Electrochemical Coating of Hyaluronic Acid on Conducting Substrates for a Potential Neural Electrode Application

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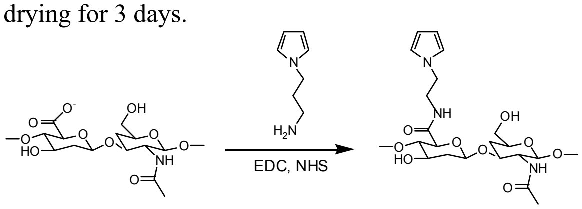
Abstracts: Neural electrodes have often faced severe loss of electrical activities after implantation in vivo. A dominating reason is the glial scar tissue formation on the surface of the implanted electrodes resulting in high electrical resistances and prevention of neuron growth near the electrodes. This glial scar formation is reported to be caused by astrocyte growth and activation similar to 'a foreign body reaction'. To make electrode surface less cell adhesive and non-immunogenic, we deposited hyaluronic acid (HA), on conducting electrode surfaces. To this end, we first synthesized a pyrrole-hyaluronic acid conjugate (PyHA) and electrochemically deposited conducting substrates whith this PyHA bearing electrically polymerizable pyrrole. HA deposition was confirmed and characterized using various immunofluorescence and spectroscopic techniques. Importantly, electrochemical impedance spectroscopy indicated that the PyHA-coating did not alter the electrical properties of the conducting substrates, ITO (indium tin oxide). In vitro culture study of rat cortical astrocytes on the substrates displayed that the PyHA-coated ITO prevented astrocyte adhesion and growth up to 3 weeks, whereas the cells adhered and proliferated very well on non-coated substrate control.

Methods: To synthesize the PyHA conjugate, coupling reaction was performed with 1 mg/ml HA, 0.8 mg/ml 3-aminopropyl pyrrole, 1 mg/ml NHS, and 2 mg/ml EDC in deionized water (pH 5.5) overnight, followed by dialysis (8 Kda molecular weight cut-off) for 3 days and freeze-drying for 3 days.



PyHA was electrochemically deposited on an ITO slide (a working electrode) using a potentiostat with the following cyclic voltammetry conditions: applied potentials of 0.0-1.0 V (vs SCE) with a Pt mesh counter electrode, a scan rate of 0.1 V/m, and 50 scans. The sample was washed extensively with water. Material characterization was performed including X-ray photon spectroscopy (XPS), water contact angle measurement, immunofluorescence with HA-binding protein, atomic force microscopy (AFM), and electrochemical impedance spectroscopy (EIS). For in vitro cell culture, rat cortical astrocytes were isolated from 2-day old rat cortex. Astrocytes were cultured on PyHA-coated ITO and non-coated bare ITO in DMEM medium supplemented with 10% FBS and 1% antibiotic solution. After incubation, the cells were fixed and stained for GFAP and nuclei. Cell morphologies and cell numbers on the substrates were assessed from optical and fluorescence images.

Results: PyHA conjugate was synthesized and used for the coating of conducting substrates. Electrically active pyrrole moieties on PyHA conjugates mediated the stable deposition on the conducting substrates, ITO. The PyHA-coated ITO displayed hydrophilic surfaces with a much smaller water contact angle than that of bare ITO. Also, AFM analysis indicated that the surfaces had smoother morphologies after the HA deposition, compared to non-coated ITO substrates. HA binding proteins and XPS studies confirmed the presence of HA on the substrate surfaces.

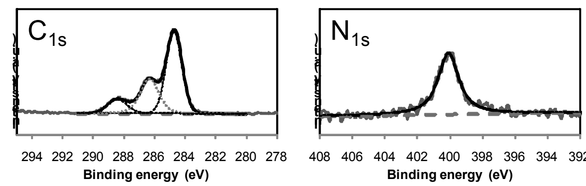


Figure 1. High resolution spectra of carbon and nitrogen atoms from the PyHA-coated ITO substrates.

The impedance spectrum, obtained from 100,000 Hz to 1 Hz, of the PyHA-coated ITO was very similar to that of non-coated ITO, indicating that no significant alteration of electrical properties was caused by the PyHA coating and that this deposition of PyHA would be a promising means to modify conventional metallic electrodes without impairing electrical electivity.

Rat cortical astrocytes did not adhere to the PyHA-coated substrates, whereas non-coated ITO allowed and supported the cell growth and proliferation. In addition, no migration to the PyHA-coated area was observed in vitro culture up t0 3 weeks.

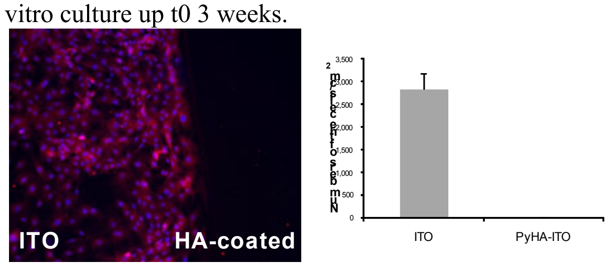


Figure 2. Rat cortical astrocytes cultured for 1 week. (Left) immunofluorescence images of astrocytes on the borders of bare ITO and HA-coated ITO stained for GFAP (red) and nuclei (blue). (Right) cell numbers on the substrates were counted after 1 week in culture.

Conclusions: Biocompatible polysaccharide, HA, was successfully deposited on conducting substrates without impairing the material's impedance. The stable ability to prevent astrocyte adhesion and migration was demonstrated. This study provides not only a technique to electrically modify conducting substrates with pyrrole-coupled biopolymers but also provide promising tools to design biocompatible electrodes for neural applications.