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The long-range effect induced by untying hydrogen bonds for single cell test using SECM

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ABSTRACT

The enhanced positive feedback current of scanning electrochemical microscopy (SECM) is observed at a large separation between the tip and the substrate surface in the case of a 3-mercaptopropionic acid (3-MPA) self-assembled monolayer (SAM). The effect that induces the large separation (named the long-range effect) is confirmed by SECM and quantitatively studied by electrochemical quartz crystal microbalance (EQCM). A key finding is that the long-range effect results from untying hydrogen bonds beforehand formed between the hydroxyl group of tip redox species and the carboxyl group of 3-MPA SAM. It is found that the long-range effect is increased with the packing density of the 3-MPA SAM. On the basis of these findings, the long-range effect is applied to the nondestructive test of living cells using SECM.

1. Introduction

Scanning electrochemical microscopy (SECM) has evolved as a successful test technique for characterization and electrochemical kinetic study of surfaces [1,2]. Enhancing the feedback current of SECM, especially the positive feedback current, is one of the main directions of research over the past decade [3,4]. The in-situ surface interrogation mode of scanning electrochemical microscopy (SI-SECM) operates a transient positive feedback current for the characterization of adsorbed species on the substrate electrodes, providing opportunities for fundamental breakthroughs in electrochemistry [5,6]. This mode of SECM requires a current impulse to activate the adsorbed species on the substrate, and the tip-substrate separation (d) is less than one radius of the tip (a), $d/a < 1$ [7]. This approach is however not ideal for surface investigations. Hence a novel approach that allows a larger tip-substrate separation and provides a sustainable positive feedback current, would have a wider significance on SECM. In these respects, it is necessary to create a new way to extend the diffusion distance of redox species and increase the concentration of redox species in front of the tip.

The self-assembled monolayer (SAM) bonded on all kinds of material surfaces, has become increasingly popular and better known over the past twenty years [8–11]. The bio-applications of SAM have been recognized and studied extensively [12–15]. This suggests enormous potential of the functional SAMs for clinical applications. The physical adsorption, covalent and covalent coordinate bond methods have been developed for molecules immobilization onto SAM [16,17], such as covalent immobilization of proteins [18]. Among the intermolecular interactions developed for molecules immobilization on SAM, those based on weak interactions still need to explore [19].

Hence a new way is desirable to take advantage of the long-range interactions (e.g. hydrogen bonding) between the tip redox species and terminal groups of SAM on the substrate electrode. In consideration of the long-range interactions, ferrocenemethanol (described as $FcCH_2OH$) is selected as the tip redox species. This redox species benefits the formation of hydrogen bonding and electrostatic attraction because of the obvious distinction of electric charge between the reduced form ($FcCH_2OH$) and the oxidized form (Fc^+CH_2OH). Besides, the hydroxyl group in $FcCH_2OH$ molecule provides the foundation for formation of hydrogen bonds [20]. It is common to graft molecules via

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intermolecular interactions with the aid of modified layers [21,22]. The 3-mercaptopropanoic acid (described as 3-MPA) is one of the popular functional molecules to graft protein molecules, and possesses a carboxyl group in the terminal end [23].

If the hydrogen bonding interaction between $FcCH_2OH$ and 3-MPA is able to improve the concentration of redox species on the substrate surface and then release the redox species into the hemispherical diffusion profile of the tip by untying hydrogen bonds, the positive feedback current will be enhanced. That is to say, the tip could be positioned further to obtain a feedback current in the same intensity. This is beneficial for nondestructive test of living cells. Therefore, it becomes a useful strategy that initially adsorbs the tip redox species by hydrogen bonds and then releases them in the SECM scanning process by disturbing the hydrogen bonds.

In the neutral medium, like pH 7.0, the ionization of the weak acid 3-MPA is feeble. The oxidation product of tip redox species, Fc^+CH_2OH, facilitates the ionization of the carboxyl group by attracting the negative charged carboxylate ions. This leads to untying of hydrogen bonds between 3-MPA and $FcCH_2OH$. Consequently, the released $FcCH_2OH$ molecules will diffuse to the tip and enhance the feedback current of the tip. For these reasons, a new mode of SECM based on SAM modified substrate electrode is applied to our work. In the SECM experiment (Scheme 1), the substrate gold electrode is modified with SAM of 3-MPA which possesses a carboxyl group in the terminal end. $FcCH_2OH$ that equips with a hydroxyl group acts as the tip redox species. Before the test of the approach curve, a certain amount of tip redox species $FcCH_2OH$ are bonded on the 3-MPA SAM by hydrogen bonds. Interestingly, we find that the positive feedback current is enhanced, resulting from untying hydrogen bonds beforehand formed between the tip redox species and the 3-MPA SAM. The ionization of the carboxyl group is driven by the electrostatic attraction between Fc^+CH_2OH and the carboxylate ions. As a result, the released redox species diffuse into the range of the hemispherical profile in front of the tip. This brings to an interesting phenomenon that the tip feedback current of approach curve continuously increases in a very large tip-substrate separation. The effect that causes the feedback current increasing from 10 to 60 times radius of the micrometer tip is defined as the long-range effect. To the best of our knowledge, this is the first work to enhance the feedback current of SECM by using a SAM. Furthermore, the resulting long-range effect is applied to the single cell test using SECM which is beneficial for cell tests without affecting the natural metabolic pathway and cell morphology.

