Acta Biomaterialia 38 (2016) 44–58

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Transplantable living scaffolds comprised of micro-tissue engineered aligned astrocyte networks to facilitate central nervous system regeneration

Carla C. Winter [a,b,c,1], Kritika S. Katiyar [a,c,d,1], Nicole S. Hernandez [a,e], Yeri J. Song [a,e], Laura A. Struzyna [a,b,c], James P. Harris [a,c], D. Kacy Cullen [a,c,e,*]

[a] Center for Brain Injury & Repair, Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States
[b] Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, United States
[c] Philadelphia Veterans Affairs Medical Center, Philadelphia, PA, United States
[d] School of Biomedical Engineering, Drexel University, Philadelphia, PA, United States
[e] Neuroscience Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 24 February 2016
Accepted 13 April 2016
Available online 29 April 2016

Keywords:
Tissue engineering
Living scaffold
Glial cell transplant
Biomaterials
Regeneration
Neurotrauma
Neurodegeneration
Traumatic brain injury
Cell migration
Axon pathfinding
Neural stem cells

ABSTRACT

Neurotrauma, stroke, and neurodegenerative disease may result in widespread loss of neural cells as well as the complex interconnectivity necessary for proper central nervous system function, generally resulting in permanent functional deficits. Potential regenerative strategies involve the recruitment of endogenous neural stem cells and/or directed axonal regeneration through the use of tissue engineered "living scaffolds" built to mimic features of three-dimensional (3-D) *in vivo* migratory or guidance pathways. Accordingly, we devised a novel biomaterial encasement scheme using tubular hydrogel-collagen micro-columns that facilitated the self-assembly of seeded astrocytes into 3-D living scaffolds consisting of long, cable-like aligned astrocytic networks. Here, robust astrocyte alignment was achieved within a micro-column inner diameter (ID) of 180 μm or 300–350 μm but not 1.0 mm, suggesting that radius of curvature dictated the extent of alignment. Moreover, within small ID micro-columns, >70% of the astrocytes assumed a bi-polar morphology, versus ~10% in larger micro-columns or planar surfaces. Cell–cell interactions also influenced the aligned architecture, as extensive astrocyte-collagen contraction was achieved at high (9–12 × 10^5 cells/mL) but not lower (2–6 × 10^5 cells/mL) seeding densities. This high density micro-column seeding led to the formation of ultra-dense 3-D "bundles" of aligned bi-polar astrocytes within collagen measuring up to 150 μm in diameter yet extending to a remarkable length of over 2.5 cm. Importantly, co-seeded neurons extended neurites directly along the aligned astrocytic bundles, demonstrating permissive cues for neurite extension. These transplantable cable-like astrocytic networks structurally mimic the glial tube that guides neuronal progenitor migration *in vivo* along the rostral migratory stream, and therefore may be useful to guide progenitor cells to repopulate sites of widespread neurodegeneration.

Statement of Significance

This manuscript details our development of novel micro-tissue engineering techniques to generate robust networks of longitudinally aligned astrocytes within transplantable micro-column hydrogels. We report a novel biomaterial encasement scheme that facilitated the self-assembly of seeded astrocytes into long, aligned regenerative pathways. These miniature "living scaffold" constructs physically emulate the glial tube – a pathway in the brain consisting of aligned astrocytes that guide the migration of neuronal progenitor cells – and therefore may facilitate directed neuronal migration for central nervous system repair. The small size and self-contained design of these aligned astrocyte constructs will permit minimally invasive transplantation in models of central nervous system injury in future studies.

* Corresponding author at: 105E Hayden Hall/3320 Smith Walk, Philadelphia, PA 19104, United States
E-mail address: dkacy@mail.med.upenn.edu (D.K. Cullen).
[1] The first two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.actbio.2016.04.021
1742-7061/

1. Introduction

Neurodegeneration due to disease, stroke, or trauma poses particularly challenging medical problems, often resulting in functional deficits that are frequently life altering and permanent due to the limited regenerative capacity of the central nervous system (CNS). For example, major CNS neurotrauma such as severe traumatic brain injury or spinal cord injury provokes multifaceted neurodegenerative cascades and complex cellular and molecular responses, potentially culminating in a significant necrotic zone surrounded by a glial scar [1–3]. The glial scar forms due to the proliferation and migration of various cell types such as microglia/macrophages, endothelial progenitors, and astrocytes, and initially plays a crucial role by sequestering acutely injured tissue from the surrounding penumbra to limit the extent of degeneration while aiding the restoration of the blood brain barrier (BBB) [2,4,5]. Beyond this initial phase, the chronic glial scar is primarily composed of tightly interwoven processes of reactive astrocytes, which also secrete growth-inhibiting molecules and extracellular matrix (ECM) molecules such as chondroitin sulfate proteoglycans (CSPGs) [2,6]. This dense meshwork of disorganized reactive astrocytes and proteoglycans is considered to be the primary physical and chemical barrier to CNS regeneration.

