Machine-Assisted Discovery of Chondroitinase ABC Complexes Towards Sustained Neural Regeneration

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Statement of Purpose: Traumatic spinal cord injuries (SCI) are life-disrupting events which cause both physical and psychological changes. Soon after the injury is sustained, a large glial scar is formed at the injury site mainly due to the cascading inflammatory events occurring in the body. This scar is composed of chondroitin sulfate proteoglycans (CSPGs), which inhibit neuronal regeneration by creating a physical and chemical barrier. The bacterial enzyme, *Chondroitinase abc* (chABC), has shown a lot of promise as a therapeutic intervention because of its ability to cleave the glycosaminoglycan side chains on CSPGs promoting neural plasticity. Unfortunately, chABC suffers from thermal instability and loses all of its activity at 37°C within a few days. ***Polymer Enzyme Complexes*** (PECs) have the ability to protect enzymes and preserve their activity under harsh environments by forming a shell. These PECs 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 speedily discover PECs to stabilize chABC for longer period of time at 37°C. The PECs have been tested *in vitro* for their bioactivity and biocompatibility.

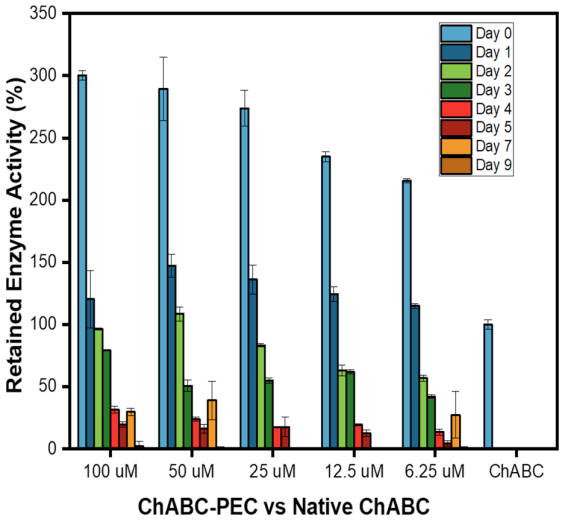

Figure 1: A stabilized polymer-enzyme complex retained enzymatic activity for up to 9 days at certain concentrations while the native enzyme died within 24 hours

Methods: To synthesize our PECs, 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 16 hrs. Polymers were then serially diluted in DMSO, transferred into artificial cerebrospinal fluid (pH~7.4), mixed with equal volume of enzyme solution (15 µL each, final enzyme concentration ~ 1 ng/µL), and placed in an incubator at 37°C for 24 hr. After incubation, 30 µL of 4 mg/mL chondroitin sulfate substrate was added and absorbance was measured using a kinetic assay at 232 nm for 60 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 504 polymers with varied characteristics. Activity data and polymer composition data was then used to optimize a Gaussian Process Regression model to correlate polymer features and activity retention. The polymers in the subsequent generations, which each had 24 polymers, were designed *in silico* and synthesized and tested for activity retention. Four exploration iterations yielded median enzyme activities of 44%, 68%, 82% and 75% respectively. All of these polymers were used to train the model allowing us to conduct an exploitation generation of another 24 polymers which had a median enzyme activity of 87%. Out of all of these polymers one was identified to be able to stabilize chABC and retain residual activity for more than a week at 37°C. The native enzyme lost all activity within the first 24 hours **(Figure 1)**. The top performing polymer construct was tested on astrocytes and found no toxicity at therapeutic doses.

Conclusions: Remarkably, we have already identified few heteropolymer compositions that retained > 100% chABC activity at the end of 24 hrs at 37°C. Our future efforts will be directed towards *in vitro* and *in vivo* studies to look at the efficacy of these polymer-enzyme constructs. 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

Acknowledgement: This work was supported by the NIH grant R35GM138296.