2. Experimental

2.1. Chemicals and materials

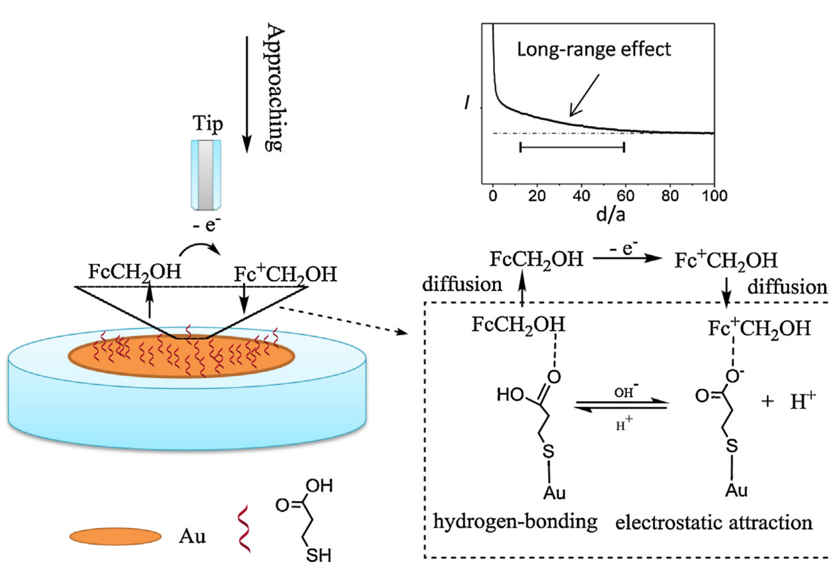
3-mercaptopropanoic acid (3-MPA), ferrocenemethanol ($FcCH_2OH$), potassium ferricyanide, potassium ferrocyanide and potassium chloride were purchased from J&K Scientific Co., Ltd., (Beijing, China). All chemicals were used with no further purification. Fetal bovine serum, Dulbecco's modified eagle's medium (DMEM) were purchased from Gibco Life Technologies (USA). Water ($18.2\,M\Omega\,cm^{-1}$) used in the experiments was generated by a Milli-Q filter (Research UV, Hetai Instrument Co. Ltd., Shanghai, China). The gold disk electrode, Ag/AgCl reference electrode and the platinum SECM tip with a radius of $5\,\mu m$ (Part number: CHI116) were purchased from CH instruments, Inc.

2.2. Preparation of the substrate electrode with the 3-MPA SAM

The polycrystalline gold disk electrodes were polished with alumina slurry to achieve a mirror-like surface and then cleaned by sonication. They were then washed with water and etched in 0.05 M H_2SO_4 solution by cycling the potential from 0 to 1.5 V (vs. Ag/AgCl, sat. KCl) at a scanning rate of $50\,mV\,s^{-1}$ and dried with pure nitrogen gas. 3-MPA SAM was prepared on clean surface of polycrystalline gold disk electrode. The procedure involved the immersion of the gold electrode into a 0.2 M solution of 3-MPA in ethanol at room temperature for 2 hours, followed by ultrasonic cleaning in water.

2.3. Electrochemical measurements

Electrochemical measurements were carried out with CHI920C scanning electrochemical microscopy. For cyclic voltammetry, a gold disk electrode was employed as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl electrode with saturated KCl solution as the reference electrode. For the SECM approach curve test, a micrometer Pt disk electrode of $10\,\mu m$ diameter was employed as the SECM tip and a 3-MPA SAM gold electrode as the substrate electrode. Electrochemical Impedance Spectroscopy measurements were performed in an



Scheme 1. Schematic representation of the long-range effect induced by untying hydrogen bonds between $FcCH_2OH$ and 3-MPA with SECM approach curve test.