Controlled Protein Release from Layer-by-Layer Coated Hydrogel Scaffolds for Nerve Repair

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Statement of Purpose: Paralysis, or the functional loss of voluntary movement resulting from damage to nerve or muscle tissue, is a devastating condition with no treatment available. Recently, agarose hydrogel nerve guidance scaffolds (NGS) have been fabricated [1] and functionalized with brain derived neurotrophic factor (BDNF), demonstrating substantial axon regeneration *in vivo* [2,4]. However, these reports supplied BDNF to promote regeneration by a cellular release method. The following work focuses on functionalizing agarose hydrogel NGS to release BDNF by utilizing an acellular mechanism, hydrogen-bonded layer-by-layer (LBL). To this end, the effects of acetic acid incorporation during assembly on release rates, cumulative quantities, and improving BDNF stability will be explored. Additionally, modifications to the agarose hydrogel framework by the presence of sucrose will have significant impacts on LBL deposition and release kinetics. From this work, incorporation and controlled release of nerve growth factors from LBL-coated NGSs will promote and sustain axon growth by an acellular method, and will provide insight toward understanding LBL deposition and release on highly porous hydrogels.

Methods: Nerve growth proteins can be incorporated into the NGS via a hydrogen-bonded, dual polymer LBL system under acidic conditions [3]. By depositing LBL on the highly porous hydrogel, the gradual release of lysozyme, a suitable analog for nerve growth factors such as BDNF, is achieved by release of polymer layers under physiological pH. This system consists of depositing alternating layers of hydrochloric acid adjusted polyacrylic acid, polyethylene glycol, and lysozyme within the hydrogel framework and measuring protein release every 24-hours. By modifying the hydrogel pore structure through sucrose presence during gelation, changes to LBL deposition and release was monitored and confirmed by nitrogen adsorption (NA) and scanning electron microscopy (SEM). In addition, changes to LBL kinetics were observed when utilizing acetic acid.

Results: Release of lysozyme, a BDNF analog, from the acellular LBL method has been shown to reach milligram/milliliter concentrations over the course of one month [3]. Recently, we have shown that augmenting the mesopore volume within the hydrogel through the addition of sucrose during gelation results in a substantial increase in release dose, exhibiting a cumulative release surpassing 2500µg/mL in some samples. These results are supplemented by modifications to the nanopore volume as seen by NA and SEM on supercritically dried agarose hydrogels. In addition to tailoring pore structure, the presence of sucrose dramatically increases hydrogel surface area; a property critical for further LBL deposition. By this technique, BDNF can be released in concentrations previously unattainable from the LBL method. Modifications to the LBL assembly may also promote enhanced BDNF release and stability. Incorporating acetic acid into the LBL process has shown controlled release of lysozyme in clinically relevant dosages on the week timescale. Acetic acid not only affects LBL kinetics, but also stabilizes proteins; an important aspect for maintaining bioactivity and broadening the spectrum of compatible drugs during acidic LBL assembly. This work, coupled with methods of producing and concentrating BDNF, has laid the foundation for releasing relevant doses of bioactive BDNF from the LBL process, and furthers our understanding of LBL deposition and release on highly porous hydrogel networks.

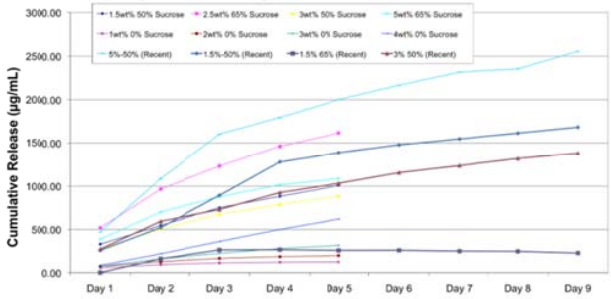

Fig 1. Lysozyme release from sucrose-modified agarose

Conclusions: Functionalized agarose NGS are considered viable for nerve regeneration studies, having shown positive results *in vivo*. However, these results encouraged regeneration by utilizing a cellular mechanism to release BDNF. LBL has been shown to be a viable alternative for acellular controlled release of proteins at clinically relevant dosages over a month timescale. This work is furthered by modifications to the hydrogel framework via sucrose addition, showing dramatic increases in release dose by tailoring of surface area and nanopore volume. Additionally, incorporating acetic acid into the LBL process substantially affects deposition and release kinetics while stabilizing proteins under acidic conditions; broadening the spectrum of LBL-compatible drugs. From this work, LBL assembly and disassembly from highly porous hydrogels is more fundamentally understood. Moreover, functionalized hydrogel NGS capable of linearly guiding and encouraging axon growth through acellular, bioactive BDNF release can be produced for *in vivo* studies, and may provide a potential therapy for paralysis treatment.

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