.buckley@tcd.ie (C.T. Buckley).

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to non-freeze dried and standard freeze-dried scaffolds. The results of this study not only highlight the importance of aligned pores of diameters ~20–60 μm on cellular infiltration, but also presents unidirectional freeze drying as a viable technique for producing this required architecture in decellularized nerves. To the best of our knowledge, this study represents the first attempt to manipulate the physical structure of decellularized nerves to enhance cell penetration which may serve as a basis for future peripheral nerve regenerative strategies using decellularized allografts.

1. Introduction

The current "gold" standard for treatment of peripheral nerve injuries (PNI), the autograft, poses several disadvantages such as high financial cost, morbidity at donor site and the requirement of two surgeries (Noble et al., 1998). Moreover, there is a frequent size mismatch of donor nerves and a low success rate of only 50%, with higher rates of failure for patients above 50 years and for defects longer than 7 cm (Lee and Wolfe, 2000). For the past two decades, several biomaterials have been developed to facilitate accelerated repair and better axon regeneration (Belkas et al., 2004; Biazar et al., 2010; Chang et al., 2007; Daly et al., 2012). Much progress has been made in identifying important characteristics necessary for success of a biomaterial as an autograft replacement and in understanding the interactions of growing axons within these biomaterials; however, the regenerative levels of the autograft remain unmatched and effective nerve regeneration is still an urgent clinical need (Gu et al., 2011; Rajaram et al., 2012).

Characteristics of an ideal nerve guidance conduit (NGC) include biocompatibility, biodegradability, semi-permeability, the existence of longitudinal channels to promote axon regeneration, the presence of necessary guidance cues from extracellular matrix (ECM)-like components in addition to long term storage and ease of handling/suturing (Deumens et al., 2010; Kehoe et al., 2012; Spivey et al., 2012). Although many natural (e.g. collagen) (Kemp et al., 2009; Li et al., 1991) and synthetic (polycaprolactone (PCL), chitosan, polyglycolic acid (PGA)) (Jansen et al., 2004; Lee et al., 2012; Meek and Jansen, 2009; Yao et al., 2009) conduits have been developed in an attempt to replace the requirement for autografts, functional recovery that matches autograft levels is seldom observed (Kehoe et al., 2012). Several studies have reported that decellularized allografts have consistently performed better than other commercially available grafts, which is most likely due to these grafts possessing the necessary architecture and guidance cues from nerve ECM components to promote axon regeneration (Karabekmez et al., 2009; Kehoe et al., 2012; Whitlock et al., 2009). A majority of protocols that decellularize nerve grafts are based on those developed by Sondell et al. (1998) and Hudson et al. (2004b) that predominantly use chemical decellularization agents. It has been shown for other tissues that enzymatic treatment with nucleases (Crapo et al., 2011; Mangold et al., 2012; Yang et al., 2010) enhances removal of immunogenic components from decellularized nerves. Hence, our first aim was to test the effects of an optimized decellularization process using chemical and enzymatic reagents on the biochemical components and nerve architecture of rat sciatic nerve sections.

While decellularized grafts perform better than other commercially available grafts, the clinical requirement is for a graft that can perform better or equal to that of an autograft to enhance regenerative levels and reduce the time taken for complete functional recovery (Spivey et al., 2012). To fulfill this requirement, additional factors have been incorporated into decellularized grafts such as cells (e.g. Schwann cells and Mesenchymal stem cells (MSC)) (Wang et al., 2008, 2012; Zhang et al., 2010), growth factors (Hagg et al., 1991; Li et al., 2012) and gels (Zhao et al., 2012). However, current decellularized grafts are limited due to the size of the basal laminal tubes (5–10 μm), which are smaller than the typical diameter of cells (10–15 μm). Moreover, while small endoneurial tubes are optimal for protecting fully grown axons, they are less ideal for regenerating axons. It has also been previously shown in a rat model that motor grafts lead to higher nerve fiber count and better recovery than sensory grafts in part due to the larger diameter of endoneurial tubes in motor nerves (Moradzadeh et al., 2008).

Previous studies using different biomaterials such as alginate, chitosan and collagen have investigated the effects of subcellular topography on axon regeneration and shown that medium sized pores (20–60 μm) along the longitudinal direction were optimal for maximum axon penetration while allowing for minimum axon misdirection (Bozkurt et al., 2009; Pawar et al., 2011). Whether such large uniform channels can be formed in decellularized nerves has not been demonstrated to date. With these questions in mind, the overall objective of this study was to modify the physical structure of decellularized rat nerves by introducing large axially aligned channels to assist in enhanced cellular penetration. We hypothesized that unidirectional freezing (which has been established as a method to produce aligned pore structures in synthetic polymers and collagen (Bozkurt et al., 2009; Hu et al., 2009)) can induce the formation of such channels. Specifically, the aims of this work were to first optimize a decellularization method to enhance cellular removal, to induce the formation of axially aligned channels in these decellularized grafts using unidirectional freeze-drying, to characterize the formation of channels and bulk mechanical properties and finally to assess cell penetration into these grafts and compare them to decellularized grafts without channels. To the best of our knowledge this present work represents the first attempt to manipulate the physical structure of decellularized nerves containing large, axially aligned and open channels.