Optimizing hydrogels *in vitro* for transplantation of iPS-NPCs *in vivo* after stroke

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Statement of Purpose: Strokes are the leading cause of adult disability. However, current treatments focused on rest and rehabilitation lack the ability to promote wound healing and tissue regeneration in the brain. While stem and progenitor cell transplantation have shown promise in promoting recovery, critical obstacles include the low survival rate post-transplantation and the lack of differentiation in the cells that do survive. Delivering cells via a biomaterial scaffold decorated with bioactive signals is an approach that can potentially attenuate these problems. The focus of this work is to 1) optimize hyaluronic acid hydrogels *in vitro* for the culture of iPS-NPCs and 2) determine the effect of our hydrogel system on transplanted cell viability and differentiation in an *in vivo* mouse stroke model.

Methods: Neural progenitors derived from induced pluripotent stem cells (iPS-NPCs) were encapsulated in three-dimensional hyaluronic acid hydrogels crosslinked with matrix-mettaloproteinase (MMP) degradable peptides *in vitro*. These hydrogels were functionalized with three peptide fragments containing the active sequences of RGD, YIGSR, and IKVAV at concentrations ranging from 0-300 μM each. The exact combination was determined from a statistical design of experiments program. Hydrogels from each peptide combination tested were collected and frozen at days 1 and 7 for analysis. The gels were degraded with Tryp-LE (Life technologies, Grand Island, NY) and cells collected via centrifugation. The DNA content was measured using a CyQuant proliferation assay (Life technologies, Grand Island, NY). For immunofluorescence staining, cells in the hydrogel were fixed, permeabilized, blocked with appropriate serums and incubated with desired primary/secondary antibodies. For *in vivo* transplantation experiments, immune-compromised mice were injected with Rose-Bengal (Sigma-Aldrich, St. Louis, MO) intraperitoneally and a light source aimed at the motor cortex of the mouse brain initiated the photothrombotic ischemia. One week later, 100,000 iPS-NPCs suspended in buffer or hydrogel were injected into the stroke cavity using a Hamilton syringe/syringe pump. Two weeks post-injection, the mice were sacrificed, brains isolated, sectioned and stained for analysis.

Results: A statistical Design of Experiments (DOE) approach was used to determine the interacting effects of three different peptide fragments derived from fibronectin and laminin on iPS-NPC proliferation *in vitro*. This multi-factorial experimental design, in which the concentrations of these three peptide sequences were systematically modulated, enabled us to optimize a specific hydrogel formulation. After three optimization iterations, we found that 100 μM RGD, 48 μM YIGSR, and 300 μM IKVAV increased proliferation by 97% compared to control hydrogels with a scrambled RGD sequence (Fig. 1A-B). Furthermore, this combination of peptides also resulted in iPS-NPC's differentiating toward a neuronal phenotype, whereas the controls did not. To determine the effect of our hydrogel on transplanted cell viability and differentiation *in vivo*, 100,000 iPS-NPCs were injected into cortically-stroked immune-deficient mice with or without a hydrogel carrier using a 30 gauge needle attached to a Hamilton syringe/syringe pump at 0.6 μL/min. One week later, staining of sections showed good endogenous cell infiltration (Fig. 1C). While the presence of the hydrogel did not significantly improve transplanted cell viability (~40% of transplanted cells survived with or without the hydrogel carrier), the hydrogel did promote differentiation. The iPS-NPCs encapsulated inside the hydrogel showed significantly higher levels of double cortin compared to the cells injected with buffer (Fig. 1D).

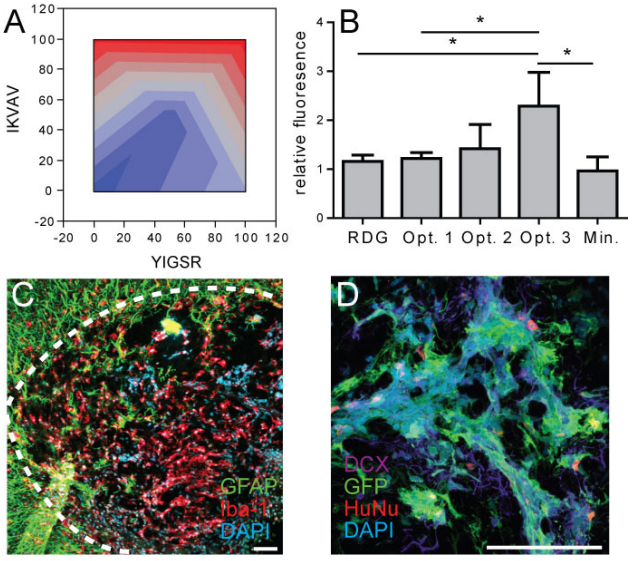

Figure 1. (A) Heat map showing effect of peptides on iPS-NPCs proliferation at a constant concentration of RGD. (B) Optimization iterations continued to improve encapsulated cell proliferation. (C) IF staining shows infiltration of endogenous mouse cells into transplanted hydrogel in stroke cavity. (D) Transplanted iPS-NPCs begin to differentiate to neurons when encapsulated inside a hydrogel *in vivo*. Scale bars = 100 μm

Conclusions: *In vitro* experiments using a DOE approach enabled us to optimize a three-dimensional hyaluronic acid hydrogel for iPS-NPC proliferation. *In vivo* mouse stroke model experiments demonstrated the ability of this hydrogel matrix to promote differentiation of transplanted cells. Since we are ultimately interested in developing a therapy that can improve functional recovery in stroke victims, behavioral studies on stroked mice with this iPS-NPC/hydrogel transplantation would be the next step.