# Modulating Stromal Derived Factor-1α Release from Nanoparticles Via Affinity-Based Matrices

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**Statement of Purpose:** Traumatic brain injury (TBI) is one of the leading causes of death and long-term disability worldwide. Although TBI mortality rates have reduced drastically, no clinical treatments address the progression of deleterious secondary injury cascades that can persist for months.[1] The response to injury also includes endogenous repair efforts where the adult brain recruits endogenous neural progenitor/stem cells (NPSCs) to the lesion area out to 4 months after injury.[2] In either case of progressive degeneration or repair signaling, temporal and spatial control of soluble signaling factors appear to be critical in regulating the injury microenvironment. The chemokine, stromal-derived factor-1α (SDF-1α), plays a vital role in the recruitment response by directing NPSC migration via a concentration gradient in the brain interstitium.[3] We are interested in exploring if NPSC recruitment may be modulated through sustained local delivery of SDF-1α for a period greater than 30 days. We have previously synthesized and characterized poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs) that release bioactive SDF-1α for ~50 days. These NPs, however also exhibit a burst release of 30-35% (cumulative release in 1day). The high initial burst release is of concern due to the supratherapeutic levels of SDF-1α that it may achieve in the peri-lesion space. To address this, we explored affinity-based interactions between SDF-1α and fibrin. Fibrin is known to have binding sites for immobilization of specific cytokines typically via heparin-binding domains (e.g.basic fibroblast growth factor;bFGF).[5] Moreover, fibrin has been utilized to slow protein diffusion through its matrix to achieve short term controlled delivery of proteins such as bone morphogenetic protein-2.[6] Thus, we hypothesize that embedding SDF-1α encapsulated NPs in fibrin matrices may be used to modulate the release profile, particularly burst release characteristics.

**Methods:** NPs were prepared using a specific water/oil/water emulsion technique.[7] The oil phase for particle synthesis was comprised of PLGA (50:50 polymer ratio; ester-terminated) in ethyl acetate. The protein load consists of SDF-1α and BSA (0.2% & 2.0% respectively; (w/w) PLGA). Tocopheryl polyethylene glycol 1000 succinate (TPGS) was used as the emulsifier. Size distributions were calculated from SEM images. Verification of SDF-1α release through the entire course of the release period was confirmed using ELISA. Bioactivity of SDF-1α was verified using the in vitro from Boyden chamber migration assays using fetal derived murine NPSCs. Standard protocols were used for the ELISAs to determine relative SDF-1α binding for fibrinogen and fibrin.[8] One-way ANOVA ($\alpha < 0.05$) analyses determined statistical significance.

**Results:** The NPs utilized in this study have a mean diameter of 290.3±21.9 nm (PDI = 0.23±.05) and the product yields ranged between 58-65%. Encapsulation efficiency of SDF-1α was 61.7% ± 2.8 and the particles demonstrated complete release in ~50 days. Boyden chamber migration assays demonstrated the release of bioactive SDF-1α from the NPs (Fig 1A). To determine binding affinities between SDF-1α and fibrinogen, plates were coated with 100nM SDF-1α, bFGF (positive binding control) or bovine serum albumin (BSA; negative binding control). After incubation with fibrinogen relative binding affinities were determined using ELISA where a significantly higher signal was observed towards SDF-1α relative to BSA (Fig 1B). In a separate experiment (not shown) plates were also either coated with heparin (positive control) or a monolayer of fibrin.[8] In this case, a primary antibody against SDF-1α indicated a SDF-1α concentration dependent binding to the fibrin-modified surface.

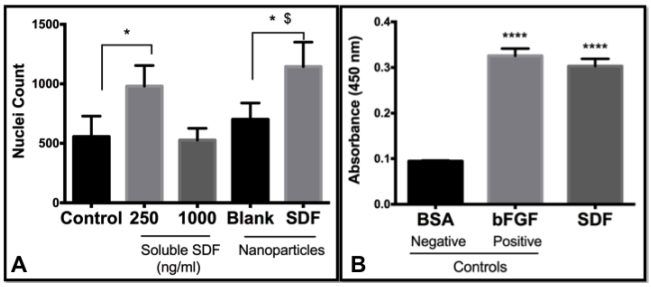


**Figure 1** – **A)** NPSC migration when exposed to basal media (control), soluble SDF-1α supplemented media (250 & 1000 ng/ml), blank NP release media and SDF-1α loaded NP release media (*$p < 0.05$ compare to control; **$** $p<0.05$ compared to blank NP release media) **B)** Fibrinogen has comparable affinity to towards SDF-1α and bFGF (****$p<0.01$ compared to BSA)

**Conclusions**: SDF-1α is known to have a biphasic cellular response as demonstrated in Figure 1A where 250ng/ml SDF-1α induces significant increase in migration response while 1μg/ml does not. Thus, the initial burst release from PLGA NPs has the potential to elevate SDF-1α beyond the effective chemotactic concentration range in the injury microenvironment. Our data suggest that SDF-1α (a heparin binding protein) participates in protein/protein interactions with fibrin/fibrinogen potentially indicating that fibrin matrices may be employed as a tool to modulate the burst release from our NPs. Future studies include the use of surface plasmon resonance to quantify SDF-1α/fibrin affinity and determine effect of fibrin on the release profile from embedded NPs.

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**References:**

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