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PEGylation of platinum bio-electrodes

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Keywords

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PEGylation of platinum bio-electrodes

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Abstract

Controlling protein interactions at the implanted electrode interface is becoming an important strategy for the management of foreign body responses that have proven to be detrimental to the long-term performance of neural prosthesis. In this study, PEGylation was conducted on platinum bio-electrodes to render the surface protein-resistant. The PEGylated electrode was investigated using a quartz crystal microbalance-dissipation, electrochemical impedance spectroscopy and cyclic voltammetry.

Keywords: PEGylation; protein adsorption; quartz crystal microbalance-dissipation; platinum electrode; neural prosthesis; cochlear implants

1. Introduction

The therapeutic efficacy of a chronically implanted neural prosthesis, such as the cochlear implant, is determined by the ability to effectively transfer charge across the electrode-tissue interface. A key factor is the host foreign body response that gives rise to fibrotic tissue encapsulation of the neural probe, consequently insulating the electrode from targeted neurons. Foreign body response typically arises from a biological cascade of non-desirable reactions, and is triggered by non-specific protein adsorption at the device interface. Controlling the protein interactions at the implanted electrode interface is thus becoming an important strategy for the management of adverse tissue responses around the device.

PEGylation is orignially a pharmeceutical term, referring to covalent modification of protein/peptide drugs with poly(ethylene glycol) derivatives (PEG, FDA-approved). There are more than 8 PEGylated phamarceuticals receiving regulatory approval by the US and/or EU.[1] PEG is well-known for low toxicity and immunogenicity; it is highly soluble in water, and forms a conformational cloud with high hydration numbers and polymer chain mobility, which is responsible for excellent protein-resistant properties.[2] In addition to drug delivery, PEG has been intensively explored as biocompatible, anti-fouling coatings for medical implants to improve the *in vivo* efficacies.[3]

The work reported here is a natural follow-up of our bioenginering effort involving the surface modification of cochlear implants for enhanced electro-neural interfacing[4]. PEGylation was conducted on platinum electrodes to render the surface protein-resistant. The resultant PEG

coating was investigated in terms of quartz crystal microbalance-dissipation (QCM-D), electrochemical impedance spectroscopy (EIS)[5] and cyclic voltammetry (CV).

2. Experimental

2.1 Materials

mPEG thiols (M_w 5000 Da) were purchased from Jenkem Technology Co., Ltd, China. Potassium hexacyanoferrate (III), potassium nitrate, bovine serum albumin (BSA) and fibrinogen from bovine plasma were from Sigma-Aldrich, Australia. Pt electrodes (0.5 mm diameter), similar size close to the electrode pad in cochlear implants, were custom-made from CH Instrument, Inc., USA. Artificial perilymph solution (ARF, pH 7.4) was prepared to simulate the body fluid in the cochlea, comprising NaCl (7.39 g/L), $KHCO_3$ (0.35 g/L), $NaHCO_3$ (2.02 g/L), $CaCl_2$ (0.08 g/L) and $NH_2C(CH_2OH)_3$ (0.61 g/L).

Pt electrodes were polished using aqueous alumina slurry, and cleaned using cyclic voltammetry in 0.5 M H_2SO_4 between -250 and 1200 mV (vs. an Ag│AgCl). The rinsed electrodes were treated with a deoxygenated mPEG thiol solution in Milli-Q water (1.0 mM) overnight at room temperature, rinsed and dried with N_2 and denoted as Pt-PEG.

2.2 QCM-D measurements

QCM-D measurements were undertaken using a Q-Sense E4 system (Q-Sense AB, Sweden) equipped with a multichannel peristaltic pump (IPC Ismatec SA, Switzerland). The QCM sensor was an AT-cut quartz-crystal with a 10 mm diameter Pt electrode with a fundamental resonance frequency of 5 MHz. For PEGylation, the Pt-coated sensor was equilibrated in Milli-Q water,

