Stabilization of Chondroitinase ABC using Single Enzyme Nanoparticles for Spinal Cord Injury Repair

Shashank Kosuri, Heloise Mugnier, Matthew Tamasi, Zachary Finkel, Isabel Perez, Li Cai, Rene Schloss, Martin L. Yarmush, Adam J. Gormley
Biomedical Engineering, Rutgers University, Piscataway, New Jersey

Statement of Purpose: Traumatic spinal cord injury (SCI) is a debilitating, life-disrupting event that results in significant physical, psychological, and socio-economic disability. Soon after the primary injury, a cascade of cellular and molecular events results in a dense network of glial scar, mainly composed of chondroitin sulfate proteoglycans (CSPGs), that act as a physical and chemical barrier to neuronal regeneration. *Chondroitinase abc* (chABC), a bacterial enzyme, has shown a lot of promise as a therapeutic intervention because of its ability to cleave CSPG side chains and promote neural plasticity. However, this enzyme suffers from thermal instability and loses all of its activity at 37°C within a few days. Current techniques to stabilize chABC include using high concentration sugar solutions (not feasible for *in vivo*) or modifying protein structure using highly cumbersome engineering techniques. Recently, ***single enzyme nanoparticles*** (SENs) are gaining increased attention because of their ability to protect enzymes and preserve their activity under harsh environments by forming a shell. These nanoparticles contain enzyme packed inside a synthetic polymeric shell that stabilizes and safeguards it from the surrounding microenvironment. Therefore, we are utilizing a ***machine learning guided combinatorial approach*** to rapidly discover SENs that can stabilize chABC under physiological conditions. The top ten performing SENs have been tested *in vitro* for their bioactivity and biocompatibility. The top three performing candidates *in vitro* have been selected to be tested *in vivo* in a rat hemisection SCI model to evaluate their therapeutic efficacy.

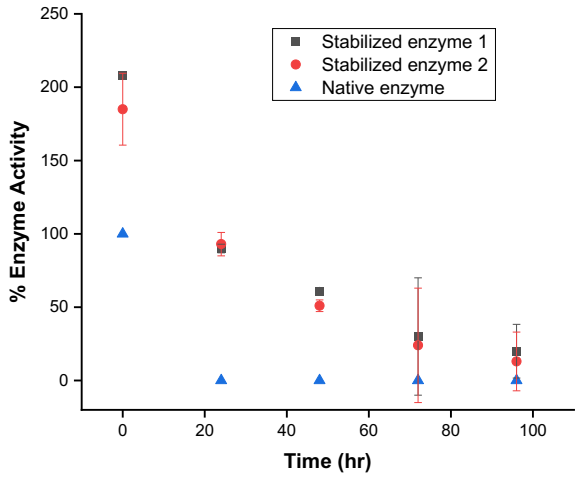

Figure 1: Stabilized polymer-enzyme complexes retain enzyme activity for up to 96 hrs

Methods: To synthesize our SENs, we used a Hamilton MLSTARlet liquid handling robot that is programmed to perform photoinduced electron/energy transfer–reversible addition-fragmentation chain-transfer (PET-RAFT) polymerization reactions in 96 well plates (1). Briefly, stock solutions of monomer (2 M), RAFT agent (50 mM) and catalyst (2 mM) were prepared in dimethyl sulfoxide (DMSO) and loaded into the Hamilton robot and automatically mixed into appropriate ratios in polypropylene 96 well plates (Greiner bio-one) using custom built software and irradiated under yellow light for 12 hrs. Polymers were then serially diluted in DMSO, transferred into phosphate buffer or artificial cerebrospinal fluid (pH~7.5), mixed with equal volume of enzyme solution (25 µL each, final enzyme concentration ~ 1 ng/µL), and placed in an incubator at 37°C for 24 hr. After incubation, 50 µL of 4 mg/mL chondroitin sulfate substrate was added and absorbance was measured using a kinetic assay at 232 nm for 30 mins. Initial velocity obtained was used to calculate specific activity. Enzyme activity at t = 0 and t = 24 hr heating served as positive and negative controls for the experiment.

Results: In order to understand the influence of design parameters such as chain length, and composition on enzyme stability and activity retention, our preliminary experiment involved testing of 150 polymers with varied characteristics. Activity data and polymer composition data was then used to train a random forest model to correlate polymer features and activity retention. Second generation polymers designed *in silico* were synthesized and tested for activity retention. Second generation polymers had a median enzyme activity of 60% and maximum enzyme activity for one sample was 97% at the end of 24 hr period. Out of the 200 polymers tested so far, we have identified two polymer combinations that stabilized chABC and retained some residual activity even at end of 96 hrs while native enzyme lost all activity within few hours **(Figure 1)**. Recently, we tested the biocompatibility of our top-performing SEN-chABC complexes with neurons in collaboration with the Yarmush/Schloss laboratories and found no signs of toxicity at therapeutic doses (data not shown).

Conclusions: Remarkably, we have already identified few heteropolymer compositions that retained >90% chABC activity at the end of 24 hrs at 37°C. Our future efforts will be directed towards analyzing this data using active machine learning techniques (Random forest, Gaussian optimization) to identify patterns and predict future designs. Our current studies include designing animal experiments to evaluate the therapeutic efficiency of these constructs in a rat hemisection model.

References:

1. Tamasi, M., Kosuri, S., DiStefano, J., Chapman, R., & Gormley, A. J. Automation of Controlled/Living Radical Polymerization. *Advanced Intelligent Systems*, 1900126.