SDF-1α-loaded Dextran Sulfate/Chitosan Nanoparticles As a Multi-pronged Approach to Enhance Neural Stem Cell Infiltration into Hydrogels for CNS Applications

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Statement of Purpose: Endogenous neural stem cells (NSCs) home towards central nervous system (CNS) lesions resulting from various injuries/diseases but may have their repair/regenerative potential hindered by an unfavorable milieu. Numerous hydrogels with well-suited properties, such as injectable gelatin-hydroxylphenyl-propionic acid (Gtn-HPA) hydrogels,[1] have therefore been engineered to provide a permissive environment for endogenous NSCs in the injured/diseased CNS. Promoting infiltration of NSCs into these hydrogels, however, remains a challenge and most likely requires the use of instructive cues. We recently developed biocompatible polyelectrolyte complex nanoparticles (PCNs) from dextran sulfate (DS) and chitosan (CS) to provide sustained release of SDF-1α, a chemoattractant for NSCs. Here, we explored their utility as a modular addition to Gtn-HPA hydrogels to enhance NSC infiltration and examined the underlying mechanisms.

Methods: Gtn-HPA conjugates were prepared as previously described.[1] SDF-1α PCNs were prepared by sequentially mixing recombinant rat SDF-1α with DS (500kDa) and CS (15kDa, ~84% deacetylation) at a ratio of 0.2:1:0.33. Blank PCNs were similarly prepared by omitting SDF-1α. Three-dimensional migration assays were performed using specially developed annulus + core (A+C) hydrogel constructs (Fig. 1A). The annulus served as a neural tissue simulant comprised of 0.8mg/ml collagen I hydrogel containing 1×10^6 aNSCs with/without 10ng/ml FGF-2. The core comprised of Gtn-HPA hydrogels incorporating soluble SDF-1α, PCNs, SDF-1α PCNs or various soluble polymers: DS, CS, low molecular weight DS (DSL; 9-20kDa) and heparin (H; 9-21kDa)]. The A+C hydrogel constructs were imaged after 7 days to quantify NSC infiltration into the Gtn-HPA hydrogel core. Proliferation was examined using the same two aforementioned hydrogels in a bilayer configuration and measured using Picogreen DNA quantification assay.

Results: Despite being permissive matrices for NSC migration, Gtn-HPA hydrogels, by themselves or even with soluble SDF-1α, failed to produce significant NSC infiltration (Fig. 1A-B). Incorporation of SDF-1α PCNs into Gtn-HPA hydrogels markedly increased NSC infiltration, highlighting the importance of sustained SDF-1α release. We examined the proliferative, chemokinetic and/or chemotactic roles played by this sustained SDF-1α release. Proliferation was found unlikely to be involved given its similar levels between PCNs and SDF-1α PCNs (Fig. 1C). Chemokinesis was also excluded based on the failure of SDF-1α PCNs to maintain any increase in NSC infiltration (Fig. 1D) relative to PCNs (Fig. 1B) when subjected to medium baths of soluble SDF-1α that disrupted concentration gradients arising from the SDF-1α PCNs but preserved chemokinetic effects. Sustained

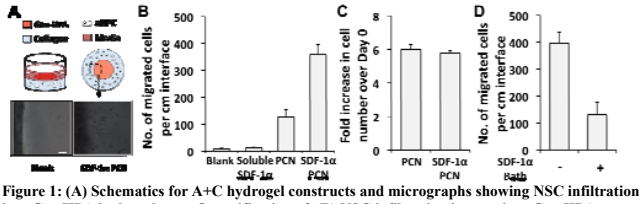

Figure 1: (A) Schematics for A+C hydrogel constructs and micrographs showing NSC infiltration into Gtn-HPA hydrogel core. Quantification of (B) NSC infiltration into various Gtn-HPA hydrogel formulations, (C) proliferation as influenced by PCNs and SDF-1α PCNs and (D) NSC infiltration with or without medium baths of soluble SDF-1α.

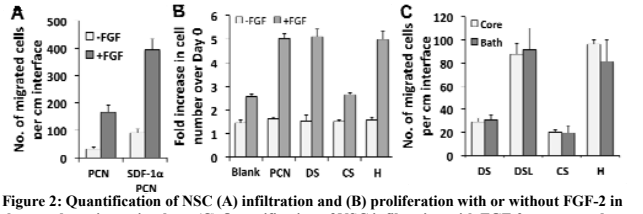

Figure 2: Quantification of NSC (A) infiltration and (B) proliferation with or without FGF-2 in the annuluar tissue simulant. (C) Quantification of NSC infiltration with FGF-2 present and with various soluble polymers loaded into the Gtn-HPA hydrogel core or the medium bath.

SDF-1α release from SDF-1α PCNs had therefore specifically promoted chemotactic recruitment to enhance NSC infiltration relative to PCNs.

Incorporation of PCNs themselves within Gtn-HPA hydrogels enhanced NSC infiltration and proliferation (Fig. 2A-B). We discovered that both effects arose from PCNs utilizing FGF-2 found *in situ* in the tissue simulant, as verified by its disappearance when FGF-2 was omitted, and could be traced to the heparin or heparin-like polymers *i.e* DS and DSL (Fig. 2B-C). Interestingly, DSL, but not DS, enhanced NSC infiltration, showing molecular weight of the polymer to be a critical parameter. We further observed enhanced NSC infiltration regardless of whether DSL or H was in the hydrogel core or medium bath (Fig. 2C). Collectively, these data suggested PCNs to have utilized FGF-2 and enhanced NSC infiltration by increasing proliferation (of NSCs already migrated into the core) and chemokinesis. Finally, NSC infiltration with SDF-1α PCN/+FGF was significantly greater than the sum of SDF-1α chemotactic recruitment (SDF-1α PCN/-FGF) and the PCN-driven increase (PCN/+FGF). It appeared likely that PCNs also exploited the ability of FGF-2 to potentiate chemotactic recruitment[2] to drive the observed difference.

Conclusions: SDF-1α PCNs released their chemokine load to drive chemotactic recruitment of NSCs into Gtn-HPA hydrogels. They could further utilize FGF-2 found *in situ* to increase proliferation, enhance chemokinesis and potentiate SDF-1α chemotactic recrtuiment. This multi-pronged approach orchestrated a greatly enhanced level of NSC infiltration and could potentially benefit hydrogels designed for various CNS applications.

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References: (1) Lim TC. Biomaterials 2012;33:3446-3455. (2) Pickering JG. Circ Res. 1997;80:627-637