Biomaterials 74 (2016) 89–98

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Functionalized composite scaffolds improve the engraftment of transplanted dopaminergic progenitors in a mouse model of Parkinson's disease

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ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form
25 September 2015
Accepted 26 September 2015
Available online 30 September 2015

Keywords:
Neural transplantation
Hydrogel
Xyloglucan
Electrospinning
PLLA
Glial derived neurotropic factor
GDNF
Dopamine
Parkinson's disease

ABSTRACT

With the brain's limited capacity for repair there is a need for new and innovative therapies to promote regeneration. Stem/progenitor cell transplantation has received increasing attention, and whilst clinical trials demonstrating functional integration exist, inherent variability between patients has hindered development of this therapy. Variable outcomes have largely been attributed to poor survival and insufficient reinnervation of target tissues due in part to the suboptimal host environment. Here we examined whether improving the physical properties of the host milieu, by way of bioengineered scaffolds, may enhance engraftment. We developed a composite scaffold, incorporating electrospun poly(L-lactic acid) short nanofibers embedded within a thermo-responsive xyloglucan hydrogel, which could be easily injected into the injured brain. Furthermore, to improve the trophic properties of the host brain, glial derived neurotrophic factor (GDNF), a protein known to promote cell survival and axonal growth, was blended into and/or covalently attached onto the composite scaffolds to provide controlled delivery. *In vitro* we confirmed the ability of the scaffolds to support ventral midbrain (VM) dopamine progenitors, and provide sustained delivery of GDNF – capable of eliciting effects on cell survival and dopaminergic axon growth. In Parkinsonian mice, we show that these composite scaffolds, whilst having no deleterious impact on the host immune response, enhanced the survival of VM grafts and reinnervation of the striatum, an effect that was augmented through the scaffold delivery of GDNF. Taken together, these functionalized composite scaffolds provide a means to significantly improve the milieu of the injured brain, enabling enhanced survival and integration of grafted neurons.

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1. Introduction

Damage to the central nervous system (CNS), as a consequence of disease or trauma, can have devastating consequences due to its limited capacity for repair. With a notable lack of therapies, the transplantation of stem cells and neural progenitors has received increasing attention. Proof of principle studies in animals and clinical trials have demonstrated that new neurons are capable of functionally integrating into the injured brain; however, hindering the development of these therapies has been the inherent variability in treatment outcomes. Key challenges facing neural transplants in the CNS have been variable graft survival, as well as inadequate integration and reinnervation of the host tissue [2,23]. In part, this has been attributed to the non-conducive environment of the adult brain – failing to provide adequate physical and trophic support for the graft, and highlighting the need for strategies to improve the host milieu.

Several lines of evidence support the importance of physical scaffolding for the growth and connectivity of new axons. In neural development, 'pioneer axons' have been observed to establish precise targeting and establish scaffolds along which later-developing axons grow [3,37]. Additionally, the presence of a number of extracellular matrix (ECM) and associated adhesion molecules in the developing brain provide critical anchoring to cells

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http://dx.doi.org/10.1016/j.biomaterials.2015.09.039
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within their appropriate location and support axonal extension [20,21]. Within the injured brain, founding work by Aguayo and colleagues demonstrated that CNS axons were capable of growing over distances in the adult brain when they were placed into the permissive environment of a peripheral nerve graft [9,38], work that was also demonstrated in the support of dopaminergic neural grafts [1], and complimented by studies using alternate support cells such as Schwann and olfactory ensheathing cells to enhance graft integration [18]. Furthermore, in more recent years, grafted neurons have been demonstrated to grow their axons along residual host axons within the designated pathway, or alternate axonal tracts, using these host axons as natural scaffolds [19,46]. Combined, this knowledge suggests that efforts to improve the physical support of grafted neurons could enhance integration.

One such strategy to optimize graft support is to engineer biomaterials for implantation [26]. In this regard, electrospun nanofibers provide some of the best examples of simulation of the brain's 3-dimensional (3D) *in vivo* environment—whereby scaffold fibers can support cell adhesion and axon extension. A number of our previous studies have demonstrated that electrospun scaffolds can influence cell proliferation, neural differentiation and axon extension *in vitro*, whilst also influencing survival and plasticity of both host- and grafted-derived neurons in the intact brain [16,28,30,31,33,50]. Despite these encouraging findings, the bulkiness of electrospun scaffolds renders them less attractive for repair of the brain and more appropriate for spinal cord or peripheral nerve injury where they can be functionalized and used to ensheath axon bundles. Consequently, increasing attention has been paid to the potential of hydrogels—particularly thermo-responsive gels that can be designed to (i) be liquid at 4 °C (enabling easy injection) yet rapidly form gels *in situ* at 37 °C and (ii) match the modulus of the host tissue [17,27,51]. This is appealing in the current context as cells can be mixed into the liquid prior to implantation. We have previously demonstrated that xyloglucan (polysaccharide) hydrogels can support the survival of neural cells *in vitro*, promote neurite growth of host neurons following implantation into the intact brain, and additionally suppress local reactive astrocytes [29,32]—all critical attributes for cell transplantation.