followed by the introduction of mPEG thiol (1.0 mM in Milli-Q water) over 4 h, and then a rinse with Milli-Q water until stabilised. For protein adsorption, both bare and PEGylated sensors were equilibrated in ARF (for BSA) or 0.9 wt% NaCl (for fibrinogen), loaded with a BSA solution in ARF (2.8 mg/mL) or a fibrinogen solution in 0.9 wt% NaCl (1.0 mg/mL) for 4 h, and then rinsed with ARF (for BSA) or 0.9 wt% NaCl (for fibrinogen) until stabilised. The Voigt model was selected to determine the hydrated layer mass (ng/cm^2) and thickness of the PEG or absorbed protein adlayers, using the 5th, 7th and 9th overtones.[6] The Q-tools software package v.3.0.10.286 (Biolin Sci, AB) was used for data modelling, with the input parameters for the layer density (1027 kg/m^3), fluid density (1020 kg/m^3), layer viscosity ($10^{-5} \leq 10^{-2}$ kg/ms), layer shear modulus ($10^4 \leq 10^7$ Pa), and mass ($10^2 \leq 10^5$ ng/cm^2).[6]

2.3 Electrochemical characterisation

A three-electrode electrochemical cell was used for all the electrochemical measurements, consisting of a Pt or Pt-PEG working electrode, a platinum mesh auxiliary electrode and an Ag | AgCl (3.0 M NaCl) reference electrode. EIS measurements were conducted on a Gamry EIS 3000^{TM}, at open-circuit potential with AC amplitude of 10 mV. CV experiments were performed using an EDAQ e-corder® electrochemical analysis system (EDAQ Pty Ltd, Australia).

3. Results and discussion

3.1 QCM-D

PEGylation of Pt electrode is realized via the formation of sulfur-platinum bonds[7]. QCM-D was employed to monitor the PEGylation process and protein adsorption behavior through changes in resonance frequency (Δf, coupled to layer hydrated mass) and energy dissipation (ΔD, coupled

to layer viscoelasticity). Injecting an mPEG thiol-containing solution onto a Pt-coated sensor elicits an immediate decrease in frequency (Fig. 1A), suggesting a rise in the layer mass attributable to the attached PEG and associated water, and the change in solute density above the sensor. This is followed by a slow small rise in frequency attributed to mass loss arising from dehydration of the resultant adlayer as a result of a further increased density of surface grafted PEG[8]. Rinsing the modified sensor with deionized water produces a further increase in frequency until it reaches a plateau, as a result of the removal of any reversibly bound PEGs, as well as the solute density change above the sensor.

The pronounced concurrent increase in dissipation upon PEGylation highlights the soft nature of the water-rich PEG coating. The dissipation for the PEG adlayer reaches its maximum after ~30 minute exposure to the mPEG thiol. The PEG adlayer is deemed a viscoelastic hydrogel due to the high degrees of hydration of PEG.[9,10] For instance, a number of studies reported on ≥ ~80 wt% water contents for surface-grafted PEGs.[11,12] By modeling the frequency and dissipation shifts using the viscoelastic Voigt model, the thickness and mass of the hydrated PEG were determined to be 8.0±0.4 nm and 822±46 ng/cm^2, respectively.

Albumin and fibrinogen were selected for protein adsorption studies. Albumin is one of the most abundant proteins in inner ear fluids[13]; while fibrinogen is a serum protein, playing a critical role in promoting inflammatory reactions to biomaterial implants[14]. The time-resolved adsorption kinetics of BSA and fibrinogen at a Pt electrode interface (Fig. 1B and C) illustrated the mass uptake took place predominantly within the first 10-15 minute protein exposure. After a thorough rinse to remove any weakly bound protein, the thickness and mass of the stabilized proteinaceous

layers are 6.3±0.4 nm and 719±43 ng/cm^2 for BSA, and 16.1±0.1 nm and 1855±1 ng/cm^2 for fibrinogen. The much higher uptake of fibrinogen, in comparison to BSA, is consistent with its larger molecular size and increased hydrophobicity. Further insight into the Pt interface modified either by the PEG or protein layer was obtained by examining the endpoint ΔD per respective adlayer mass (Δm), ΔD/Δm. In comparison to that of the PEG layer, the endpoint ΔD/Δm value decreases by a factor of 2.6 for the BSA layer, and by a factor of 3.0 for the fibrinogen layer (Fig. 1D). This implies that the adsorbed BSA or fibrinogen on Pt surface constitute a much stiffer layer, possibility with a lower water content.[15]

The PEGylated Pt interface demonstrates excellent resistance to both BSA and fibrinogen adsorption (Fig. 1B and C), with a typical endpoint Δf of <~2 for BSA uptake, and ~7.2 Hz for fibrinogen uptake. When fitted using the Voigt model, these correspond to a mass below ~27 ng/cm^2 for the absorbed BSA, and ~157 ng/cm^2 for the absorbed fibrinogen, indicating ≥~92% inhibition of protein adsorption at the electrode interface by PEGylation. However, these values serve only as a rough estimation, given the difficulty to attain accurate mass for modeling such small frequency changes.

