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Biocompatible molecularly imprinted polymers for the voltage regulated uptake and release of L-glutamate in neutral pH solutions

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ABSTRACT

A biocompatible device for the voltage dependent uptake and release of the neural transmitter L-glutamate in neutral pH solutions is demonstrated. The device consists of a gold electrode coated with molecularly imprinted, overoxidised polypyrrole (oPPy). It is shown here that oPPy can behave as an anion exchanger in neutral pH. The voltage dependent uptake and release of glutamate from the oPPy as well as the enantioselectivity of the polymer layer for L-glutamate over D-glutamate are investigated in neutral pH solutions using electrochemical quartz crystal microbalance techniques. The biocompatibility of the oPPy layer is demonstrated using retinae from young rats. The retinae were isolated and the dissociated cells were kept in culture for up to 1-week. The cells were exposed to the oPPy layers for 3 days, and there is no significant difference in the survival rate between the cells cultured on the oPPy layers and the control samples. Additionally the cell–polymer interface from cells grown directly on the oPPy layers is investigated using electron microscopy.

1. Introduction

An electrochemical device for the controlled uptake and release of biomolecules in physiological solutions is of great interest for application in biology and medicine. In this study a biocompatible "molecular switch" for the trafficking of biomolecules in neutral pH solutions is demonstrated. The device functions like a sponge, selectively taking up or releasing the target biomolecule as the result of an electrical signal, thereby varying the concentration of the biomolecule in the solution when the device is switched. No changes to the biomolecule occur as a result of the switching. The device has a simple structure, consisting of an electrode with a molecularly selective coating.

L-Glutamate was the target molecule for the molecular switch investigated in these studies. L-Glutamate is the primary neural transmitter for brain function. It is a small amino acid with zwitterionic properties: at pH values below 2.2 the amino group is protonized and the net charge on the molecule is positive. At pH values between 2.2 and 4.4 L-glutamate exists as a zwitterion. At higher pH values, the OH groups are dissociated and there is a net negative charge on the molecule. Extended elevated levels of L-glutamate are toxic to neurons, and so a device which regulates the concentration of L-glutamate should perform reproducibly and reliably for in vito applications. Fig. 1 shows the chemical structure of L-glutamate (right) and of D-glutamate (left). The molecules differ only in chirality, and the D-isomer was used in this study in order to test the selectivity of the molecular switch for L-glutamate.

The conjugated polymer polypyrrole (PPy) was investigated as the candidate material for the electrode coating in the "glutamate switch". PPy can be synthesised chemically or electrochemically by oxidation of the pyrrole monomer. When electrochemically synthesized, anions from the electrolyte solution are incorporated into the PPy backbone to preserve the charge neutrality of the polymer chain. The doped polymer is conductive and adheres to the surface of the working electrode (Smela, 1999; Vernitskaya and Efimov, 1997). Electrically degrading (overoxidising) the PPy after deposition leads to the expulsion of the anions from the polymer backbone, replacing them with carbonyl groups (Beck et al., 1987; Otero et al., 2004), resulting in a porous and insulating layer. In the literature it has been shown that overoxidised PPy (oPPy) has molecularly selective properties, and the voltage regulated enantioselective uptake and release of amino acids at low pH has already been demonstrated (Deore et al., 1999; Syritski et al., 2008; Kong et al., 2010).

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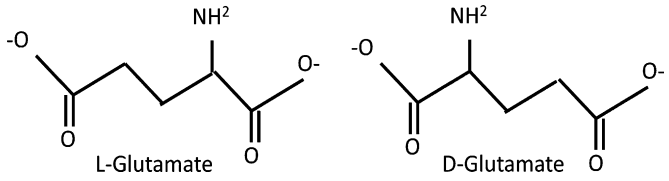

Fig. 1. The chemical structure of the enantiomers L-glutamate (left) and D-glutamate (right). At pH values below 2.2 the amino group is protonized and the molecule has a net positive charge. At pH values between 2.2 and 4.4 L-glutamate exists as a zwitterion. At pH values above 4.4 the OH groups are dissociated and the molecule has a net negative charge.

Biocompatibility is a major criterion to be satisfied by novel biomaterials. Conducting polymers, such as polypyrrole and poly(3,4-ethylenedioxythiophene) (PEDOT), have been receiving increasing interest in the last few years due to the high potential for this material to be used in bioapplications (Wang et al., 2004; Richardson-Burns et al., 2007; Wallace et al., 2009). Recent work has investigated the properties of PPy doped with biomolecules (Lui et al., 2009; Gelmi et al., 2010) including how variations in the preparation of the PPy influence the resulting biocompatibility of the material (Rammelt et al., 1999).

The characteristics of polypyrrole doped with biomolecules are dependent on the fabrication conditions as well as the dopant molecule. In previous studies we investigated the mechanism of the electrochemical deposition of polypyrrole doped with L-glutamate (von Hauff et al., 2008; Meteleva-Fischer et al., 2009). Additionally the proof of principle of the uptake and release of L-glutamate from molecularly imprinted polypyrrole (Fuchs et al., 2009) was shown. In this study we investigate the feasibility of molecularly imprinted oPPy as a glutamate switch in physiological conditions. The enantioselectivity of oPPy for L-glutamate over D-glutamate in neutral pH solutions is demonstrated. Biocompatibility tests with young rat retinae were performed and the results indicate a good toleration of oPPy by the cells. These results indicate that oPPy is an interesting candidate material for use in bionic devices and chemical implants.

