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A field effect transistor biosensor with a γ-pyrone derivative engineered lipid-sensing layer for ultrasensitive Fe^{3+} ion detection with low pH interference

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ABSTRACT

Field effect transistors have risen as one of the most promising techniques in the development of biomedical diagnosis and monitoring. In such devices, the sensitivity and specificity of the sensor rely on the properties of the active sensing layer (gate dielectric and probe layer). We propose here a new type of transistor developed for the detection of Fe^{3+} ions in which this sensing layer is made of a monolayer of lipids, engineered in such a way that it is not sensitive to pH in the acidic range, therefore making the device perfectly suitable for biomedical diagnosis. Probes are γ-pyrone derivatives that have been grafted to the lipid headgroups. Affinity constants derived for the chelator/Fe^{3+} complexation as well as for other ions demonstrate very high sensitivity and specificity towards ferric ions with values as high as 5.10^{10} M and a detected concentration as low as 50 fM.

1. Introduction

The development of point-of-care and self-monitoring approaches in medical practices underline the needs of reliable real time sensing devices and require the use of non-intrusive technologies such as a wireless sensor network. Indeed, up to now, most of biomedical tests are being realized in hospitals and require large and complex equipments. Operation of such complicated systems usually requires high skilled technicians and can be time consuming. All those factors lead to a high cost for medical tests that prevent a large population from having adequate health care service. This problematic has lead to the development of emerging techniques with highly specific features (Walker et al., 2000). The development of field effect transistors based sensors has risen as one of the most promising approaches for the future of biomedical diagnosis and monitoring (Kuila et al., 2011; Li et al., 2013; Lerner et al., 2013). They fulfil the requirements in terms of fabrication cost and size, as well as, in term of easiness to use (Bernards et al., 2008; Khodagholy et al., 2012; Wijaya et al., 2010). All these features make them suitable for high throughput fabrication and individual use by patients.

Sensing field effect transistors are currently based on metal-oxide-semiconductor field effect transistors (MOSFET) technology (Chen et al., 2011a; Collet and Vuillaume, 1998; Bergveld, 1986). Instead of a metal layer used as gate in MOSFET, a BioFET sensor operates with a reference electrode dipped into an electrolyte solution (Schöning and Poghossian, 2002). Nevertheless, for both types of devices, the electrical field across the gate dielectric controls the conductivity in the transistor channel, between source and drain. In classical MOSFET, this electric field is controlled by application of a potential difference between the gate and the source (or drain). First transistor-based sensors were developed in the mid 1960s for the detection of ions and have been especially used as pH sensors (Chang et al., 2010). These so called ISFETs (Ion-Sensitive Field Effect Transistors) are usually comprised of an oxide gate dielectric bearing hydroxyl groups at the metal–solution

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interface. Their sensing properties rely on the amphoteric property of the OH functions that can depending on the pH, either gain or lose reversibly a proton into $-OH_2^+$ or $-O^-$ respectively, at the metal–electrolyte interface, causing therefore strong changes of the density of surface charge of the gate dielectric. The response mechanism to changes in ion concentrations of such system is mainly attributed to the influence of ion concentration on the potential drop across the electrolyte within the diffuse layer, and it can be identified as the surface potential of the oxide layer on the electrolyte side, ψ_0. These surface potential fluctuations will lead therefore to both a modulation of the electric field across the oxide layer and of the conductivity of the channel that can then be easily measured (Lin et al., 2013a; Presnov et al., 2013; Bae et al., 2013). More recently, graphene based ISFET have also been developed for pH sensing. In such device the sensing performances are related to the changes in graphene conductivity when the pH of the buffer is changed (Kiani et al., 2013; Ohno et al., 2010). Moreover, ISFETs turned out to be very useful in biology to measure for instance the biological activities of cells (Lee et al., 2009a or enzymes (Lin et al., 2013b). As exemplified hereby, Sakata et al. (2011) have measured in real-time the respiration activity of fertilized ova using such device. Indeed, the carbon dioxide produced by the cell metabolism is released in the extracellular medium and combines with water to form carbonic acid which freely dissociates into $H^+ + HCO_3^-$ resulting in a protonation of the hydroxyl groups at the surface of the oxide gate dielectric and a conductance modulation. However, despite their interest of use in research labs, this device configuration has a number of potential limitations that are unsuitable for the development of sensors with high specificity and sensitivity. De facto, in monitoring applications, very small variations of the pH analyte (blood, urine, plasma), which is very likely to happen when a patient is being treated with drugs, will induce undesirable changes of the channel conductivity. Additionally, this type of device is therefore not advisable for the detection of specific objects (molecules, ions) in a solution of unknown composition and some alternatives have to be found. To overcome and solve such drawbacks, technological solutions have been envisioned and tested like the, immobilization of specific probes and/or biomolecules onto the gate-dielectric surface. This alternative approach was successfully developed for the fabrication of bio-FETs (biologically modified field-effect transistors) with the use and grafting of biomolecules (Uslu et al., 2004; Wu et al., 2011; Tatikonda et al., 2013; Hammock et al., 2013; Lee et al., 2009b), leading to sensors with higher specificity and selectivity. Although the use of such probes will lead to an increased selectivity, their unique presence is insufficient to counter-act the effect of pH as they are often themselves sensitive to pH. To circumvent these side effects and gain stability in a wide range of pH we propose to change the chemical properties of the gate dielectric. Accordingly, we demonstrate herein that the use of a monolayer of engineered lipids as gate dielectric instead of an oxide (Nguyen et al., 2013 which was further stabilized by a two-dimensional polymerization in the plane of the layer (Charrier et al., 2010; El Zein et al., 2012) leads to a non-sensitive pH dielectric compared to other systems. Furthermore, electrical characteristics measurements have shown that such monolayer of thickness ~2.7 nm behaves as a good dielectric, with low leakage current and relatively high voltage electrical breakdown (Dumas et al., 2011). Therefore, these unique properties make this dielectric a system of choice for the fabrication of reliable and specific sensors. In this study the sensor was developed for the detection of ferric ions (Fe^{3+}), and the specificity of the detection is ensured by the grafting of specific probes to the lipids, in this case a γ-pyrone derivative chelator.

