



Real time assessment of surface interactions with a titanium passivation layer by surface plasmon resonance

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ABSTRACT

Due to the high corrosion resistance and strength to density ratio titanium is widely used in industry, and also in a gamut of medical applications. Here we report for the first time on our development of a titanium passivation layer sensor that makes use of surface plasmon resonance (SPR). The deposited titanium metal layer on the sensor was passivated in air, similarly to titanium medical devices. Our “Ti-SPR sensor” enables analysis of biomolecule interactions with the passivated surface of titanium in real time. As a proof of concept, corrosion of a titanium passivation layer exposed to acid was monitored in real time. The Ti-SPR sensor can also accurately measure the time-dependence of protein adsorption onto the titanium passivation layer at sub-nanogram per square millimeter accuracy. Besides such SPR analyses, SPR imaging (SPRI) enables real time assessment of chemical surface processes that occur simultaneously at “multiple independent spots” on the Ti-SPR sensor, such as acid corrosion or adhesion of cells. Our Ti-SPR sensor will therefore be very useful to study titanium corrosion phenomena and biomolecular titanium–surface interactions with application in a broad range of industrial and biomedical fields.

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1. Introduction

Thanks to its high strength to density, excellent corrosion resistance, decreasing cost, and increasing availability, titanium and its alloys enjoy widespread industrial applications in a wide variety of highly corrosive environments, including sea water, bleaches, alkaline solutions, oxidizing agents, and organic acids [1]. These excellent properties mean that titanium is widely used in industries including aerospace, marine, power generation, and desalination plants, for instance [2–5]. Its extremely high corrosion resistance results from the formation of a very stable, continuous, highly adherent, and protective oxide film on the titanium surface, formed spontaneously and instantly once fresh metal surfaces are exposed to air or moisture.

Titanium is also commonly used to fabricate a variety of medical devices such as hip and knee joints, bone screws and plates, dental implants, stents, pacemaker cases and centrifugal pumps in artificial hearts [6–8]. Due to rapidly aging populations, especially in developed countries, national health care costs are escalating. In

particular, the increased incidence of hard tissue and cardiovascular diseases such as periodontitis, osteoarthritis, and arteriosclerosis is strongly correlated with the rapidly growing elderly population. Therefore, the development of innovative treatment techniques for functional repair or complete cure of these diseases is highly desirable. In attempts to improve the healing potential of such medical device, much research has been devoted to titanium surface modification methods that enable controlled adsorption of biomolecules and ions or regulated drug release [9–12]. In biomaterial sciences the strategic importance of fundamental research in nanobiotechnology has recently been acknowledged [13]. The development of highly sensitive methods that can monitor the interaction of biomolecules at titanium surfaces are therefore needed.

Surface plasmon resonance (SPR) can offer real time and label-free analysis of the interfacial events that occur on the surface of a metal layer under physiological conditions [14,15]. Recently, the technique of SPR imaging (SPRI) has been developed and applied to monitor the adsorption of organic materials and biomolecules at multiple independent spots [16]. In this study we report for the first time on our development of a titanium passivation surface sensor chip for SPR [17]. There are few reports of titanium SPR (Ti-SPR) sensors in which the titanium metal layer was passivated

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in air. Although many studies of a TiO₂-coated sensor for SPR have been reported [18–23], their sensors were directly coated with TiO₂ and titanium metal was not used. In medicine and dentistry the titanium metal surface of dental implants and artificial bones oxidize in air. Our Ti-SPR sensor has a titanium passivation layer, closely resembling the conditions under which titanium medical devices are normally used during clinical treatment.

2. Experimental section

2.1. Materials

Bovine serum albumin was purchased from Sigma–Aldrich Japan K.K. (Tokyo, Japan). γ -Globulin was purchased from Nacalai Tesque Inc. (Kyoto, Japan). bFGF (recombinant human basic growth factor, KCB-1) was kindly donated by Kaken Pharmaceutical Co. Ltd. (Kyoto, Japan). Dulbecco's phosphate buffered saline without calcium and magnesium (pH 7.4) (PBS) was purchased from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan). Dodecylphosphate (DDP) was purchased from Alfa Aesar (Ward Hill, MA). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). All chemicals were used as received without any additional purification. Glass plates made of S-LAL10 (refractive index 1.72, diameter 15 mm, thickness 1 mm) were purchased from Arteglass Associates Co. (Kyoto, Japan).

2.2. Surface plasmon resonance instruments

We constructed a SPR instrument which determines the SPR spectrum and the SPR angle shift [24,25]. The SPR instrument, constructed with reference to Knoll's method, utilized the Kretschmann configuration in which the metal was in the form of a thin film mounted directly onto a S-LAL10 glass plate coupled to an S-LAL10 dispersing prism with an index matching fluid [14,26]. An SPR chip was attached to the SPR flow cell, which was 10 mm in length, 1 mm in width, and 1 mm in thickness. Solutions were allowed to pass through the flow cell [25]. The He–Ne laser light ($\lambda = 632.8$ nm) was linearly *p*-polarized using a Gran–Thomson prism and then passed through a non-polarizing cube beam splitter. The sample surface was exposed to the *p*-polarized light through the prism. The intensity of the reflected light was determined by a photodiode detector. A computer was used to control a biaxial rotation stage and to process the intensities of the incident and reflected light as a SPR spectrum. At the angle at which there is a dip in the spectrum the light resonated surface plasmons on the metal layer. This angle is called the SPR angle [14,15].