3.2 Electrochemical characterization

Fig. 2A-D compares the CVs of bare and PEGylated Pt electrodes in 10 mM $K_3Fe(CN)_6$ before and after protein adsorption. The bare electrode CV shows quasi-reversible oxidation and reduction of the $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ at +285 and +150 mV, respectively (Fig. 2A). PEGylation of the electrode surface results in a small decrease in the peak currents (e.g., $i_{pcPt\text{-}PEG}/i_{pcPt} = 0.93$) and an increase in peak separation, reflecting an increased charge transfer resistance surface due

to the presence of PEG layer. The shape of the CV then remains largely unchanged upon exposure to BSA or fibrinogen (Fig. 2B). By contrast, the CV of the bare Pt electrode after exposure to BSA or fibrinogen becomes plateau-shaped with a pronounced reduction in the currents, emphasizing a strong blocking effect of the adsorbed proteinaceous layer (Fig. 2C). The retained electron transfer capability of the PEGylated electrode surface in a protein-rich environment as demonstrated in Fig. 2D, highlights the anti-fouling nature of the modified electrode interface, which is in good agreement with our QCM-D results. It is worthwhile to mention that the electrochemical behaviour of a redox probe chosen will depend on the chemical interactions of both redox states with the electrode surface.[16,17] The use of ferri-ferrocyanide as a redox probe in this study is justified as it allows comparison with a number of other studies that use the same probe[18,19].

PEGylation of the Pt electrode produces a moderate increase in impedance over a broad frequency range below ~10^4 Hz (Fig. 3A), and a negative shift in phase angle at 10^2–10^5 Hz (Fig. 3B). PEGylation also causes a small decrease in phase angle in the lower frequency region of 0.1-10 Hz. These characteristics are consistent with the presence of PEG at the electrode surface that reduces both the double layer capacitance and electrode conductance. For instance, PEGylation results in a decrease of ~54% at 1 kHz and ~40% at 10 kHz in double layer capacitance, and a decrease of ~49% at 1 kHz and ~59% at 10 kHz in electrode conductance. For cochlear implant electrodes, high-frequency impedance (≥ 1 kHz) is of importance as auditory neural stimulation wherein biphasic current pulses with 25-100 μs pulse width can be employed.[20] In this study, PEGylation elicits a more notable increase in the high-frequency impedance than protein adsorption, e.g., an increase of ~90% in impedance at 1 kHz and ~30%

at 10 kHz by PEGylation, as opposed to an increase of ~21% at 1 kHz and ~11% at 10 kHz by fibrinogen adsorption (Fig. 3C). The PEGylated electrode shows negligible changes in impedance after exposure to fibrinogen (Fig. 3D). Therefore it seems that the protein-resistant property of the PEGylated electrode is achieved with compromised impedance. However, this conclusion was drawn based on the result obtained in a much simplified *in vitro* condition. For cochlear implants, the *in-vivo* impedance has shown to be governed predominantly by the biological environment surrounding the electrode, such as fibrotic tissue response, and increase up to 6-7 folds after a 12-week implantation.[21,22] The strategy of PEGylation reported here provides some control over the evolution of the electrode-tissue interface by minimizing non-specific protein adsorption and subsequent foreign body response. Since the nature of this interface is critical to longer-term performance *in-vivo* studies are warranted in the future.

4. Conclusions

A simple method was developed for PEGylation of Pt bio-electrodes. The immobilized PEG coating is highly hydrated and permeable, with a thickness of ~ 8 nm, and can inhibit ≥ ~92% adsorption of both BSA and fibrinogen. Optimisation of the coating properties in terms of the *in-vivo* electrode performance is required in the future, and the knowledge gained will provide a basis to facilitate the ongoing development of cochlear implants, as well as other types of neural prostheses for improved electro-neural interfacing.

