Using Biomedical Sensor—Reflectometry Interference Spectroscopy for Evaluation of Biocompatibility of Biomaterials

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Abstract: Using a biomedical sensor setup RIfS we have investigated the kinetic behavior of bovine serum albumin (BSA), human fibrinogen (FIb) and human IgG adsorbed onto surfaces of hydroxyapatite (HA) and polyurethane (PU) H50-50. According to the operation principle of RIfS and the molecular dimensions of three kinds of proteins, a formula to calculate the adsorbed layers of proteins onto the surface of HA and PU H50-50 has been introduced. The results show that the adsorbed layers of three kinds of proteins on the surface of HA are 1.0751 for IgG, 0.9684 for BSA, 0.7464 for FIb and that of PU H50-50 are: 0.8199 for IgG, 0.7964 for BSA, 0.6120 for FIb. It is shown that RIfS can perform a kinetic, real time and in situ analysis of plasma proteins adsorbed on a surface of biomaterials. From this study the potential application of RIfS as a new analytical tool in the evaluation of the biocompatibility of biomaterials was confirmed and the experiences of preparing the suitable nanograde film from in- and organic biomaterials for a RIfS experiment were accumulated.

Keywords: Biomedical sensor, RIfS, biomaterials, biocompatibility, protein adsorption

1. Introduction

Reflectometry Interference Spectroscopy (RIfS) was based on the white-light interference effects occurring on a thin transparent film and has been systematically developed by Gauglitz et al. (1). RIfS was utilized as transducer in biomolecular interaction analyses some years ago (2, 3).and shows high precision and high stability in monitoring the change of optical thickness owing to interfacial molecular interaction. For probing surface interaction, the use of RIfS is as advantageous as surface plasmon resonance (SPR). It is able to rapidly monitor any dynamic process, such as adsorption or degradation, to a wide range of biomedically relevant interfaces in real time without the need to label the adsorbate and without the need for complex sample preparation. It can rapidly obtain information on the rate and extent of adsorption, enabling the determination of the association/dissociation kinetics and the affinity constants of specific ligand ligate interaction. RIfS has been proved to be a useful approach for the detection of triazine herbicides in river water, hydrocarbon in air, ammonia in the gas phase, protein interactions in covalently attached dextran layers and in clinic detection of HbsAGb (4-8). However, the application of RIfS for evaluation of the biocompatibility of biomaterials, to the best of our knowledge, has not been reported yet.

The protein adsorption on the surface of biomaterials is an important factor for the adhesion of platelet and biocompatibility. Therefore, the characterization of surface–adsorbed proteins has been extensively investigated by means of different techniques: immunoassays, radio-labeled proteins, ultraviolet spectroscopy, Fourier transform infrared spectroscopy (FTIR), SPR (9-13), and so on. This paper explores the potential application of RIfS as an analytical tool for studying protein adsorption on the surface of biomaterials. This is a part of a wide-spectral program investigating the biocompatibility of different biomaterial surfaces.

2. Materials and Methods:

2.1 RIfS Apparatus

The RIfS setup used has been developed in our laboratory (14). The main elements of the setup were a CCD spectrometer with a bifurcated optical fiber and an adsorption cell whose volume is about 30 μl. The bifurcated optical fiber was used to illuminate the transducer chip and to collect the light reflected from the chip. The chip is a glass substrate on which a 10-nm Ta_2O_5 film was deposited in order to enhance the interference contrast. A self-designed software was used to control the collection of full spectra, to analyze the experimental data in real-time and to draw the experimental curves on-line.

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Abstract

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2.2 Preparation of HA and PU Films

Thin films of a synthetical HA were coated on the sensor chips (4 mm^2) with an electron beam evaporation technique. The thickness of the films was ~650 nm.

The thin films of polyurethane exposed to proteins were prepared using a spin-coating method. At first, the test polyurethane was cleaned by PBS and cut down into small pieces. These pieces were then immerged in tetrahydrofuran (0.235g/10 ml). After the polyurethane had been dissolved completely, the solution was filtrated with a two-double nylon filter. Then, 50-μl polyurethane's solution was pipetted out and dropped onto the surface of a transducer chip (4 mm^2). A PU film was spin-coated on the transducer chip using a spinning-machine at 1700 rpm. The thickness of the films was ~650 nm.

2.3. Proteins

Three kinds of proteins, namely bovine serum albumin (BSA), human fibrinogen (Fib) and IgG (Sigma Mo) were used. For each measurement a protein solution was prepared freshly in phosphate buffer saline (PBS pH=7.4) of a concentration of 0.1 mg/ml. Deionized water was used to prepare the aqueous solutions.

