Biomaterials 142 (2017) 31–40

Contents lists available at ScienceDirect

Biomaterials

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

Electrical preconditioning of stem cells with a conductive polymer scaffold enhances stroke recovery

Paul M. George [a, b, c, **], Tonya M. Bliss [b, c], Thuy Hua [b, c], Alex Lee [d], Byeongtaek Oh [a], Alexa Levinson [a], Swapnil Mehta [b], Guohua Sun [b, c], Gary K. Steinberg [a, b, c, *]

[a] Department of Neurology, Stanford University School of Medicine, Stanford, CA, USA
[b] Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA, USA
[c] Stanford Stroke Center and Stanford University School of Medicine, Stanford, CA, USA
[d] Department of Psychiatry, Stanford University School of Medicine, Stanford, CA, USA

ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form
3 July 2017
Accepted 10 July 2017
Available online 12 July 2017

Keywords:
Conductive polymer
Stroke recovery
Neural stem cell
Electrical stimulation
Tissue engineering
Cell transplantation

ABSTRACT

Exogenous human neural progenitor cells (hNPCs) are promising stroke therapeutics, but optimal delivery conditions and exact recovery mechanisms remain elusive. To further elucidate repair processes and improve stroke outcomes, we developed an electrically conductive, polymer scaffold for hNPC delivery. Electrical stimulation of hNPCs alters their transcriptome including changes to the VEGF-A pathway and genes involved in cell survival, inflammatory response, and synaptic remodeling. In our experiments, exogenous hNPCs were electrically stimulated (electrically preconditioned) via the scaffold 1 day prior to implantation. After *in vitro* stimulation, hNPCs on the scaffold are transplanted intracranially in a distal middle cerebral artery occlusion rat model. Electrically preconditioned hNPCs improved functional outcomes compared to unstimulated hNPCs or hNPCs where VEGF-A was blocked during *in vitro* electrical preconditioning. The ability to manipulate hNPCs via a conductive scaffold creates a new approach to optimize stem cell-based therapy and determine which factors (such as VEGF-A) are essential for stroke recovery.

1. Introduction

Stroke remains a leading cause of morbidity and long-term disability [1]. While acute stroke treatments exist within a narrow time window, no approved medical therapies for stroke recovery are available [2,3]. Stem cells have emerged as a potential stroke therapeutic. Human neural progenitor cells (hNPCs) are a type of stem cell derived from embryonic cells to have a neural fate [4,5]. The critical mechanisms of action and optimal delivery methods of stem cells required for efficacy remain incompletely understood. The current thinking is that exogenous hNPCs likely improve functional outcomes through neurotrophic effects of secreted factors that increase synapse formation, angiogenesis, dendritic branching and new axonal projections, as well as modulating the immune system [6–9]. However, the precise molecular details remain to be elucidated.

Biomaterials offer a unique method to interact with stem cells and manipulate their properties. Biopolymers have provided protection for stem cells implanted into the harsh stroke milieu and increased survival [10,11]. Because previously studied polymers are not responsive to external stimuli (eg electrical stimulation), the environment is controlled by inherent properties of the polymer alone. Conductive polymers, on the other hand, provide a platform to interact with stem cells through electrical stimulation [12]. Unlike inert polymer scaffolds, conductive scaffolds allow for manipulation of the stem cells after seeding of cells on the scaffold. Electrical fields influence differentiation, ion channel density, and neurite outgrowth of stem cells and other cell types [13–15]. The effect of this stimulation on subsequent stem cell performance remains unexplored.

To allow for greater control and understanding of the optimal conditions for stem cell-enhanced stroke recovery, we have derived

* Corresponding author. Department of Neurosurgery, Stanford University School of Medicine, 300 Pasteur Drive R 281, Stanford, CA 94305-5327, USA.
** Corresponding author. Department of Neurology and Neurological Sciences, Stanford University School of Medicine, 300 Pasteur Drive MC5778, Stanford, CA 94305-5778, USA.
E-mail addresses: pgeorge1@stanford.edu (P.M. George), gsteinberg@stanford.edu (G.K. Steinberg).

http://dx.doi.org/10.1016/j.biomaterials.2017.07.020
0142-9612/

a scaffold made of the conductive polymer, polypyrrole (PPy), which has advantageous mechanical and conductive properties for neural implantation [16]. The conductive scaffold allows for *in vitro* electrical stimulation and subsequent implantation of hNPCs onto the peri-infarct cortex while on the scaffold. For this study, we electrically preconditioned hNPCs on the scaffold with a short period of electrical stimulation prior to implantation onto the cortical surface. Subsequently, the conductive scaffold carrying the hNPCs is removed from the cell chamber system and implanted intracranially using a minimally invasive method of simply placing the scaffold on the brain surface of stroke-injured rats. Using RNA sequencing (RNAseq) analysis we investigated changes in gene expression in the hNPCs induced by electrical stimulation and examined how the host rat brain responded to the stimulated hNPCs, to explore the molecular pathways of hNPC-induced post-stroke recovery. Furthermore, our results show that these electrically preconditioned hNPCs, with this novel transplantation paradigm, improve post-stroke neurologic function.

