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Different synthesis methods allow to tune the permeability and permselectivity of dopamine–melanin films to electrochemical probes

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ABSTRACT

Different methods to prepare melanin films by dopamine oxidation are compared with respect to their permeability to electrochemical probes being either neutral or carrying a positive or a negative charge. To examine charge-dependence of the permeability differently charged probes of similar size (hexaammineruthenium, ferrocenemethanol and hexacyanoferrate) are employed. We deliberately not investigated the permeability of metal cations through the melanin films owing to their possible complexation by the catechol groups of melanin. The films prepared by solution oxydation methods (O_2 or Cu^{2+} as oxidants) are impermeable to hexacyanoferrate as soon as their thickness reaches 5–10 nm but remain permeable to hexaammineruthenium and ferrocenemethanol up to thickness reaching 25–40 nm before becoming also impermeable. The melanin films prepared by electrochemical deposition display a marked difference; they remain permeable to hexacyanoferrate up to 35 nm in thickness and to hexaammineruthenium and ferrocenemethanol up to the maximal thickness that can be reached by this deposition method. When the maximal film thickness of 45 nm is reached the film becomes also impermeable to these two redox probes. These results are explained on the basis of structural differences of the melanin films at the molecular level. The permselectivity of the melanin coatings is related to their negative surface charge density at the working pH of 7.5.

1. Introduction

Biomaterials coatings are of fundamental importance for the development of new biomedical devices, because surface interactions often determine the success of a material in contact with a biological environment. In this context we have investigated the properties of synthetic melanin films made by dopamine oxidation, a new bio-inspired coating method first described in 2007 [1]. Compared to other coating techniques for biomedical applications, dopamine–melanin films have the advantage to be easily prepared in a single step and allow to coat the surface of almost all classes of known materials [1]. Furthermore the chemical groups present at their surface allow for various secondary functionalisations [1], for example amine binding to catechol groups [2]. The present communication treats the permeability of dopamine–melanin coatings to electrochemical probes at physiological pH. The permeability properties of such coatings are important for a wide range of possible applications from separation membranes in fuel cells [3] to electrochemical dopamine detection [4] and for testing if melanin coatings could be used in the inhibition of corrosion. It would be desirable that the melanin coatings produced through different deposition methods display some permselectivity. It is the aim of this communication to demonstrate that melanin films produced using oxygen, Cu^{2+} or the working electrode of a cyclic voltammetry setup are impermeable to the negatively charged hexacyanoferrate and become impermeable to the positively charge rutheniumhexaamine and the neutral ferrocenemethanol only for much higher film thicknesses. Our experiments show also that the films produced by electrodeposition need to be much thicker than those produced using oxygen or Cu^{2+} as oxidants to become impermeable to hexacyanoferrate. Finally the melanin film prepared by electrodeposition become impermeable to rutheniumhexaamine and ferrocenemethanol only when the film growth stops, namely at around 45 nm. Our data show that the permeability of melanin coatings is largely dependent on the deposition method even if all the deposits have a similar surface morphology as measured by Atomic Force Microscopy.

2. Experimental

All solutions are prepared using ultrapure water with a resistivity of 182 kΩ m from a Milli Q Plus water purification system

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(Millipore, Billerica, Massachusetts, USA). The pH of the solutions is measured with a HI8417 pH-meter (Hanna Instruments, Woonsocket, Rhode Island, USA) and adjusted by addition of hydrochloric acid solution (37%, Sigma–Aldrich, St. Louis, Missouri, USA, ref. 258148).

The permeability of dopamine–melanin deposits to the neutral ferrocenemethanol ($C_{11}H_{12}FeO$, Sigma–Aldrich, ref. 335061), the anionic hexacyanoferrate ($K_4Fe(CN)_6$, Sigma–Aldrich, ref. P9387) and the cationic hexaamineruthenium ($Ru(NH_3)_6Cl_2$, Sigma–Aldrich, ref. 303690) is determined by cyclic voltammetry (CV) in a conventional three electrode set-up (CH Instruments, Austin, Texas, USA, model CHI 604B) as described previously [5,6]. The reference and counter electrodes are an Ag/AgCl electrode (CHI 111) and a platinum wire (CHI 115). The working electrodes are made of amorphous carbon (CHI 104). They are polished on γ-alumina powder with a particle diameter of 50 nm (Buehler, Lake Bluff, Illinois, USA, ref. 40-6325-008). Three successive polishing steps of 2 min are performed, separated by rinsing with water. Then the electrodes are sonicated twice during 3 min in a Transonic TI-H-50 sonicator (Laval Lab, Laval, Canada) at a frequency of 130 kHz in water.

The capacitive and faradaic currents are measured by cycling the potential versus Ag/AgCl between −0.1 V and 0.65 V at a scanning rate of 0.05 V/s. This is a potential range allowing the detection of all three redox probes in aqueous solution at pH 7.5. We avoid working at higher potentials even in the presence of a melanin coating at the electrode in order to avoid the oxidation of water. Note that we choose iron complexes and a ruthenium complex as redox probes rather than the corresponding cations, Fe^{2+} or Ru^{2+}, owing to the known ability of melanin to complex metal cations [7]. Such a complexation process could influence their redox behaviour and hence the apparent film permeability.