2.4 Protein Adsorption Measurement

The protein adsorption measurement has been performed as follows: the sensor chip coated with HA/PU film was mounted into the adsorption cell carefully and fixed with a clip. Then, the peristaltic pump was started to drive PBS flow into the adsorption cell. The PBS flow cleaned the surface of the HA/PU film drove out air bladders appearing potentially in the adsorption cell. Most importantly, it produced a physical PH-environment for the sorption of proteins. After this treatment, the protein solution flowed into the adsorption cell at a rate of 200 μl/min and the kinetic adsorption process of proteins on the surface of the HA/PU film can be oberseved in situ on the monitor of the computer. Until the kinitic adsorption curve of proteins become stabil, the PBS was driven into the adsorption cell again to wash the proteins which didn't adsorpted fixly on the material surface. The wash heading would get a response on the adsorption curve. The mark on the Y axis of the Fig.1-5 give the thickness of the proteins adsorbted on the HA/PU film. For each kind of proteins, at least three tests were performed (n=3).

2.4 Calculation of the Adsorbed Layer of Protein

Since the molecular dimensions of various proteins are different (12), a normalized treatment of the experiment data was performed using the formula we have defined in order to obtain the actual adsorbed layer number L_p.

$$L_p = \frac{\overline{x_p}}{D_p}$$

with D_p being the molecular dimension of a single protein and $\overline{x_p}$, the average thickness of each kind of proteins on the surface of the material to be probed.

2.5 AFM Imaging

The microstructure of the HA film and PU film and the surfaces with adsorbed proteins were imaged with a commercial atom force microscope (AFM). The instrument was operated in Tapping Mode using silicon cantilevers oscillating with average amplitude of 100 nm and a resonance frequency between 200 and 450 kHz. The scanning rate selected was 1 Hz. All of the images presented in this paper were obtained repeatedly and were stable under the experimental conditions.

3. Results and Discussion:

3.1 Protein Adsorption Kinetics

The kinetic adsorption curves of BSA, human IgG and Fib. on the surface of a HA/PU film were shown in Fig.1. and Fig. 2. The curves show that the adsorption behavior of HA/PU film to three kinds of proteins are similar: At first the processes of all three kinds of proteins increase quickly in the initial stage and reach to their stable values after about 500 second. Secondly, after washed with PBS the curves response in different tendencies: the curves of IgG and fibrinogen fall in evidence, what means some loosely adsorbed molecules were removed from the HA/PU film. Nevertheless, the curve of BSA shows almost no response, what shows the adsorption process of BSA onto the surface of HA/PU film has not been affected.

Fig. 3-5 are the comparing of the adsorbed three kinds of proteins respectively on the surface of HA and PU film. They show that on the surface of HA film adsorbed more proteins than on the surface of PU film in all of three kinds of proteins.

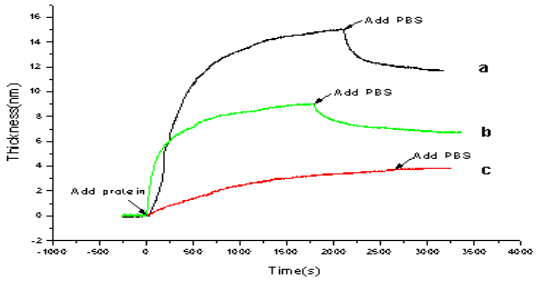


Fig. 1 Kinetic curves of adsorbed proteins on the surface of HA Film. a) IgG, b) Fib, c) BSA

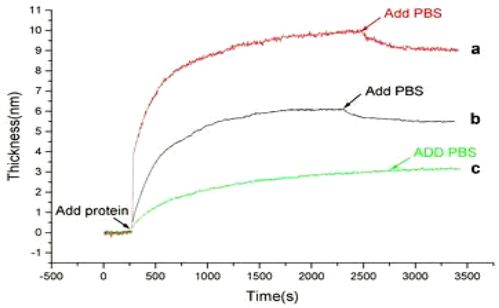


Fig. 2 Kinetic curves of adsorbed proteins on the surface of PU film. a) IgG, b) Fib, c) BSA

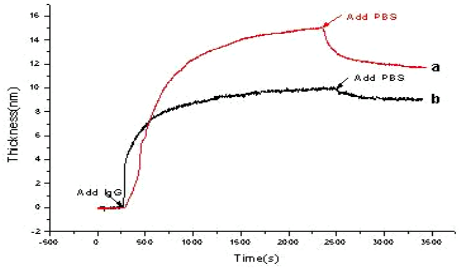


Fig. 3 Adsorbed IgG on the surfaces of HA and PU a) HA, b) PU.

