Priming Neural Stem Cell Transplants to Dynamically Respond to Brain Injury Induced SDF-1α Gradients

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Statement of Purpose: Traumatic brain injury (TBI) afflicts over 1.7 million Americans annually and its associated costs create a $77 billion strain on our healthcare system and economy[1]. The primary, mechanical insult of TBI results in a secondary, biochemical insult that is largely responsible for long-term deficient associated with TBI (e.g. chronic traumatic encephalopathy, motor impairment)[2]; however, no clinical treatments directly target the underlying pathologies of TBI. The potential of pre-clinical neural progenitor/stem cell (NPSC) transplantation has not yet been realized due to low cell viability and engraftment[3]. However, despite the many cytotoxic signals of the secondary injury, the local activated glia release the chemokine stromal cell derived factor 1-α (SDF-1α), which promotes chemotactic migration of endogenous NPSCs through interaction with its receptor, CXCR4[4]. Therefore, NPSC responsiveness to SDF-1α may be improved through upregulation of CXCR4, enabling transplants to dynamically respond to injury-relevant signals. CXCR4 upregulation has been observed in other cell types as a result of interaction with the proteoglycan hyaluronic acid (HA)[5]. Therefore, we propose that HA hydrogels will promote NPSC CXCR4 overexpression. However, it is well known that HA does not promote adhesion and migration (behaviors critical to engraftment) as effectively as other extracellular matrix proteins. Previously, our lab found crosstalk between CXCR4 and laminin (Lm) synergistically increases NPSC migration[6]. Therefore, we hypothesize that a dual-purpose HA-Lm hydrogel will 1) increase NPSC responsiveness to SDF-1α and 2) provide an infrastructure that promotes NPSC migration to ultimately enhance neural transplant engraftment.

Methods: Our HA hydrogel system is based on thiol chemistry and uses poly(ethylene glycol)-divinyl sulfone (PEG-DVS) as its crosslinker. Laminin was chemically tethered via free thiols interacting with PEG-DVS at room temperature prior to gelation; confirmed by NMR. HA was thiolated according to methods by Shu et al.[7] and reacted with PEG-DVS-Lm solution at 1.75 wt% (0.01wt% Lm). Storage modulus and gelation time were determined by rheology; microstructure was visualized by scanning electron microscopy. After gelation, NPSCs were seeded on top of the gel. At 72 hours, NPSC density and viability were visualized by live/dead assay. Temporal CXCR4 expression was analyzed by western blotting after 24, 48, and 72 hours of culture on HA-Lm gel or poly-L-lysine (PLL) and normalized internally to β-actin expression and externally to non-adherent NPSC culture. A transwell assay (Figure 1C) was used to observe NPSC chemotactic migration through the gel in response to SDF-1α at 24 and 48 hours. One-way ANOVA was used to determine statistical significance.

Results: Covalent laminin tethering to PEG-DVS was evidenced by the appearance of laminin peaks and the reduction of vinyl groups in the NMR spectra of PEG-DVS after reacting with laminin. HA-Lm gel was determined to have a physiologically relevant storage modulus of 1.02 kPa. The gel was highly porous with pore size ranging from 2-17 μm. CXCR4 expression was significantly increased on HA-Lm gels compared to PLL at 48 hours (Figure 1A,B). Significant NPSC migration in response to an SDF-1α gradient on the HA-Lm gel was observed at 48 hours compared to 24 hours and to a uniform concentration of SDF-1α and no SDF-1α at both 24 and 48 hours (Figure 1D).

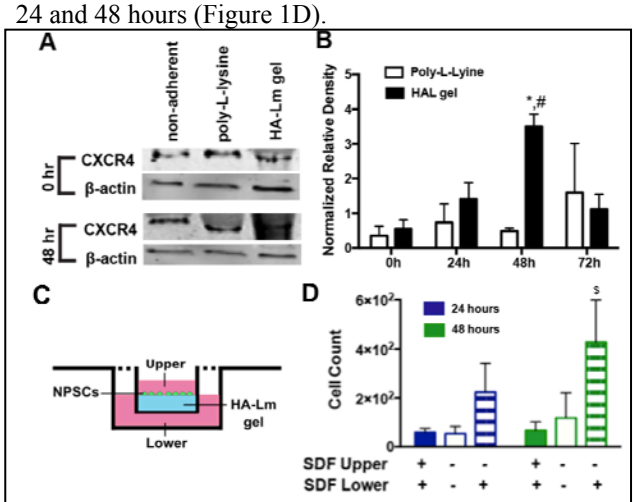

Figure 1: CXCR4 expression is significantly increased after 48 hr of culture on HA-Lm gel compared to PLL (A,B).*p<0.05 compared to all PLL groups, #p<0.05 compared to all other HA-Lm groups. HA-Lm gel supports significant NPSC chemotactic migration in response to SDF-1α gradients (C,D). $p<0.05 compared to all other conditions and time points.

Conclusions: Culture on the HA-Lm hydrogel significantly upregulates CXCR4 in NPSCs, which serves to biochemically prime NPSCs for the SDF-1α rich injury microenvironment. Moreover, HA-Lm hydrogel supports NPSC chemotactic migration in response to the injury-relevant chemokine SDF-1α. Mechanistic studies are currently underway to further investigate the regulation of CXCR4 expression and the NPSC chemotactic response. Therefore, this hydrogel delivery system may be a viable option for improving NPSC engraftment in neurotransplantation paradigms.

References: [1]CDC, 2010(17):1-74. [2]Nolan A, *Crit Care Nurs Q*, 2005;28(2): 188-94. [3]Harting MT, *Neurosurg Focus*, 2008;24(3-4):E18. [4]Kokovay E, *Cell Stem Cell*, 2010;7(2):163-73. [5]Sbaa Ketata E, *Cell Stem Cell*, 2002;20(6):585-7. [6]Addington CP, *Biomaterials*, 2014;35(10):3263-72. [7]Zheng Shu X, *Biomaterials*, 2004;25(7-8):1339-48.

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