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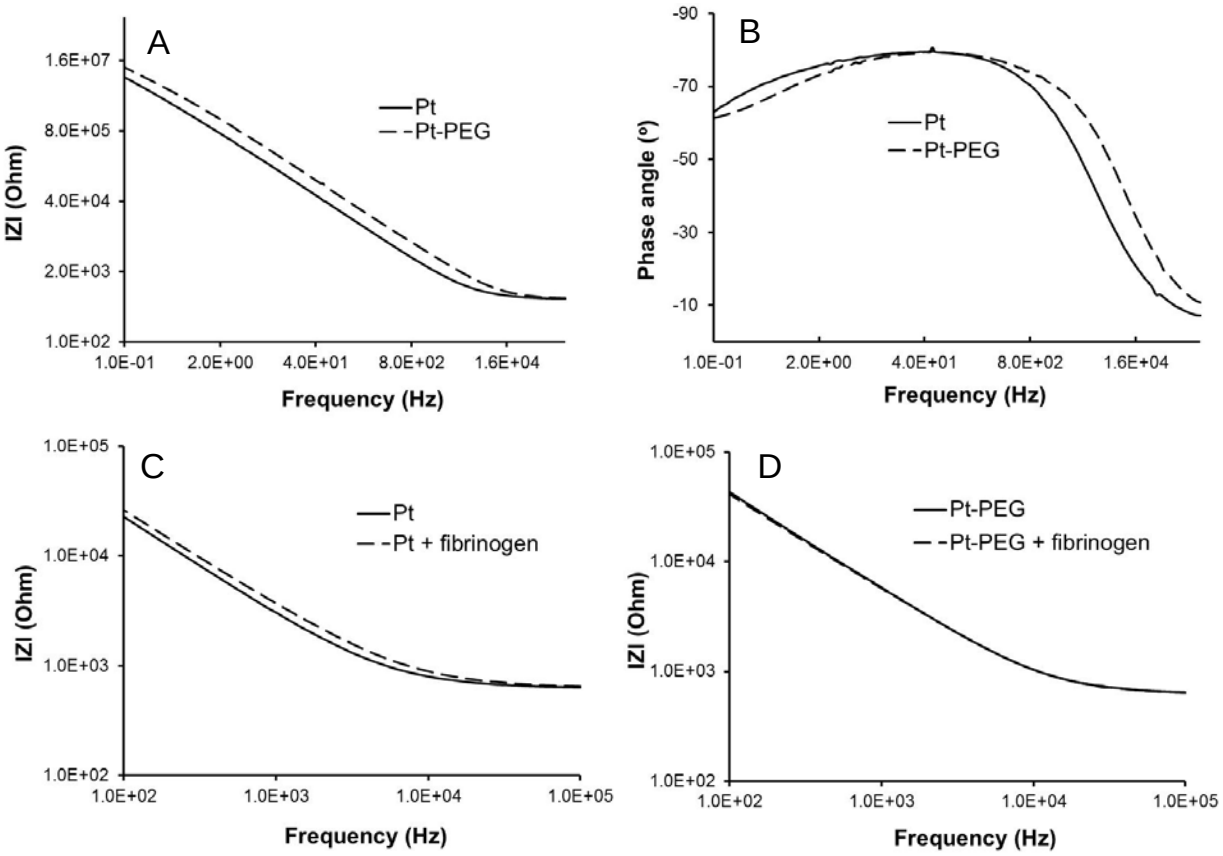

Fig. 3. The |Z| (A) and phase angle (B) plots of bare and PEGylated Pt electrodes; (C) comparison of the impedance of Pt before and after fibrinogen adsorption (Pt + fibrinogen); (D) comparison of the impedance of PEGylated Pt electrode before (Pt-PEG) and after fibrinogen adsorption (Pt-PEG + fibrinogen). EIS was undertaken at open-circuit potential in air-purged ARF at room temperature, over the frequency range of 0.1-100 kHz with AC amplitude of 10 mV. Protein adsorption procedure: Pt/PEG-Pt electrode was soaked in a fibrinogen solution (1.0 mg/mL) in 0.9 wt% NaCl for 2 h, and then rinsed with deionized water and dried with N_2.

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