Corticosteroids Functionalized Poly(*N*-vinyl pyrrolidone) as a pH-sensitive Drug Delivery System at Neural Interface

Yu Cao, Wei He
Department of Materials Science and Engineering, University of Tennessee, Knoxville, 37996

Statement of Purpose: Neuroinflammation compromises the longevity of implanted neural electrodes by forming a tight encapsulating glial scar around the implant, and decreasing the population of healthy neurons at the interface.[1] It is highly desirable to mitigate neuro-inflammation with localized pharmacological inter-ventions without interfering with the electrical properties of the implant. The objective of this study was to synthesize an anti-inflammatory prodrug that can be "ferried" to the neural tissue around the interface using the implant as a temporary support, and can delivery anti-inflammatory drug based on the extent of inflammation. This report summarizes the synthesis, characterization, release study, and the bioactivity of the prodrug *in vitro*.

Methods: The anti-inflammatory corticosteroid drug, prednisolone, was selected for this study. The prodrug was synthesized using a carboxylated poly(*N*-vinyl pyrrolidone) and characterized with ^{1}H NMR, FTIR, and UV-Vis spectroscopy. To demonstrate that the prodrug can be temporarily integrated with the neural implant, a layer-by-layer (LBL) approach was applied. Using silicon substrates to mimic the neural implant, a prodrug containing film was prepared on the surface by alternating incubation of the substrates at pH = 3 in solutions of polyacrylic acid (PAA) and prodrug, respectively. Dissociation of the prodrug from the substrate was demonstrated under physiological condition, pH = 7.4. Bioactivity of the prodrug and its released drug molecule was evaluated in vitro by activating macrophages to simulate inflammation. RAW 264.7 macrophages were cultured and stimulated with 100 ng/mL lipopolysaccharides (LPS) (Sigma) to generate nitric oxide (NO). Upon stimulation, the cultures were simultaneously supplemented with either prodrug or released drug. The amount of NO production after 24 hr was determined by measuring the accumulated levels of nitrite in the culture medium with Griess reagent (Promega).Statistical analysis was performed using SAS software and significance was assigned for $p < 0.05$.

Results: Structure of the prodrug was shown in Figure 1. The synthesis scheme can be easily adapted for other drugs in the corticosteroids family, such as dexa-methasone and methylprednisolone, and the versatility in drug loading capacity can be achieved by adjusting the degree of functionalization. In the present study, the degree of drug conjugation was found to be 16% from the results of ^{1}H NMR and UV absorbance.

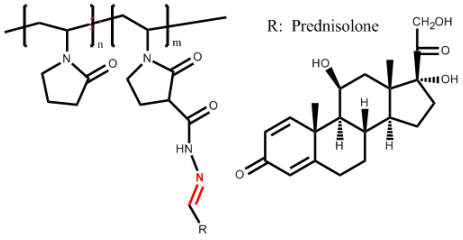

Figure 1. Structure of PNVP prodrug

Because of the repeating lactam functionality in the polymer structure, LBL assembly of prodrug can be prepared using hydrogen bonding. A linear growth of the multilayers was observed at pH = 3, where 8 bilayers of PAA/prodrug were 220 nm thick. At physiological pH, the layers disintegrated, releasing the prodrug and the underlying surface was recovered. Such feature will be beneficial to preserve the sensing function of the implants and can ease the concern of drug loading decreasing conductivity.

Bioactivity study using activated macrophages indicated whether the released drug retained its activity, and activity of the prodrug. Figure 2 showed the level of NO production, an important physiological messenger and effector molecule in neuronal tissue, from LPS activated macrophages. Day 0 data suggests that the prodrug exhibited anti-inflammatory effect, though the potency was lower comparing to treatment with similar level of free drug. When the prodrug was incubated at pH = 5.0, simulating tissue acidosis caused by severe inflammation, drug release was enhanced. NO production of cells treated with prodrug incubated for 10 days was significantly less than that of cells with 0 day sample, as the hydrazone bond between polymer and prednisolone would dissociate under acidic environment, leading to release of the more potent free drug.[2] Furthermore, no significant release was observed for physiological pH 7.4, shown from similar level of NO of day 10 sample and day 0 sample.

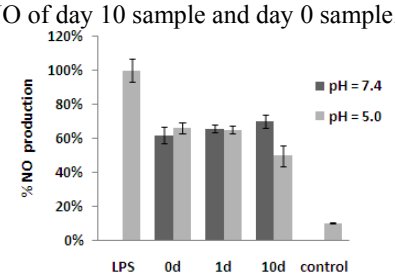

Figure 2. NO production by LPS activated macrophage with and without treatment of prodrug solutions incubated up to 10 day at pH 5.0 and 7.4. Cells without activation served as control.

Conclusions: Our study suggests corticosteroid functionalized PNVP prodrug can serve as a promising candidate for coating implants with dual pH-dependency system, in which the coating could dissociate from surface at physiological pH of 7.4 and dissociated prodrug could release drug in inflamed tissue (pH = 5.0). This novel dual pH sensitive strategy could possibly resolve the dilemma of increasing drug loading capacity and preserving conductivity of electrode in the treatment of neuroinflammation against implanted neutral electrodes.

References:

1. Polikov, V. S.; Tresco, P. A.; J. Neurosci. Methods 2005, 148 (1), 1-18.
2. Wang, D.; Miller, S. C.; Liu, X. M.; Anderson, B.; Wang, X. S.; Goldring, S. R., Arthritis Res. Ther. 2007, 9 (1),