

Monitoring of hydroxyapatite crystal formation using field effect transistor

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Abstract

In this study, hydroxyapatite (HAP) crystal formation was monitored using extended-gate field effect transistors (FETs) modified with a variety of alkanethiol self-assembled monolayers (SAMs) on the gate surface. As a result of that, the gate surface potential at the carboxyl and amino terminated SAM-gated FETs increased in the simulated body fluids (SBF) as the HAP crystals were growing on the gate surface. Moreover, in the carboxyl terminated SAM-gate FET, the increasing rate and shift of gate surface potential of FET were found to depend on the concentration of calcium in SBF. It was suggested that the process of HAP crystallization at the SAM-modified surface could be detected by the FETs. Thus, the FET device, which enables to easily detect ionic charges in a real-time and label-free manner, is useful as a method to evaluate biomaterials based on biomineralization such as bone regeneration process.

1. Introduction

A field effect transistor (FET) is able to detect the ion changes at gate surface so that has been studied as sensitive biosensor with detecting small organic molecules or ions [1-4]. The electrical events at the gate/solution interface are amplified and transduced as the shift of gate surface potential owing to chemical or biochemical events. Ion-sensitive FET (ISFET) is cited as the most representative FET device, because the gate insulator such as Si_3N_4 and Ta_2O_5 has hydroxyl groups in a solution resulting in the equilibrium reaction with hydrogen ions. Thus, ISFET was often used as a pH sensor. Moreover, the extended-gate FET has been developed so as to construct the gate materials freely with separating from semi-conductive device [5]. We previously reported ion-sensitive extended-gate FET using alkanethiols self-assembled monolayers (SAMs), which had carboxyl and amino terminal groups, and the SAM-coated gate FET showed pH response close to the Nernst equation [6].

Hydroxyapatite (HAP), expressed as $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ in a chemical formula, is well known as the main composite of bone and teeth. In the biological process, it is recognized that HAP is crystallized from Ca^{2+} and PO_4^{3-} in aqueous solution by ionic organic molecules as initiator of crystal nucleation. In bone and teeth formations, acidic proteins play an important role of crystal nucleation, thus it might

be necessary for some polar groups to attract Ca^{2+} and PO_4^{3-} strongly.

In the present study, we observed HAP formation on some functional groups of SAM for initiating crystal nucleation and crystal growth. At the same time, the ionic change between SAM and ions in the solution was monitored using extended-gate FET for understanding the fundamental mechanism of biomineralization.

2. Methods and Results

2.1. HAP crystallization on SAM-coated gate surface

Four alkanethiols, of which their terminal groups were methyl ($-\text{CH}_3$), amino ($-\text{NH}_2$), and carboxyl ($-\text{COOH}$), were used as the SAM formation, respectively. Au thin film (100 nm) was sputtered on the glass substrate following Cr thin film (15nm) as an adhesive layer, and the Au substrate was immersed in the ethanol solution including alkanethiol of 1 mM for 24 hours. SAMs were formed utilizing the covalent bonding of gold and thiol compounds. The wettability had changed after modifying each alkanethiol as previously reported. With each of these SAM-coated Au substrate, the extended-gate FETs were constructed as shown in Fig. 1 and HAP formation was observed by monitoring gate surface potential changes using SBF fluid (pH 7.4) containing 3.75 mM of CaCl_2 at 37 °C for 7 days. As shown in Fig.2, the deposition of HAP crystal was observed at the carboxy-

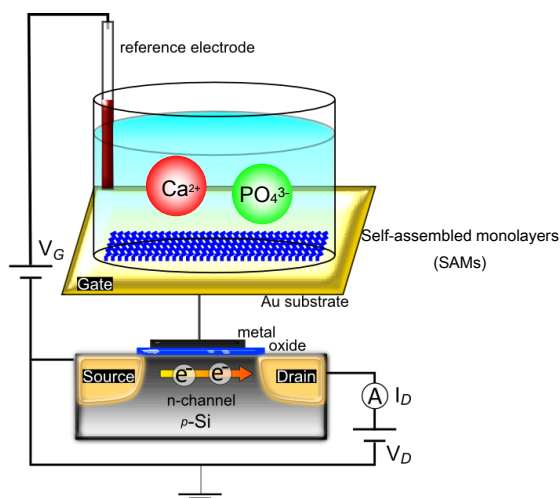


Fig. 1 The diagram of SAM coated extended-gate FET for crystallization of HAP.