The SPR-1000 SPRI apparatus (UBM, Kyoto, Japan) employed in this study was developed with reference to our SPRI apparatus [16]. The SPR chip with arrayed spots was mounted on an S-LAL10 dispersing prism with index matching fluid. The flow cell was constructed using a washer made of silicone and a vinyl chloride lid with an inlet and outlet. The back of the chip was illuminated by *p*-polarized, collimated, and polychromatic white light through the prism. The reflected light was passed through an interference filter and collected by a CCD camera. The data was acquired using our in-house designed software.

The Ti-SPR sensor chip can be used in both instruments. All experiments were carried out at 25 °C.

2.3. Development and evaluation of the titanium SPR sensor

2.3.1. Design of the titanium SPR sensor

General SPR sensor chips are based on gold-coated glass substrates. The Ti-SPR sensor chips were prepared by depositing titanium metal on the contamination-free gold surface of the SPR

sensor. To design an optimal Ti-SPR sensor it was necessary to consider the thickness and oxidation of the deposited titanium layer and the detection of protein adsorption in water solutions. So the SPR spectra of the Ti-SPR sensor were simulated using the Fresnel equation of reflection and transmission using a prism, glass plate, Cr, Au, Ti, TiO₂, protein, and water multilayer (Fig. 1) to estimate the optimal layer thickness of the deposited titanium [26,27]. When the amount of protein adsorption or the thickness of the TiO₂ layer is changed, the SPR angle is shifted. So the shift in the SPR angle (degree) can be calculated as the amount of protein (4.02 ng mm⁻² degree⁻¹) or the thickness of the etched TiO₂ layer (0.77 nm degree⁻¹).

The designed Ti-SPR sensor chips were prepared by Osaka Vacuum Industrial Co. Ltd. (Osaka, Japan). These chips were prepared by depositing Cr, Au, and Ti on S-LAL10 glass plates under 2.0×10^{-2} Pa using an electron beam evaporation method.

2.3.2. Characterization of the titanium layer on the Ti-SPR sensor

A transmission electron microscopy (TEM) cross-section of the developed Ti-SPR sensor chip embedded in epoxy resin was prepared using an Ion Slicer (EM-09100IS, JEOL), and imaged using a JEM-3010 (JEOL) microscope operated at 300 keV. The surface elemental composition of the Ti-SPR sensor was determined using X-ray photoelectron spectroscopy (XPS) (AXIS-HS, Kratos, Manchester, UK) in vacuo at less than 10^{-7} Pa. We used AlK α monochromatic X-rays with a source power of 150 W (acceleration voltage of 15 kV and filament current of 10 mA) and measured the elemental composition ratio of Au, Ti, and O at photo-electron take-off angles of 90°, 60°, 45°, 30°, and 15°. The layer thicknesses of the titanium and the oxidized titanium on the Ti-SPR sensor were estimated from the SPR spectrum of this sensor and the relationship between the amount of protein adsorbed and the SPR angle shift was determined.

2.4. SPR measurements of acid etching and biomolecule adsorption

2.4.1. Effect of acid etching on the titanium surface

The effect of acid etching the titanium surface was determined by SPR. Phosphoric acid solutions were prepared by dropping 85 wt.% phosphoric acid into pure water to obtain pH values of 1.8, 1.9, 2.0, and 3.0. The running solution used was pure water.

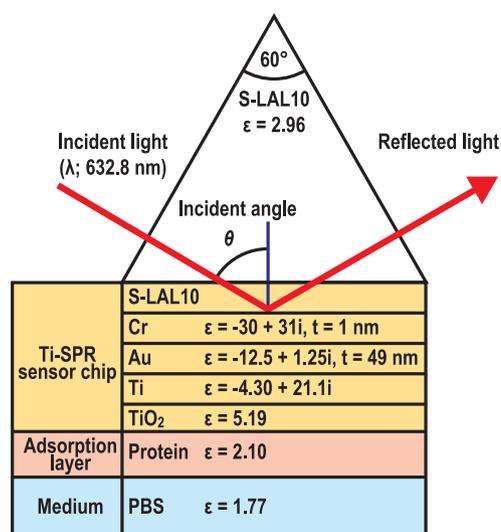


Fig. 1. Schematic illustrating the Ti-SPR sensor with the coupled prism for SPR. The deposited titanium metal layer is easily oxidized by air and its thickness changed. The thickness and dielectric constant are indicated for each coated/adsorbed substance (S-LAL10 glass, Cr, Au, Ti, TiO₂, protein, and PBS).

| ID | Title | Pages |
|-----|---|-------|
| 796 | Real time assessment of surface interactions with a titanium passivation layer by surface plasmon resonance | 7 |

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