Rationally Identified Affinity Peptides for Local Delivery of Nerve Growth Factor

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Introduction: The objective of this research was to identify peptide sequences with varying affinities for a nerve growth factor (NGF)and investigate the effects of incorporating these peptides in a fibrin-based delivery system. Affinity-based delivery systems have been used to deliver basic fibroblast growth factor (bFGF), NGF and other growth factors.[1,2] These systems use the interaction between heparin and a growth factor to sequester growth factor within a delivery system. We identified peptide sequences with unique affinities for NGF and covalently link them to fibrin to constitute a delivery system. The peptides bind NGF via noncovalent interactions to the delivery system. This reduces the amount of NGF in the diffusible form, thus slowing its release from fibrin matrices. Using an *in vitro* model we evaluated the ability of the identified peptides to modulate the delivery of NGF from the fibrin matrices and evaluated the activity of NGF release from the delivery system.

Materials and Methods: NGF affinity peptide sequences were identified by screening Ph.D.-12™ Phage Display Library (New England Biolabs) against NGF-conjugated chromatography resin. After washing the column, phage were eluted using buffers with successively lower pH (6.0, 4.5, and 2.8). The phage were amplified, and their DNA was isolated and sequenced. The results were used to derive consensus NGF-binding peptides (NBPs)(Table 1). Additionally, a transglutaminase substrate (italics) was added to the consensus sequence to allow covalent incorporation of peptide into fibrin matrices.[3]

Table 1: NGF –binding Peptide (NBP) Sequences

Elution Conditions	Sequence
pH 2.8	*NQEQVSPG*SVSVKAKKSVNR
pH 4.5	*NQEQVSPG*QMRAPTKLPLRY
pH 6	*NQEQVSPG*NQSPNHTQNRAY

Fibrin matrices (4mg/mL, Sigma) were made with varying concentrations of NGF (25, 50, 100, and 250 ng/mL) as described previously.[3] Control matrices were also made (unmodified fibrin and NGF without NBP at the varying NGF concentrations).

To determine the activity of NGF released from the delivery system, fibrin matrices were washed 4 times with TBS over 23 hr, and once with media for 1h prior to cell implantation. Dorsal root ganglia (DRGs) were dissected from day 8 chick embryos and placed into the fibrin matrices with 1 mL of modified neural basal media (Invitrogen).[4] Some of the unmodified fibrin matrices were supplemented with 20ng/mL of NGF in the media and used for normalization. Brightfield images of the DRGs were taken at 48 hrs and analyzed to determine the average neurite extension, defined as the radius of the annulus between the DRG body and the halo of neurites.

To determine the dissociation equilibrium constant (K_D) of NGF from each NBP, release studies were performed. 400 µl of TBS + 2% BSA was added to each matrix after polymerization, and the matrices were allowed to equilibrate for 48 hr. The washes were removed, and the matrices were extracted to remove the remaining NGF. The washes and matrix-extracted NGF were analyzed using an ELISA (R&D Systems,). The results were used to calculate K_Ds for each peptide. With these constants, a mathematical model modified from a previous studies[2] was solved using finite element modeling software (Femlab, Comsol).

Results: The effect of NBPs with varying affinities on NGF activity was evaluated using DRG neurite extension (Figure 1). Neurite extension was significantly greater than fibrin with NGF in media for pH 2.8 and pH 4.5 NBPs at 100ng/mL NGF, and for the pH 4.5 NBP at 50ng/mL and 250ng/mL. The K_D for each peptide was found from the release studies; pH 2.8 $K_D = 1.83 \times 10^{-4}$ M, pH 4.5 $K_D = 1.32 \times 10^{-4}$ M, and pH 6 $K_D = 2.14 \times 10^{-4}$ M. The results suggest that the identified peptides have varying affinities for NGF and demonstrate the ability to retain NGF. The mathematical model exhibited good agreement when compared to a release assay for the fibrin systems.

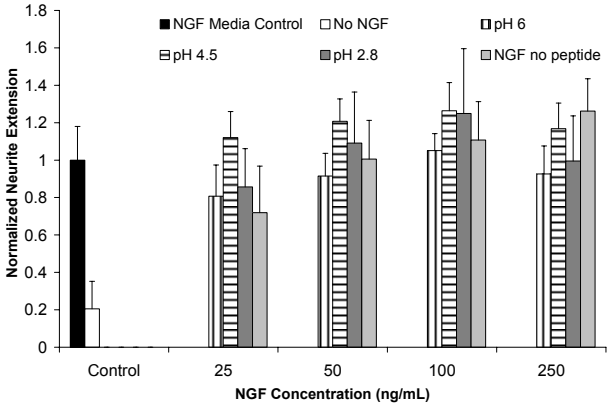

Figure 1 *Effect of NBP affinity on DRG neurite extension through fibrin matrices with NGF. NGF media control consists of an unmodified fibrin matrix with 20 ng/mL NGF in the culture medium. No NGF control consists of unmodified fibrin matrix with no NGF in the cell culture medium. Affinity peptides (pH 2.8, 4.5, & 6) matrices contain NBPs with varying concentrations NGF incorporated into the matrix.*

Conclusions: These results have presented a method for identifying peptide domains with varying affinity for a target protein. The affinities of these identified domains have the ability to be tailored in intensity for their target. As an example multiple peptide domains were identified for the sustained release of NGF from a fibrin matrix. The identified affinity peptides modulated the active release of NGF *in vitro.* A mathematical model, which accurately described the system, was developed and may be used in future work to estimate release of growth factors in varying environments.

References

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