Before the permeability measurements, all the solutions are deoxygenated by means of nitrogen bubbling for at least 5 min. CV is first performed on the pristine electrode to measure the capacitive currents in buffer solution (10 mmol/L Tris + 0.15 mol/L $NaNO_3$, pH 7.5). Faradaic currents are measured in the same buffer solution in presence of 1 mmol/L of the selected electrochemical probe. Then the working electrode is coated with melanin and rinsed with buffer solution before performing again CV as on the melanin coated electrode.

The permeability of melanin films is measured by the relative oxidation and reduction peak currents of the electrochemical probes. Therefore the peak currents after melanin deposition are divided by the peak currents measured on the same electrode before melanin deposition.

Melanin films are deposited by four different methods, which differ mainly in the way melanin formation is initiated by the oxidation of dopamine. Oxygen (methods *A* and *B*), copper(II) ions (method *C*) or the working electrode of an electrochemical cell (method *D*) serve as oxidising agents:

In method *A* [5,6], the substrates are successively immersed for $n \times 5$ min in freshly prepared dopamine hydrochloride (Sigma–Aldrich, ref. H8502) solutions at a concentration of 2 g/L in 50 mmol/L Tris buffer (tris(hydroxymethyl)aminomethane, Sigma–Aldrich, ref. T1503) at pH 8.5 in contact with ambient air without stirring. This method has been shown to allow for the deposition of melanin film which continues to grow linearly with the number of deposition steps up to at least $n = 85$ (thickness of 45 ± 5 nm) [8].Method *B* [1,7] is similar to *A* but the substrate is immersed in only one single dopamine solution continuously aerated with an aquarium pump (RENA Air 50, Mars Fishcare, Metz, France).In method *C* [9], 30 mmol/L of copper(II) sulphate ($CuSO_4$, Sigma–Aldrich, ref. 61230) are added to the dopamine solutions that are not aerated but deoxygenated before and kept under nitrogen during the deposition experiment. Due to the addition of copper sulphate the pH of the solutions decreases to about 4.5 and is not readjusted.

In deposition methods *A*, *B* and *C*, melanin deposits form progressively at the substrate/solution interface at the same time as melanin grows in solution. Anyway the solution undergoes a progressive colour change from colourless to pink (in about 1 min in oxygenated solutions) to brown and finally to black. To avoid the possible role of melanin growth in solution on the deposition of melanin at the substrate/solution interface, we also deposited melanin through an electrode selective electropolymerization, namely by method *D*.

In method *D* [10] dopamine–melanin is electrodeposited within the electrochemical cell. The potential (vs. a Ag/AgCl reference electrode) is cycled between −0.4 V and 0.3 V at a speed of 0.01 V/s in a deoxygenated dopamine hydrochloride solution (0.5 g/L in 10 mmol/L Tris, pH 7.5).

This potential window was selected owing to the electrochemical activity of dopamine, as shown in the results section and in accordance with the experiments of Yao and co-workers [10]. The solution contains additionally 150 mmol/L sodium nitrate ($NaNO_3$, Sigma–Aldrich, ref. S5506), and it is kept under nitrogen for the whole deposition time to avoid spontaneous oxidation of dopamine in solution. Under these conditions, and contrarily to deposition methods *A*, *B* and *C*, no melanin is formed in the bulk of the solution as judged by its transparency during the whole duration of the experiment. These films were characterized by topography images in contact mode with a Nanoscope IV instrument (Veeco, Santa Barbara, California, USA) at a scanning frequency of 2 Hz. The employed cantilevers (Veeco, ref. MSCT-AUWH) have a nominative spring constant of 0.01 N/m and are terminated with a silicon nitride tip having a nominative radius of curvature of 10 nm.

For methods *A*, *B* and *C* the thickness of the melanin deposits is calculated from the reaction time using data from previous publications [5,6,8]. The growth kinetics was investigated by means of ellipsometry (PZ 2000, Horiba-Jobin Yvon, France) after drying the films under a stream of nitrogen. The ellipsometric thickness was found to be consistent with scratch height measurements performed with Atomic Force Microscopy [5,6].

To determine the thickness of melanin deposits by method *D*, experiments are carried out in the electrochemical module (QEM 401, Q-Sense, Göteborg, Sweden) of a quartz crystal microbalance with dissipation monitoring (QCM-D, model E4, Q-Sense). Here, a gold-covered quartz crystal serves as a working electrode and the counter electrode is a platinum plate parallel to the crystal surface. The reference electrode is an Ag/AgCl electrode. The changes in oscillation frequency of the quartz crystal are followed at its third, fifth and seventh overtone (i.e. close to 15 MHz, 25 MHz, 35 MHz). Since the reduced frequency changes $\Delta f_\nu/\nu$ (ν: overtone number) overlap and the dissipation remains small, the Sauerbrey approximation [11] is used to calculate the adsorbed mass per surface area Γ:

$$\Gamma = -C \cdot \frac{\Delta f_\nu}{\nu} \tag{1}$$

where the value of the proportionality constant *C*, is given by QSense to be equal to 16.6 ng cm^{-2} Hz^{-1}.

Furthermore Γ and the density ρ of the deposit (previously determined as (1.2 ± 0.1) g/cm^2 [5]) are used to calculate the film thickness:

$$d = \frac{\Gamma}{\rho} \tag{2}$$

Impedance spectra of the melanin films were measured on the amorphous carbon working electrode after a given deposition time (for melanin films made according to methods *A*, *B* and *C*) or after a given number of CV cycles (for melanin films made according