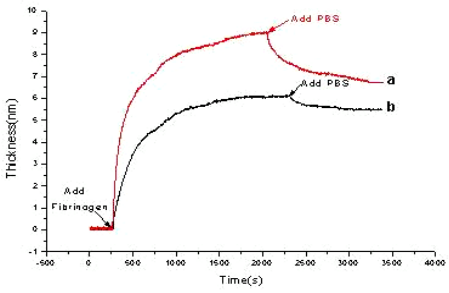


Fig. 4. Adsorbed FIb on the surfaces of HA and PU. a) HA, b) PU

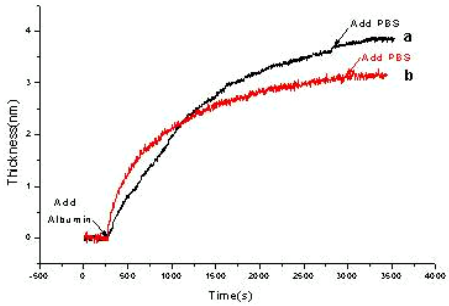


Fig. 5. Adsorbed BSA on the surfaces of HA and PU. a) HA, b) PU

3.2 Adsorbed Layer of Three Proteins on the HA and PU Films

Tab.1 shows that the RIfS thickness of three kinds of proteins on the HA surface has a decreased order of IgG (11.826nm) > Fib. (6.717nm) > BSA (3.873nm). However, the adsorbed layer number, calculated using the formula, has the series of IgG (1.075) > BSA (0.968) > Fib (0.746).

Tab.2 shows that the RIfS thickness of three kinds of proteins on the HA surface has a decreased order of IgG (9.019nm) > Fib (5.5083nm) > BSA (3.1855nm). However, the adsorbed layer number, calculated using the formula, has the series of IgG (0.819) > BSA (0.796) > Fib. (0.6120).

Tab.1 RIfS thickness and adsorbed layer number of three kinds of proteins on the HA films

thickness / proteins	$\bar{x}$ (n=3)	SD	adsorbed layer
IgG	11.8265	0.0487	1.0751
BSA	3.8734	0.0266	0.9684
Fib.	6.7175	0.456	0.7464

Tab.2 RIfS thickness and adsorbed layer number of three kinds of proteins on the PU films

thickness / proteins	$\bar{x}$ (n=3)	SD	adsorbed layer
IgG	9.0196	0.0940	0.8199
BSA	3.18555.	0.1205	0.7964
Fib.	5.5083	0.0471	0.6120

3.3 AFM

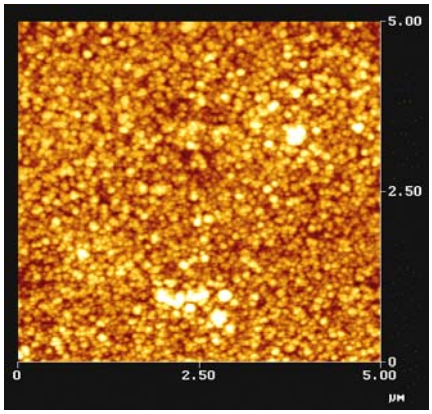
AFM analysis was recommended to probe the lateral distribution of the protein molecules with the monolayer and confirmed indirectly the presence of the passively adsorbed protein on the HA surfaces. The AFM image in

Figure 6(a) is the microstructure of the HA surface and in Figure 6(b) that with adsorbed protein IgG.

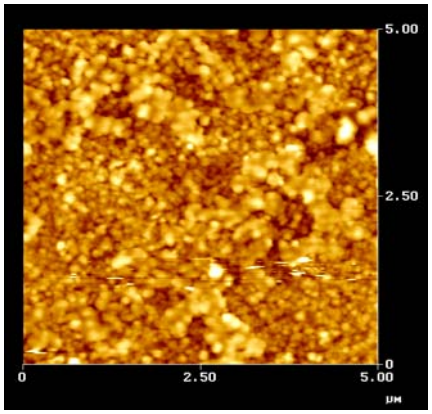
Figure 6(a) shows a smooth surface of the HA coating with the character of grain form. The AFM images of IgG coated HA surface in Figure 6(b) reveal a densely packed surface complete coverage of the proteins, which is congruously with the calculated adsorbed layer in Tab. 1.

Conclusion:

1. The potential application of RIfS as a new analytical setup for biomolecular interactions on a surface of biomaterial is confirmed.
2. The adsorbed layers of three kinds of proteins on the surface of HA and PU H50-50 are same series: IgG > BSA > Fib
3. On the surface of HA film more proteins can be adsorbed than on the surface of PU film in all of three kinds proteins.



(a)



(b)

Fig. 6 AFM image of micro structure of an HA surface (a) and with adsorbed IgG (b)

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