Restoring functionality following major CNS injury will require techniques to promote regeneration across an injury site, likely including both local neural cell replacement and reestablishment of afferent and efferent axonal tracts. In particular, various cell-based and biomaterial strategies have been pursued to promote regeneration and, in some cases, to overcome the inhibitory nature of the glial scar. For example, there have been notable efforts to directly transplant cells following spinal cord injury [7–10] and brain injury [11,12]. In addition, the use of template biomaterial scaffolds to enhance organization and regeneration across an injury site has been pursued [13,14]. Despite decades of research, however, there remains no effective treatment that can facilitate the reestablishment of neural cell populations and axonal regeneration across the glial scar.

Building on these efforts, neural tissue engineering offers tremendous promise to reestablish lost cellular structure and neural pathways by direct replacement and/or by assisting endogenous regeneration. Here, we are pursuing the creation of tissue engineered "living scaffolds" to facilitate nervous system regeneration [15–21]. Living scaffolds derive from a combination of biomaterial and cell-based techniques to create preformed constructs consisting of living cells in a defined, often anisotropic, three-dimensional (3-D) architecture [15,16]. Of note, the engineering of living constructs with defined 3-D cytoarchitecture is what differentiates this approach from more conventional "cell seeding" strategies. In general, living scaffolds are designed to mimic developmental and otherwise physiologically robust mechanisms to facilitate long-distance axonal pathfinding and reformation of complex neural tissue structure. Unlike conventional cellular or biomaterial approaches for neuroregeneration, living scaffolds can simultaneously provide defined direction-dependent structural and neurotrophic support to actively drive endogenous neural cell migration and axon regeneration, rather than simply being permissive substrates. Living scaffolds are also differentiated from acellular biomaterial approaches by providing a degree and mechanism(s) of engagement that may be actively modulated via signaling feedback from the host based on the extent and progression of regenerative processes over time [15,16].

The objective of the current study was to employ micro-tissue engineering techniques to create a new class of living scaffolds consisting of 3-D longitudinally aligned astrocytic networks contained within miniature transplantable hydrogel micro-columns. This work builds on our previous efforts to create a range of tissue engineered living scaffolds comprised of neurons and long axonal tracts to facilitate regeneration and functional recovery following nervous system trauma or degeneration [15–20,22,23]. Our long-term objective is to utilize engineered micro-tissue comprised of aligned astrocytic networks as a living scaffold to promote neuron migration and axon regeneration across mammalian glial scars (Fig. 1). The rationale for such micro-constructs is based on the capacity of astrocytes to serve as favorable substrates for neuron migration during development as well as the organized astrocytic framework that permits axon regeneration in nonmammalian glial scars. For instance, during development, precursor cells called radial glia serve as natural living scaffolds for neurodevelopment in both the brain and spinal cord by providing processes for immature neurons to physically attach to and migrate along [24–26]. Additionally, during postnatal development some radial glia mature into astrocytes to form longitudinally oriented microstructures called "glial tubes" that direct migrating neuroblasts in the rostral migratory stream (RMS) leading from the subventricular zone (SVZ) [27,28]. Further inspiration for an astrocyte-based living scaffold comes from the capacity of lesioned axons to naturally regenerate through the glial scar in nonmammalian vertebrates [29,30]. A critical feature of the nonmammalian glial scar is an organized cellular framework that guides regenerating axons into and through the glial scar, suggesting that at least some axons have the intrinsic ability to regenerate across a lesion provided there is a permissive, organized cellular environment [30]. These studies and developmental mechanisms suggest that tissue engineered living scaffolds comprised of aligned astrocytes could be used as favorable substrates for neuronal migration and axonal pathfinding, provided that the astrocytes present pro-regenerative structural and soluble cues.

The current studies build on our previously reported micro-tissue engineering techniques to create miniature transplantable micro-columns composed of tubular agarose hydrogels with a bioactive collagenous matrix interior. Here, we systematically tested the effects of micro-column biomaterial scheme (e.g. concentration, composition, and geometry) and cell seeding parameters (e.g. astrocyte density and neuronal presence) in order to achieve optimal 3-D aligned astrocyte network formation. These studies revealed that micro-column physical cues such as the radius of curvature dictated astrocyte morphology and the extent of alignment; whereas astrocyte seeding density determined the compaction of the resulting aligned networks. Moreover, co-seeding with neurons revealed permissiveness for neuronal adhesion and neurite extension, which occurred directly along the aligned astrocytes in the micro-constructs. This is the first report of living scaffolds consisting of dense, cable-like 3-D "bundles" of aligned bi-polar astrocytes measuring <150 μm in diameter yet extending to a remarkable length of over 2.5 cm. Although beyond the scope of the current manuscript, future studies will assess the neuroregenerative efficacy of this strategy, whereby we predict that the small size and self-contained design of these cable-like aligned astrocytic networks will permit minimally invasive transplantation to orchestrate neural progenitor migration and/or axonal pathfinding in models of CNS injury.

2. Materials and methods

2.1. Hydrogel micro-column fabrication

All supplies were from Invitrogen (Carlsbad, CA), BD Biosciences (San Jose, CA), or Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Three-dimensional hydrogel micro-columns were designed and fabricated to induce alignment of astrocytes within the central