In the following, we focus on the properties of our sensor and present results on sensitivity and specificity towards Fe^{3+} ions. We first demonstrate that the engineered sensing layer constituted of the lipid layer and the hydroxyl γ-pyrone derivative is not sensitive to pH change in a wide range of pH therefore fulfilling the conditions for high sensitivity and specificity measurements. Accordingly, we show a very high sensitivity of the sensor which we attribute to a high affinity constant of the chelation process between chelators immobilized on the lipids headgroups and ferric ions. Finally, the specificity towards Fe^{3+} ions is also investigated.

2. Materials and methods

Sub-sections regarding material, device fabrication, and the formation of the lipid monolayers are to be found in the supplementary information file.

2.1. Preparation of the modified lipids with γ-pyrone

Under physiological conditions, γ-pyrone derivatives (L) are known to show high affinity towards iron ions (Kontoghiorghes, 1988; Moggia et al., 2006, 2009; Santos, 2002; Liu and Hider, 2002; Thirumurugan and Perumal, 2011). Indeed, they have the ability to form five-membered chelate rings with iron in which the metal (M) is coordinated by two vicinal oxygen atoms leading to neutral and stable [3L:1M] complexes. The synthesis and characterization of the modified lipid with γ-pyrone was described in detail elsewhere (Nguyen et al., 2013) (Fig. 1a). First, the phosphatidylcholine head-group of the commercially available DC8,9PC **1** was selectively cleaved with phospholipase C affording **2** in quantitative yield. The later was then reacted with the γ-pyrone derivative **3** leading to the end-capped lipid **4** in almost quantitative yield. Compound **3** was synthesized in 2 steps from commercially available kojic acid using standardized procedures (Imafuku et al., 1979; Ma et al., 2004; Ellis et al., 1996.

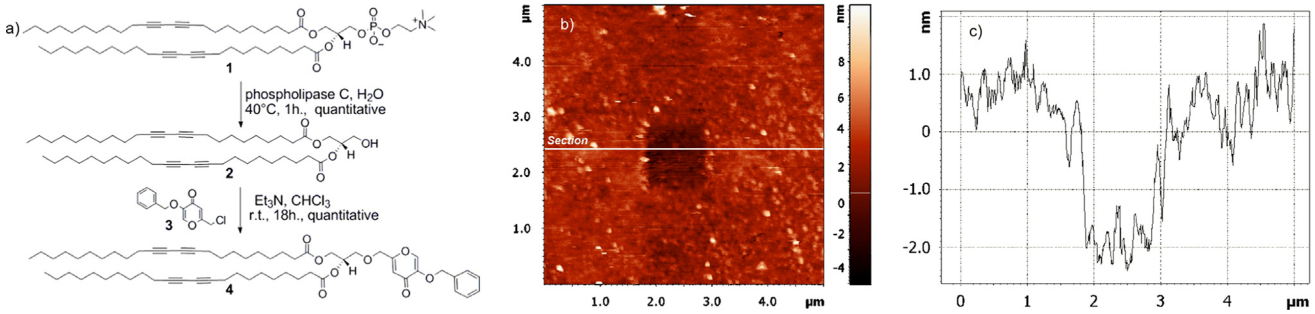

Fig. 1. (a) Synthesis of the modified lipid **4**, (b) AFM image of a lipid **4** monolayer after making a hole with an AFM tip. The cross-section of the hole (c) allows the estimation of the monolayer thickness to ~2.7 nm.