

# Stromal Derived Factor-1 $\alpha$ Release from Poly(Lactic-co-Glycolic) Acid Nanoparticles Induces Neural Stem Cell Migration

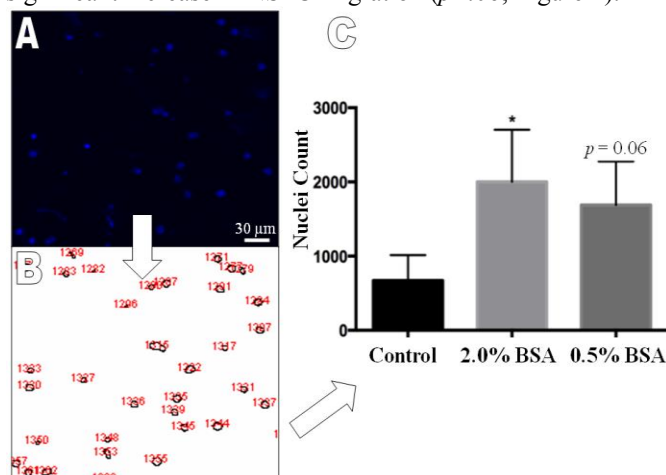
Dipankar Dutta, Chase Fauer, Rachael W. Sirianni, Sarah E. Stabenfeldt  
Arizona State University, Tempe, Arizona

**Statement of Purpose:** Traumatic brain injury (TBI) is a leading cause of death and long term disability worldwide. TBI is characterized by the primary injury (damage from a mechanical insult) and the secondary injury (prolonged damage from resulting biochemical processes).<sup>1</sup> Advances in treatment have reduced TBI fatality rates, but addressing its sub-acute and chronic effects remains a challenge.<sup>1</sup> Progression of injury microenvironment is largely mediated by temporal and spatial orchestration of cytokine signaling.<sup>2</sup> A number of these factors act as neuroprotective agents and/or activate the endogenous regeneration response that involves trafficking of neural stem/progenitor cells (NSPCs).<sup>3</sup> After injury, NSPC proliferation increases within neural niches, while migrating NSPCs selectively accumulate near the injury area.<sup>3</sup> The chemokine, stromal-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ), is implicated in this recruitment response through its ability to induce NSPC chemotaxis mediated by its concentration gradient in the brain interstitium.<sup>3</sup> Thus, we hypothesize that controlled delivery of SDF-1 $\alpha$  after TBI will amplify NSPC recruitment to the peri-lesion area in focal cortical injury models. In this study, we focused on fabrication and characterization of SDF-1 $\alpha$  encapsulated poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs). We report successful synthesis of PLGA NPs and encapsulation of bioactive SDF-1 $\alpha$  in PLGA NPs as demonstrated by NSPC migration *in vitro*. Moreover, the excipient concentration (bovine serum albumin; BSA) significantly affected encapsulation efficiency of bioactive SDF-1 $\alpha$ .

**Methods:** Particle size distribution, encapsulation efficiency, model protein (insulin) release profiles, and *in vitro* NSPC migration were quantified. NPs were prepared using standard water/oil/water emulsion techniques.<sup>4</sup> The oil phase for particle synthesis was comprised of 100 mg/mL PLGA (50:50 polymer ratio; ester-terminated) in ethyl acetate. The water phase contained insulin or SDF-1 $\alpha$  and 0.5% or 2.0% (wt.% of PLGA) BSA. Ultra-sonication of the above and 5% (w/v) d- $\alpha$  tocopheryl polyethylene glycol 1000 succinate (TPGS) yielded the second emulsion. After a 3 hr incubation in 0.4% TPGS, the particles were size fractionated centrifugally, washed and recovered through freeze-drying. Size distributions were calculated from 8-10 SEM images with >70 sampling points each. Migration was measured using the Boyden chamber assay in the presence of resuspended NPs (2mg/mL for all groups; n=3). After a 3-day incubation, the nuclei of migrated cells were stained, imaged and quantified using a particle count algorithm (Figure 1). Equal variance Student's *t*-test was used for statistical analysis where a probability value (*p*) of < 0.05 was considered statistically significant.

**Results:** The particles have a mean diameter of 290.3 $\pm$ 21.9 nm (PDI = 0.23 $\pm$ .05) nm and the product

yields range between 51-56%. Overall mean particle diameter and PDI were tailored by adjusting synthesis parameters and employing centrifugal size fractionation (data not shown). Encapsulation efficiency of the model protein, insulin, was 61.7%  $\pm$  2.8 and the particles demonstrated 90% cumulative release in ~40 days. Migration assays indicated a significant increase over controls (particles without SDF-1 $\alpha$ ) only for particles synthesized using 2.0% BSA excipient (*p* < 0.05). Reducing the BSA excipient to 0.5% did not elicit a significant increase in NSPC migration (*p*=.06; Figure 1).



**Figure 1** – NSPC Migration. A: Raw image of DAPI stained nuclei. B: Nuclei count. C: Results demonstrated 2.0% BSA has a significant effect on NSC migration.

**Conclusions:** In this study, we demonstrated a 61.7% encapsulation efficiency of insulin within the PLGA NPs. Migration assays from SDF-1 $\alpha$  loaded particles indicated successful encapsulation of bioactive SDF-1 $\alpha$  in both formulations of BSA excipient concentrations (Figure 1). However, 0.5% BSA formulation failed to induce a statistically significant increase in migration (*p*=.06), implying a susceptibility of free SDF-1 $\alpha$  to denaturation, likely due to harsh synthesis conditions (water/organic interfaces, shear stresses, heat, etc.).<sup>5</sup> Current studies are focused on quantifying bioactive SDF-1 $\alpha$  release profiles and evaluation of NP SDF-1 $\alpha$  release within an *in vivo* TBI model.

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## References:

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