In addition to the physical support necessary for transplanted neurons, numerous studies have highlighted the capacity of trophic proteins and guidance cues to enhance graft survival and integration [10,19,24,35,46]. During embryonic development, establishment of neural circuits relies on the precise temporal and spatial expression of axonal growth and guidance cues. Following establishment of circuitry many of these cues are down regulated, with this loss of expression believed to be one of the major contributors to the adult brain's poor regenerative capacity. Re-expression of these trophic cues can greatly increase the survival and integration of residual host and newly transplanted neurons. This has been most notably demonstrated through the delivery of various neurotrophins known to promote neuronal survival and axonal plasticity [22,24]. However, to date, delivery of trophic proteins into the brain relies on implantation of cells that over-express the protein, invasive pumps for protein infusion *in situ*, or injection of viral constructs to induce local cells to produce proteins. Each of these approaches is clinically problematic and new efforts for providing a prolonged, yet controllable, trophic environment to promote the integration of grafted cells is required. Using biomaterials, we have recently developed methods to prolong the presentation of proteins *in vitro* and in the intact brain [16,49,50]. Covalent tethering of proteins onto biomaterials prolongs their presentation by preventing endocytosis. Previously we have demonstrated that prolonged presentation of brain derived neurotrophic factor (BDNF) or GDNF onto electrospun fibers resulted in improved survival, proliferation, differentiation, and neurite growth of cells *in vitro* and *in vivo* following implantation into the uninjured brain [16,49,50].

In recent years, a number of studies have examined the potential of biomaterials to deliver GDNF and/or cells in an effort to improve graft integration. This has included the use of various hydrogels to deliver stem cells including those over-expressing GDNF [15,52], as well as microcarriers/spheres to deliver GDNF with and without fetal progenitors [6,45]. Unfortunately, the success of these approaches have been largely suboptimal due to the bulkiness of the biomaterials together with cells, and duration of peak GDNF dose delivery. Resultant outcomes have positively described no change or reduced host inflammatory responses yet only modest increase in graft survival, and little to no improvement in graft plasticity.

In the present study, we sought to develop a more sophisticated composite scaffold, demonstrating the benefits of electrospun scaffolds delivered as short nanofibers within a thermo-sensitive xyloglucan hydrogel. These composite scaffolds were further functionalized to prolong the delivery of the GDNF, thereby providing an improved physical and trophic niche environment for the survival and engraftment of new neurons. As a feasibility and efficacy study, we elected to examine the potential of these biomaterials in a rodent model of Parkinson's disease, a neurodegenerative disorder characterized by the loss of dopamine neurons, that has shown the greatest progress in the field of neural transplantation [2,23].

2. Materials & methods

2.1. Preparation of xyloglucan and poly-L-lactide acid scaffolds

Poly(L-lactic acid) (PLLA) was obtained from Absorbable Polymers, Inc (AL, USA). A polymer solution of 10% (w/v) was prepared for electrospinning by dissolving the PLLA in 10 mL of acetone and chloroform (Merck Pty Ltd, Australia) at a ratio of 1:3 (v/v) with 1 mM Dodeoyltrimethyl-ammonium bromide (DTAB, Sigma). A custom built electrospinner consisting of a syringe pump (KD-100, KD Scientific, Holliston, USA) and an adjustable DC voltage power supply (Model RR 50–1.25R/230/DDPM, Gamma High Voltage Research, Ormond Beach, FL, USA) was used with a voltage of 22 kV and a 21 G needle. A flow rate of 1.0 mL/h, a working distance of 10 cm from a 13 cm diameter spinning mandrel rotating at 2000 rpm was employed. The collected PLLA scaffolds were dried in a vacuum oven (Labec) overnight at 30 °C. Aligned PLLA scaffolds were embedded in cryo-mounting media (TissueTek OCT, Fisher Scientific, USA), sectioned on a freezing microtome into short fibers ranging from 2 to 10 μm in length, and collected into deionized water (dH_2O). The short fibers were washed in dH_2O, ultrasonicated and centrifuged 5 times to remove residual OCT. PLLA short fibers for *in vitro* and *in vivo* use were aminolyzed in 0.5% (v/v) ethylenediamine (ED, Sigma–Aldrich, USA) in isopropanol (IPA, Caledon Laboratories, Canada) for 15 min at 20 °C. The inclusion of amine moieties on the surface of the PLLA was necessary for the subsequent attachment of the cross-linker sulfo-4-(N-maleimidomethyl) cyclohexane-1-carboxylic acid (SMCC) and GDNF tethering, described below.

Xyloglucan was prepared by enzymatic modification from tamarind seed xyloglucan, as previously described [43]. In brief, the xyloglucan was purified by dissolving 1 wt.% in dH_2O with a magnetic stirrer at 0–5 °C. The resultant solution was then precipitated out in 60% ethanol and washed with 60% ethanol through a sintered glass filter and flask, attached to a vacuum pump. An additional acetone wash was conducted. The precipitate was then dried at room temperature for two days in a vacuum oven.

Poly-D-Lysine (PDL) was coupled to xyloglucan as previously described [29]. Briefly, 54 mg poly-D-lysine (PDL) (Sigma–Aldrich) and 58 mg 4-azidoaniline (Sigma–Aldrich) were covalently bound

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