Release Rate Controls Biological Activity of Nerve Growth Factor Delivered from Fibrin Matrices Containing Affinity-based Drug Delivery Systems

Matthew D. Wood, Shelly E. Sakiyama-Elbert
Department of Biomedical Engineering, Washington University, St. Louis, MO 63130

Statement of Purpose: Affinity-based delivery systems containing the polysaccharide heparin take advantage of the interaction between sulfated groups on heparin and basic domains on proteins (e.g. growth factors). Previously, we used combinatorial techniques to identify peptide sequences exhibiting high, medium, and low affinity for heparin.[1] An affinity-based delivery system was developed consisting of peptides (of varying heparin affinity) covalently bound to fibrin matrices, heparin, and nerve growth factor (NGF), which binds to heparin with moderate affinity. The goal of this research was to determine how peptide affinity for heparin and the molar ratio of peptide to heparin in the delivery system affected the release rate of NGF and the biological activity of NGF. We also sought to understand whether peptide affinity for heparin modulated biological activity independent of release rate.

Methods: A mathematical model was developed to understand how varying peptide affinity for heparin and the molar ratio of peptide to heparin affected the concentrations of matrix-bound species NGF within fibrin matrices at equilibrium and NGF release from fibrin matrices. Peptides of varying heparin-binding affinity were synthesized by standard solid phase Fmoc chemistry. Fibrin matrices (4.0 mg/mL, Sigma) were prepared as previously described,[1] where the delivery system was prepared by incorporating heparin-binding peptide, heparin, and human β–NGF. To characterize NGF release rates, fibrin matrices were washed with aqueous media, and the amount of NGF released and remaining in the fibrin matrices was quantified by an ELISA for NGF (R&D systems). To measure the biological activity of NGF, fibrin matrices were washed for 24 h followed by implantation of embryonic chick dorsal root ganglia (DRGs). DRGs were cultured in fibrin for 48 h, and the level of neurite extension was then measured. Neurite extension was normalized to extension in fibrin matrices with NGF added to the media for the same experiment.

Results/Discussion: From the mathematical modeling, an optimal ratio of peptide to heparin was determined for each peptide. Conditions for different affinity peptides to retain similar levels of NGF after 24 h were identified by varying the peptide to heparin ratios. NGF retention after 48 h was dependent upon peptide affinity and the molar ratio of peptide to heparin. Use of the delivery system slowed NGF release over 7 days, and release rate was dependent on the peptide affinity for heparin (Fig. 1) and the molar ratio of peptide to heparin. The delivery system incorporating any peptide with 100 ng/mL of NGF promoted increased or equivalent neurite extension compared to NGF in the media (Fig. 2). Furthermore, extension between groups with similar release rates in the 7 day release study demonstrated similar levels of neurite extension, suggesting that the release rate is the major mechanism controlling NGF biological activity.

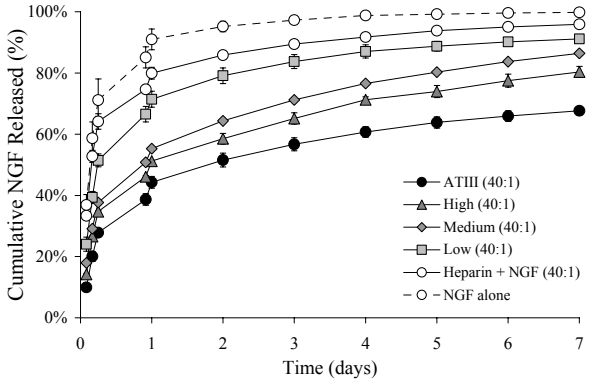

Figure 1 NGF release over 7 days depends on the peptide affinity for heparin and the ratio of peptide to heparin (not shown) in the delivery system. At the 40:1 ratio, the delivery system incorporating any peptide retained higher levels of NGF. Data represented by mean ± standard deviation (S.D.), and statistical significance was considered $p<0.05$ compared to fibrin matrix alone or delivery system incorporating only heparin at a similar concentration.

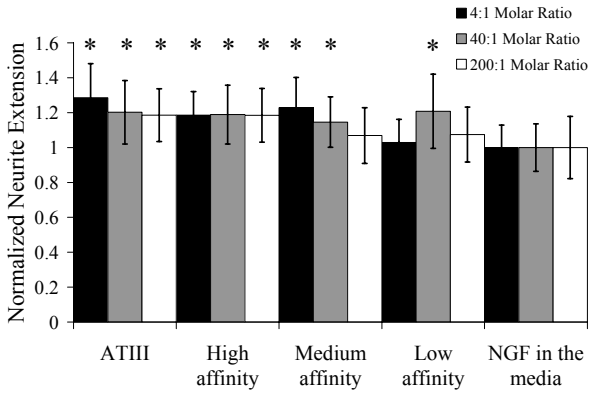

Figure 2 The delivery system incorporating any peptide with 100 ng/mL of NGF caused increased or equivalent neurite extension at 48 h compared to NGF in the media. Data represents mean ± S.D. and * indicates statistical significance ($p<0.05$) compared to NGF in the media.

Conclusions: The rate of NGF release was modulated by varying heparin-binding peptide affinity and the ratio of peptide to heparin. Biologically active NGF was delivered to DRGs, and release rate appears to be the main mechanism controlling the biological activity of released NGF, regardless of peptide affinity. This delivery system has the unique ability to allow us to study the effects of growth factor release rate on nerve regeneration *in vivo*.

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References:

1. Maxwell DJ. Acta Biomaterialia 2005;1:101-113.