Affinity Peptide for Surface Modification of Polypyrrole

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Statement of Purpose:

Polypyrrole (PPy) is an electrically conductive biomaterial with applications in peripheral nerve regeneration and in coatings for neural probes. Our aim was to complement the utility of PPy by decorating the surface with biological molecules in a specific and non-covalent fashion so as not to impair the bulk properties, including conductivity, of PPy.

In previous work (Sangvi et al., 2005), a 12 amino acid sequence (T59) was identified using phage display to selectively bind chlorine-doped PPy (PPyCl; *Figure 1*). Here we discuss our efforts in quantifying the interaction of T59 with PPy and discuss the in vivo tissue response to PPy implants treated with T59.

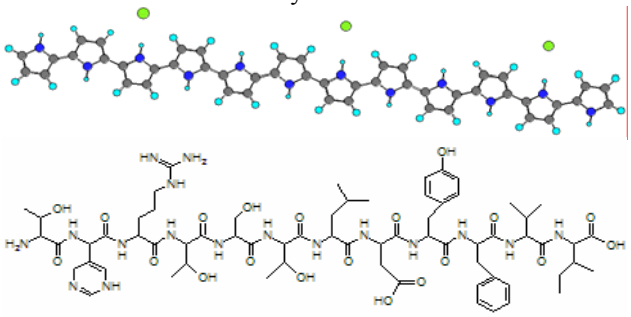


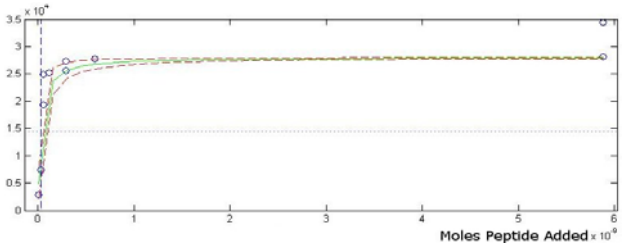
Figure 1: PPyCl (top) and T59 Peptide (bottom)

Methods:

Materials used include: PLGA (85/15), hemotoxylin, eosine, pyrrole (Sigma), QuantaBlu Kit (Pierce), NeutrAvidin-HRP conjugate, NeutrAvidin-Alexaflour 350 (Invitrogen), isofluorane, buphrenex, male Sprague Dawley rats (ARC Univ. of Texas).

A modified version of an ELISA was used to quantify the binding of T59 peptide to the PPy surface. We incubated a biotinylated version of the peptide on a PPyCl film and interacted that with a NeutrAvidin©-HRP conjugate and measured the activity via a fluorescent product, QuantaBlu Kit (Pierce).

Subcutaneous implants of PPyCl were laminated with PLGA on one side for mechanical strength and treated with the biotinylated T59 peptide. 10 μg/ml, 1 μg/ml, and untreated implants were used as well as a sham surgery. Male Sprague Dawley rats were each given three implants and one sham surgery. The implanted tissues were harvested at 1, 2, and 8 weeks. The tissue samples were fixed, sectioned, and stained with H &E.



Results/Discussion:

Shown are the results of a binding assay (*Figure 2*). Increasing amounts of biotinlyated T59 are added to a given area of PPyCl. These results are then analyzed by a non-linear fit and the disassociation constant is extracted from the fitted binding isotherm. The results for biotinlyated T59 attached to PPyCl yielded a disassociation constant (k_d) of ~50 nM. This suggests a strong interaction, only an order of magnitude weaker than many monoclonal antibody-ligand interactions.

Figure 2: T59/PPyCl Binding Curve

In vivo results (for 10 μg/ml T59) are included in the form of micrographs of H & E stained tissue (*Figure 3*). Full blinded grading and analysis is pending. However, an unblinded comparison shows no qualitative differences in inflammatory response or encapsulation of the implant, and there did not appear to be any obvious external inflammation greater than that observed in the sham surgeries.

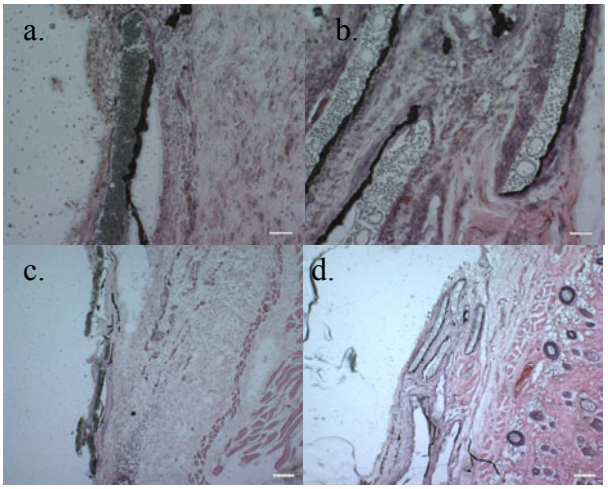


Figure 3: Subcutaneous implants of T59-modified PPy (a, c) and unmodified PPyCl (b, d). Scale bars = 20 μm (a,b) and 100 μm (c,d). The black material in the images is the PPy material.

Conclusions:

We describe quantification of the binding interaction between T59 and the conducting polymer PPy. We also show in our initial results that the T59-treated PPy is non-toxic and not inflammatory relative to an untreated PPy implant. These results support the use of specific peptides selected using phage display as a means to surface modify polymers, without altering the bulk properties of the polymers. Future work will focus on evaluating these materials for nerve regeneration.

References:

Sangvi, Miller, Belcher, and Schmidt. 2005. Biomaterials functionalization using novel peptide that selectively binds to a conducting polymer. *Nature Biomaterials* 4:496-502.