# Incorporation of Double-Walled Microspheres into Polymer Nerve Guides for the Sustained Delivery of Neurotrophic Factors

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**Statement of Purpose:** There have been many tissue engineered nerve guidance tubes developed to release growth factors for improved regeneration in long peripheral nerve defects. Growth factors, such as glial cell line-derived neurotrophic factor (GDNF), have been shown to have a significant impact on nerve regeneration in animal models and have the potential to overcome disappointing results following nerve repair with conduit based therapies. Previous studies report improved nerve regeneration following implantation of nerve guides delivering GDNF when compared to negative controls; however, results did not meet nerve autograft standards of repair.[1] The aim of this study was to fabricate a nerve guide that delivers GDNF for a period of 60 days, approximately the amount of time needed for the regenerating nerve to cross a defect of 1.5 cm in the rat animal model. It is hypothesized that in vivo studies in which GDNF is delivered throughout the entire regeneration period will result in an increase in axon elongation rate and myelination.

**Methods:** To create a sustained GDNF delivery system, double-walled microspheres of poly(lactic-co-glycolic acid) (PLGA) / poly(lactide) (PLA) were prepared using an oil-oil-water emulsion solvent evaporation technique. Permeable nerve guides were created by dissolving poly(caprolactone) in an inorganic solvent, and adding sodium chloride in a specified v/v% amount. Double-walled microspheres were incorporated into the inner lumen of the nerve guide wall by rolling the nerve guide mandrel with semi-hardened PCL slurry in a known weight of microspheres on parchment paper. To determine the protein release kinetics from double-walled microspheres, samples were incubated in buffer, centrifuged and assayed at specified time points through BCA analysis. Bioactivity of release samples was confirmed through change in absorbance ($A_{450}$) readings for 5 min. of solutions containing microsphere releasate with micrococcus lysodeikticus.

**Results:** Double-walled microspheres were prepared with a core of PLGA and a PLA shell. Microsphere core and shell material compositions were confirmed using a solvent-specific dissolution study (Figure 1A). The mode diameter of microspheres was between 50-75 μm. Release studies indicated a sustained lysozyme delivery of over 120 days (Figure 2). Lysozyme bioactivity was confirmed to 98 days of release, beyond which point bioactivity was no longer detectable. Double-walled microspheres were easily incorporated into the PCL nerve guide wall and maintained the original spherical geometry (Figure 1B), due to solvent specificity.

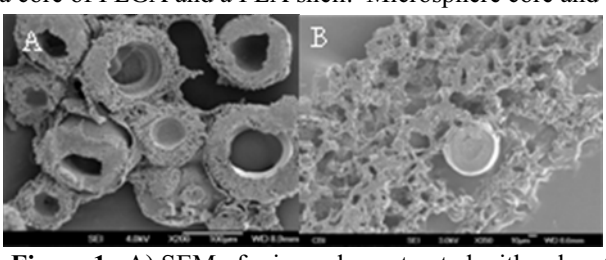


**Figure 1.** A) SEM of microspheres treated with solvent that dissolves PLGA core and not PLA shell. B) Double-walled microsphere incorporated into the PCL nerve guide wall.

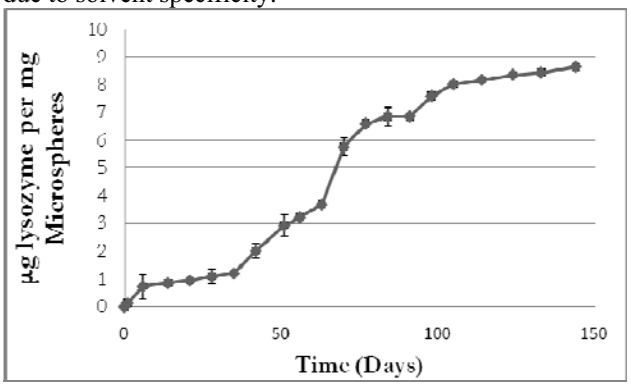


**Figure 2.** Cumulative Lysozyme Release from Double-walled Microspheres

**Conclusions:** The purpose of this study was to fabricate a nerve guide prototype of a growth factor delivering vehicle that is capable of maintaining a sustained release of protein for over 60 days. Encapsulation of proteins within polymeric microspheres is a well documented method of controlled drug delivery. However, typical microsphere release kinetics include an initial burst of protein release within the first 24 hours of placement in aqueous solution either in vitro or in vivo. To this end, the PLGA microspheres have been coated with an additional layer of polymer, creating a double-walled microsphere, which protects the encapsulated growth factor in the core of the two-layered core-shell microsphere structure. Release studies with a model protein, lysozyme, showed that microspheres delivered measurably bioactive protein for ~100 days. Furthermore, because the microsphere shell was composed of PLA, which has low solubility in the solvent ethyl acetate, proteins encapsulated within the microsphere core were protected from dissolution upon contact with the PCL solution. Current in vivo studies that compare the regenerative efficacy of double-walled nerve guides encapsulating neurotrophic factors in long gap sciatic nerve defects to single walled PLGA microsphere delivery systems are underway.

**References:** [1] Fine EG, et al. GDNF and NGF released by synthetic guidance channels support sciatic nerve regeneration across a long gap. European Journal of Neuroscience 2002;15(4):589-601.