2. Experimental

2.1. Fabrication and structural investigations of oPPy layers

Pyrrole (Py) (99%, extra pure, Acros organics and 98%, F.C.C., Sigma–Aldrich), L-glutamic acid monosodium salt monohydrate (≫98%, Fluka and ≥99%, Sigma), D-glutamic acid (≥99% (TLC), Sigma–Aldrich), and phosphate buffer (Merck, composition: $c(KH_2PO_4) = 0.026$ mol/l, $c(Na_2PO_4 = 0.41$ mol/l, pH = 7)) were commercially obtained. The pyrrole was vacuum distilled before use. Water used in this study was purified by a Milli-Q water system ($R = 18.2 \times 10^6\ \Omega$ cm). The solutions were flushed with argon for at least 5 min prior to use.

Glass electrochemical cells (three electrodes) were used for the investigations. The reference electrode used for all experiments was Ag/AgCl in 3 M KCl (aq) (CH Instruments) and all potentials in this paper are given in reference to this reaction. The counter electrode was platinum wire. Au plated glass substrates with a Cr adhesion layer were used as the working electrode for investigating polymer morphology. For EQCM investigations a Maxtex RQCM Quartz Crystal Microbalance Research System was used with gold-coated Maxtek 5 MHz-crystals as the working electrodes. As a retention system for the crystal the CHC-15 crystal holder and the GC-15 Glass Cell from Inficon were used.

The PPy was potentiostatically deposited at different potentials and all experiments were carried out in ambient temperature ($22 \pm 2\,^{\circ}C$) using a 0.5 M NaGlu(L) and 0.4 M pyrrole aqueous solution. To create L-glutamate complementary cavities in the electrochemical deposited PPy, the films were overoxidised galvanostatically in the phosphate buffer at a constant current of $I = 0.025$ mA/cm^2 until a potential of 1.2 V was reached (Syritski et al., 2008).

For polymer deposition and cyclic voltammetric investigations, an electrochemical workstation (Model 660C from CH Instruments) with accompanying software was used. For the EQCM studies a Maxtex Quartz Crystal Microbalance Research System with accompanying software was employed. For EQCM measurements, the electrochemical cell was assembled, and the system was allowed to come to equilibrium (30 min to 1 h) before measurement to guarantee frequency drifts below <1 Hz. Drifts in frequency due to temperature effects are negligible.

The morphology of the obtained films before and after the deposition was analysed with the scanning electron microscope Model Quanta 200 3D from FEI Company.

2.2. Retinal cell preparation

Wistar rat puppets (postnatal day 3–10) were decapitated in accordance with national and international guidelines and carefully enucleated. The removed eyeballs were hemisected, the retinae were detached from the pigment epithelium and incubated for 10–15 min in ice cold extracellular buffer solution (135 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.5 mM $MgSO_4$, 1 mM $NaHCO_3$, 25 mM Glucose, 10 mM 2-(4-(2-Hydroxyethyl)-1-piperazinyl)-ethanesulfonic acid (HEPES), 100×10 ml/l penicillin/streptomycin, pH adjusted with NaOH to 7.4, flushed with 5% CO_2/95% O_2). The solution was replaced by an enzymatic digestion solution containing: 137 mM NaCl, 10 mM $NaHPO_4$, 2 mM KH_2PO_4, 11 mM HEPES, 14 mM glucose, 1 mM EGTA, 2 mM DL-cysteine, 100×3.6 ml/l penicillin/streptomycin, 20 kU/l papain, and the retinae were incubated at 37 °C for 15 min. Thereafter the retinae were transferred into a Minimum Essential Medium Eagle (MEM) solution containing: 0.6 mg/ml DNAse, 3 mg/ml Ovomucoid, and in 3.5 mM $MgSO_4$, 25 mM HEPES, and carefully mechanically triturated. The dissociated cells were seeded in Petri dishes containing gold plated glass cover slips, each with 50% coverage of PPy and borosilicate glass blocks coated with PPy, respectively. The culturing medium consisted of MEM with: 40 ml/l horse serum, 40 ml/l foetal calf serum, 100×10 ml/l penicillin/streptomycin, 10 ml/l glutamine, and 6.5 μM insulin, and kept in an incubator for 10 days (37 °C, 5% CO_2). On culturing day 4 (DIV 4) arabinosylcytosine (1 μM) was added to suppress excessive growth of glia cells.

2.3. Biocompatibility tests

For biocompatibility tests the dissociated cells were kept in culture for 4 days in poly-L-lysine (1 mM) coated Petri dishes prior to exposure to PPy. Borosilicate glass blocks were coated with PPy on the bottom, carefully placed on top of the cultures and cultured for additional 3 days, allowing only a minimal cleft (<1 mm) between the cells and substrate.

After 3 days the cells were carefully enzymatically detached from the culture dish using accutase. To mark dead cells trypan blue was added to the cell suspension and the ratio between surviving and dead cells was quantified optically. As control a subset of the cultures was grown on uncoated glass blocks and processed identically using accutase and trypan blue staining.

2.4. Sample preparation for electron microscopy

To investigate the degree of contact between cell and PPy surface the cultured cells were plated directly on glass cover slips coated with gold and PPy, respectively, and cultured for 1-week.