2. Materials and methods

2.1. Fabrication of the conductive scaffold system

PPy (Sigma-Aldrich, St. Louis, MO) was electroplated onto indium tin oxide (ITO) slides (Delta Technologies, Loveland, CO) as described previously [16]. After removal from the ITO, the conductive scaffold was clamped between pieces of polydimethylsiloxane (PDMS; Sylgard, Dow, Auburn, MI) with a chamber slide forming cell chambers (Lab-Tek, Thermo Fisher, Waltham, MA; Fig. 1A). Wires were attached to the conductive scaffold outside of the chambers. For implantation, the cell chambers and PDMS were unclamped and separated from the conductive scaffold. Wires were also removed from the conductive scaffold prior to implantation. The dimensions of the implanted scaffolds were approximately 1 × 3 × 0.25 mm.

2.2. In vitro hNPC electrical stimulation

All stem cell procedures were approved by Stanford's Stem Cell Research Oversight committee. As previously described [17], hNPCs, passages 17–22, were used in these experiments and kept in DMEM-F12 media with 2% B27 and 1% N2 supplements along with LIF (10 μg/ml), EGF (20 μg/ml), and βFGF (10 ng/ml, all Invitrogen, Waltham, MA except for EGF and LIF from Millipore, Darmstadt, Germany). Briefly, the hNPCs were originally derived from the H9 human embryonic stem cell line (WiCell Research Institute). These cells were differentiated into hNPCs using serum free medium containing EGF, bFGF, and LIF. Cells were harvested from spheres that formed over multiple passages and upon passage 5–6 spheres were dissociated into a single cell suspension using trypsin-EDTA to form monolayers. In our previous work, these cells were further characterized to show that if the mitogenic factors were withheld, the hNPCs could differentiate into neurons, astrocytes, and oligodendrocytes (immunostaining at 10 days: Tuj1 62.5 ± 2.8%, Nestin 36.6 ± 2.7%, GFAP 1.9 ± 0.3%, and galactocerebrocide for oligodendrocytes 7.1 ± 0.4%) [17]. hNPCs were plated onto the PPy scaffold on Day 1 (125,000 cells/cm^2). On Day 2, media was changed for both electrically preconditioned and non-stimulated cell groups. Electrically preconditioned cells received a +1 V to −1 V square wave at 1 kHz for 1 h. The current was delivered through the PPy scaffold with wires attached to either side of the PPy scaffold outside of the cell chamber. For the bevacizumab (Avastin®, Genentech, San Francisco, CA) groups, bevacizumab was added to the media (0.5 mg/ml) 1 h before stimulation with the media changed on Day 2. The animals did not receive any bevacizumab. On Day 3, cells and supernatant were collected for analysis, or the PPy scaffold with or without hNPCs was washed with phosphate buffered saline (PBS) and implanted. The PPy scaffold-alone control samples were treated the same as the stimulated implants. For analysis of the duration of VEGF-A upregulation, cells were sampled on Day 5 and Day 7 for qPCR analysis.

2.3. In vitro immunostaining

In vitro immunostaining was performed on Day 3. Cell survival was determined by a Live/Dead kit (Life Technologies, Waltham, MA). Four random, representative 0.34 mm × 0.45 mm areas were analyzed, and alive and dead cells on the conductive scaffold were counted by a blinded-individual with results averaged across the four areas (cells/mm^2).

Cell differentiation was assessed with nestin, neuronal, glial, and oligodendrocyte markers. Primary antibodies were anti-Nestin (1:1000, Cat. ABD69, Millipore), anti βIII-tubulin (1:500, Neuromics, Edina, MN), anti-glial antifibrillary protein GFAP (1:500, Abcam, Cambridge, United Kingdom), and Anti-NG2 (1:500, Invitrogen). Secondary antibodies were from Life Technologies and DAPI (1:1000, Sigma-Aldrich). Four random, representative 0.34 mm × 0.45 mm areas were analyzed, and a blinded individual counted total cell, glial cell, and neural cell markers.

2.4. RNA − seq

In vitro preconditioned and unstimulated hNPC cDNA was isolated 24 h following electrical stimulation as described above ($n = 4$ per group). Peri-infarct rat cortical tissue that was implanted with

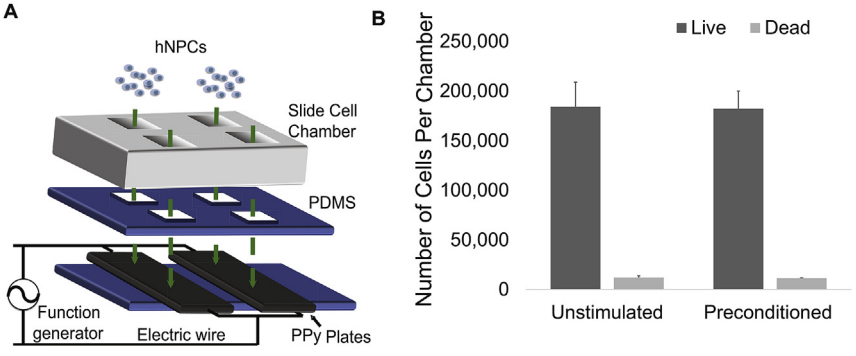

Fig. 1. In vitro PPy hNPC scaffold system for electrical stimulation. (A) Conductive scaffold system with hNPCs plating (PDMS, polydimethylsiloxane). (B) Live/dead assay results showing average number of living and dead cells (error bars show SE, n = 4, two-tailed Student *t*-test).