Synthetic Ligand-Receptor Binding for Targeted Delivery to the Injured Spinal Cord

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Statement of Purpose: In tissue engineering, targeted nanoparticle (NP) delivery is a viable option for selectively delivering a loaded therapeutic payload to a tissue of interest. Often, targeted NPs are functionalized with a surface ligand that binds to a receptor on the cell or tissue level.[1] Unfortunately, these natural-based targeting systems can fall short in that receptor molecules are susceptible to tissue remodeling. This is especially true in the case of spinal cord injury (SCI) where a dynamic immune response results in a complex cascade of cells infiltrating the injury and constantly remodeling the tissue.[2] To overcome the challenges associated with targeting a remodeling tissue, we shifted away from a natural-based targeting system towards a synthetic targeting system. By using synthetic biomaterials, we know that our target is specific to our injury and is not susceptible to changes as remodeling occurs. Using this system, we control therapeutic delivery *in vivo* to accumulate wherever we deploy our binding depot, a significant step forward in targeted delivery strategies that can be broadly applied across tissue engineering problems.

Methods: The synthetic system presented here takes advantage of the biotin/streptavidin complex for direct targeting of an implantable biomaterial beacon. Poly(ethylene glycol) (PEG) tubes were modified to contain biotin residues and serve as an implantable biomaterial targeting beacon. Biotin-PEG tubes were fabricated via an intermediate microsphere stage using a water/oil emulsion to create microspheres of approximately 50 µm in diameter. Microspheres were then shaped in a mold and UV annealed into a tube structure. Targeting NPs were fabricated using a nanoprecipitation where poly(lactic-*co*-glycolic acid) (PLGA)-PEG was dissolved in dimethylformamide and dropped into distilled water. Streptavidin was subsequently conjugated onto the surface of the formed NPs using carbodiimide chemistry to create targeting ligand nanoparticles (LNPs). Biotin-PEG depots were assayed for biotin concentration over the course of 12 weeks using a biotin quantitation kit (Thermo Fisher, Waltham, MA). LNPs were characterized with dynamic light scattering (DLS) with a Zetasizer Pro (Malvern Panalytical, Westborough, MA). *In vitro* LNP binding to biotin-PEG depots was quantified by incubating fluorescent LNPs with biotin-PEG depots in PBS. Daily samples were spun down, supernatant collected, and fresh PBS was added. Supernatant was analyzed via fluorescent plate reader to quantify LNP release. *In vivo* a C5 lateral hemisection injury was given to Balb/c mice followed by immediate implantation of a biotin-PEG depot. On day 6 post-injury, LNPs were administered intravenously, and on day 7, LNP localization was analyzed via In Vivo Imaging System (IVIS; PerkinElmer, Waltham, MA). Mice were sacrificed after IVIS imaging and organs were isolated for *ex vivo* quantification. A subset of mice were not sacrificed, and imaged on days 8-12 to assess LNP retention.

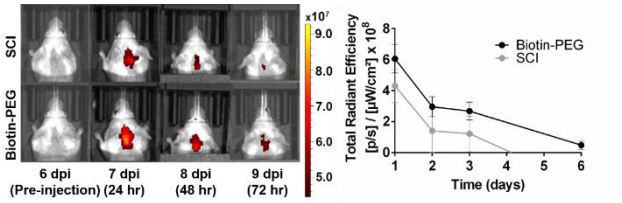

Figure 1. Fluorescent LNPs are retained longer when administered to mice receiving biotin-PEG implants compared to injury only control mice as evaluated via IVIS. Mean ± SEM.

Results: Biotin was quantified in the biotin-PEG depots for 12 weeks with no significant decrease in concentration, and all time points were in range of the calculated expected value of 32.9 pmol/mg of microsphere. LNP analysis via DLS demonstrated that LNPs had an average diameter of 70.5 ± 1.9 nm, polydispersity index of 0.224 ± 0.002, and Zeta potential of -28.2 ± 2.2 mV. *In vitro* binding characterization demonstrated significantly more LNPs were retained in the biotin-PEG depot compared to all other control conditions. After initial wash (1 hr. post-incubation) 77.4 ± 0.4% of the administered LNP dose was retained compared to 15.3 ± 3.9%, 18.4 ± 2.5%, and 24.7 ± 1.7% for PEG with LNPs, biotin-PEG with non-targeted NPs, and PEG with non-targeted NPs, respectively. After 5 days of incubating, 59.7 ± 0.3% of the LNP dose was still retained in the biotin-PEG condition. Furthermore, the rate at which initially bound NPs were released was significantly slower in the LNPs + biotin-PEG condition compared to all others. In biodistribution analysis, mice receiving biotin-PEG implants and LNPs exhibited an increase in LNP accumulation as evidenced via IVIS, and this was paired with a signal retention increase observed at the final collection point (6 days post-LNP administration). *Ex vivo* analysis also demonstrated uptake in expected filtering organs like the livers, kidneys, and spleens.

Conclusions: The synthetic system presented here demonstrates a significant increase in targeted LNP accumulation and the first use of a binding depot system for targeted delivery to a SCI. The control we have over therapeutic accumulation wherever we deploy our beacon will ultimately allow us to expand the use of this targeting system beyond SCI and broadly across tissue engineering applications wherever a depot can be used.

References: [1] Kamaly N. Chem. Soc. Rev. 2012; 41(7):2971-3010. [2] Donnelly D.J. Exp. Neurol. 2008; 209(2):378-388