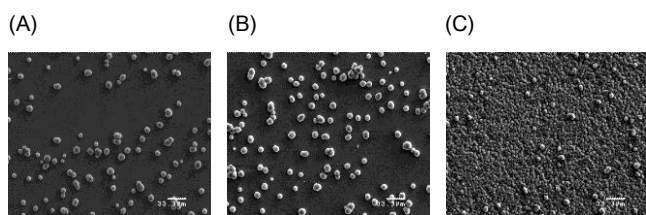
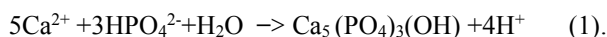


Fig. 2 SEM images of HAP crystals on each functional group terminated SAM coated gate electrode. (A): -CH₃ terminal group of SAMs, (B): -NH₂ terminal group of SAMs, (C): -COOH terminal group of SAMs.

and amino- terminal group-based SAM gate surface indicating that biomineralization was promoted by SAMs with polar group because they were able to strongly attract the ions to the substrate.

2.2. Real-time monitoring of HAP formation

Figure 3 shows the result of real-time monitoring of gate surface potential changes in the process of HAP formation on each SAM-coated gate surface. The gate surface voltage increased clearly until 72 hours using the COOH-terminated SAM-coated gate FET, and also increased using the NH₂-terminated SAM-coated gate FET in retard, while such a signal was not observed so much for non-polar terminated (-CH₃) SAM-coated gate FET during incubation. In addition, the result that the voltage shift in carboxyl-SAM coated gate FET was larger than that in amino-SAM coated gate FET seemed to be correlated to the appearance of the density of HAP crystals, considering SEM images in Fig. 2. From these result, it was possible that FET had detected the increase of hydrogen ion at the interface of gate electrode associated with HAP crystal formation, indicating the chemical formula (1),



Moreover, the generated hydrogen ions may have induced

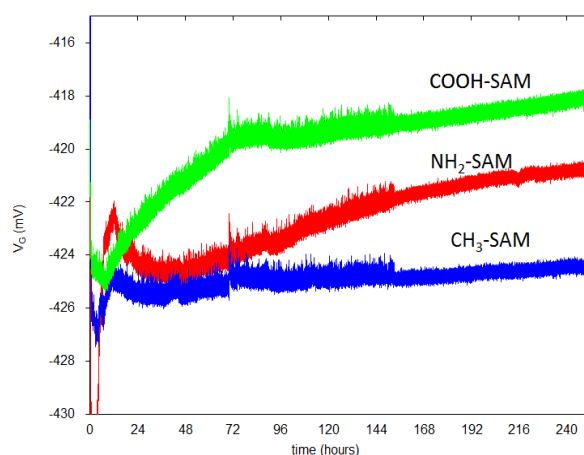


Fig. 3 Time course for gate surface potentials modified with each functional group of SAMs. Blue: -CH₃ terminated SAMs, Red: -NH₂ terminated SAMs, Green: -COOH terminated SAMs.

charge change at the gate on the basis of the equilibrium of carboxyl or amino terminal end.

Finally, we confirmed the Ca²⁺ concentration dependency for HAP crystal conformation on the COOH-SAM coated gate FET. In the result, the gate surface voltage raised lately at lower Ca²⁺ concentration compared with higher concentration, while almost no gate surface voltage change was observed without Ca²⁺ ion. It is certainly expected that the rate and amount of biomineralization would be depend on the Ca²⁺ ion, and the phenomenon was able to be monitored using the SAM coated gate FET in this experiment. To obtain the real-time information of HAP conformation would be important to control the biomineralization in vivo, thus this experiment is useful for an evaluation method of biomaterials in the future.

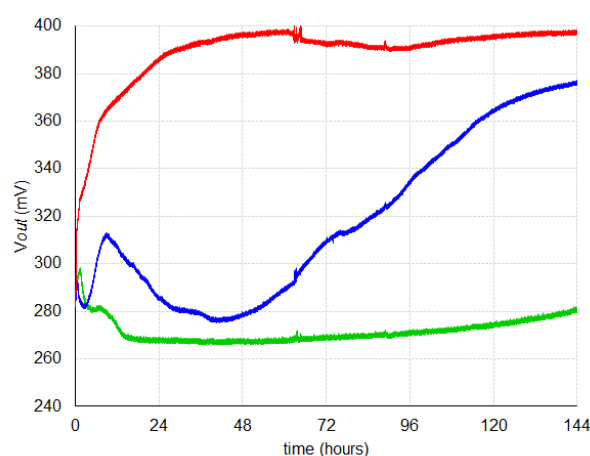


Fig. 4 Time course for surface potential changes of carboxy terminal end SAM-gated FET at different concentration of calcium ions (Green: [Ca²⁺]=0 mM, Blue: [Ca²⁺]=3.00 mM, and Red: [Ca²⁺]=3.75 mM).

3. Conclusions

HAP formation was monitored in real-time using ion sensitive extended-gate FET. The carboxy- and amino-terminal group-based SAM gate FETs showed the gate voltage shifts in accordance with HAP crystal formation on the gate surface. Moreover, the rate and amount of HAP crystal formation was monitored depending on Ca²⁺ concentration by use of the carboxy terminal end group coated gate FET. The FET would be useful as an evaluation device in the biomaterials and medical fields in the future, such as bone regenerative medical care.

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