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Surface plasmon resonance in monitoring of complement activation on biomaterials ☆

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ABSTRACT

When artificial materials come into contact with blood, various biological responses are induced. For successful development of biomaterials used in biomedical devices that will be exposed to blood, understanding and control of these interactions are essential. Surface plasmon resonance (SPR) spectroscopy is one of the surface-sensitive optical methods to monitor biological interactions. SPR enables real-time and *in situ* analysis of interfacial events associated with biomaterials research. In this review, we describe an SPR biosensor and its application to monitor complement activation onto biomaterials surface. We also discuss the effect of surface properties of the material on complement activation.

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1. Introduction

When artificial materials come into contact with blood, various biological responses, such as adsorption of proteins, activation of the complement and coagulation systems, platelet activation, inflammatory reactions and cell adhesion are induced. Control of these interactions is essential for the development of biomaterials used in

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the preparation of biomedical devices. Thus, extensive studies have been performed to understand the interactions of blood with artificial materials.

The complement system is a cascade of enzyme reactions consisting of approximately thirty fluid-phase and cell-membrane bound proteins. It plays an important role in the body's defense systems against pathogenic xenobiotics [1,2] and is also activated by artificial polymeric materials. For example, hemodialysis membranes made of cellulose or its derivatives strongly activate the complement system and this process has been extensively studied [3,4]. Information on the complement activation by artificial materials also has accumulated from various clinical settings, such as open heart surgery, blood transfusion medicine, and extracorporeal immunotherapies [5–7]. Understanding of complement activation on the surfaces of artificial materials is important to the rational design of biocompatible surfaces of synthetic materials.

Complement fragments, such as C3a, iC3b, C5a, and SC5b-9, are released when artificial surfaces are exposed to serum. The interaction of complement proteins with material surfaces has been studied using enzyme-linked immunosorbent assay (ELISA) for these complement fragments study [8–14]. Recently, various analytical techniques have been examined to study biomaterials, but few of these are able to monitor dynamic interactions under physiological conditions. Surface plasmon resonance (SPR) is a surface-sensitive optical technique that allows us to follow refractive index changes near a metal surface and thus has been widely used for real-time investigations of various dynamic biological processes, such as protein–protein, DNA–DNA and protein–DNA interactions, without the need to label the sample [15–19]. For complement research, SPR has been applied to monitor individual biomolecular interactions between complement components and thus has been used to determine their rate and affinity constants. It has been also applied to examine the formation of complement complexes such as C3 and C5 convertases. Application of SPR for these purposes has been reviewed by Ricklin and Lambris [20]. SPR also allows us to study interfacial events relating to biomaterial research since various model surfaces can be prepared on an SPR sensor chip.

In this review, we describe an application of the SPR method to study interactions of the complement system with biomaterials.

2. Surface plasmon resonance (SPR)

2.1. Principle

A surface plasmon is a longitudinal charge density wave that is propagated in a parallel manner along the interface of two media, where one surface is a metal and the other is a dielectric layer [21]. Metal is an essential component since the metal must exhibit free electron behavior as described by the free electron model [22]. Thus, metals like gold, silver, copper and aluminum are good candidates for optical excitation of surface plasmons, and so far most of the experimental work has been performed on gold and silver. In SPR sensors, the surface plasmon is excited by a light wave. Two different settings of optical units for the excitation of surface plasmons have been reported by Otto [23] and Kretchmann [24]. The Kretchmann configuration based on the total internal reflection (ATR) has been widely used for development of SPR instruments.

In the Kretchmann configuration (Fig. 1 (a)), a beam of *p*-polarized light (light that is polarized in a plane parallel to the plane of the incident light) is used to illuminate the back side of a gold thin film (typically ~50 nm) on glass through a prism, and the front side of the film faces air or a solution of interest. When the incident angle of *p*-polarized light exceeds the critical angle, the incident light is totally reflected. A small part of the light penetrates outside the glass, and this part of the light, the so-called evanescent wave, allows us to monitor events occurring on the metal/ambient interface. The wave vector of the evanescent field (k_{ev}) is given by

$$k_{ev} = \frac{\omega}{c}\varepsilon_g^{1/2}\sin\theta \tag{1}$$

where ω is the frequency of incident light, c is the speed of the light, ε_g is the dielectric constant of glass ($\varepsilon_g^{1/2}$ corresponds to refractive index of glass), and θ is the incident angle. The dispersion relation for a surface plasmon propagating along the interface between a metal with a complex dielectric function $\varepsilon(\omega)=\varepsilon'(\omega)+i\varepsilon''(\omega)$ and a dielectric with dielectric constant ε_a can be written as [22]

$$k_{sp} = \frac{\omega}{c}\sqrt{\frac{\varepsilon'(\omega)\varepsilon_a}{\varepsilon_a+\varepsilon'(\omega)}} \tag{2}$$

where k_{sp} is the wave vector for the propagating surface plasmon along the interface.

The evanescent wave of the incident light is able to couple with a surface plasmon at a specific incident angle, θ_{SPR}, where $k_{sp}=k_{ev}$, resulting in the energy loss of the incident light to the metal film and is observed as a minimum in the reflected light intensity (Fig. 1 (b)). Therefore, the SPR angle (θ_{SPR}) depends on the refractive index of the medium in the vicinity of the metal film. Changes in the refractive index above the metal surface caused by various biological processes, such as adsorption of proteins, result in an increase of θ_{SPR}.

Although various SPR experimental settings have been proposed including use of a wavelength-variable light source and a diffraction grating to couple incoming light with surface plasmon [18,19,21], the electromagnetic field of a surface plasmon is confined at the metal–dielectric boundary and decays exponentially with ~200 nm of a typical penetration depth in common. SPR spectroscopy is a sensitive methodology to monitor changes in the refractive index near the metal surface and has the advantage that analytes do not need to be labeled.

2.2. Real-time monitoring

The optical construction of an SPR instrument is simple, as shown schematically in Fig. 1 (c). The SPR sensor is a glass plate coated with gold (~50 nm) with an underlayer of chromium (~1 nm) as an adhesive layer. The plate is optically coupled to a glass prism using an index-matching fluid. A *p*-polarized laser beam is directed to the back side of a sample plate through a glass prism and reflected light intensity is monitored. By changing the incident angle of the laser beam, we can determine the SPR angle (θ_{SPR}) as the minimum in reflectance (Fig. 1 (b)). If substances are deposited onto the sensor surface, a shift in θ_{SPR} occurs ($\Delta\theta_{SPR}$) in a manner dependent on the amount of the deposited substances.

For real-time monitoring, the reflected light intensity is detected with respect to time (Fig. 2). Initially, a buffer solution is infused into a flow cell which is set up on the sensor surface to obtain a baseline (Fig. 2, process I). When a protein solution is subsequently circulated, a large increase in reflectance is observed due to the change in the refractive index of the solution (bulk effect) and the adsorption of proteins (Fig. 2, II). After circulation of the protein solution for a predetermined period (Fig. 2, III), a buffer solution is infused to wash the protein solution out and to remove loosely bound proteins (Fig. 2, IV). An increase in reflectance (ΔR) can be converted to the SPR angle shift ($\Delta\theta_{SPR}$) and the amount of adsorbed proteins is calculated from $\Delta\theta_{SPR}$. The thickness of the protein layer, the amount of protein adsorbed, was calculated from the shift in the SPR angle ($\Delta\theta_{SPR}$) using Fresnel fits for the system glass/Cr/Au/SAM/protein/water [25,26].