Simultaneous atomic force microscope and quartz crystal microbalance measurements: Investigation of human plasma fibrinogen adsorption

K.-H. Choi,[a] J.-M. Friedt, F. Frederix, A. Campitelli, and G. Borghs
MCP/ACSS, Interuniversity Micro-Electronics Center (IMEC) vzw, Kapeldreef 75, B-3001 Leuven, Belgium

(Received 5 February 2002; accepted for publication 20 June 2002)

We have combined the tapping-mode atomic force microscope (AFM) and quartz crystal microbalance (QCM) for simultaneous investigation of human plasma fibrinogen adsorption on a metallic surface using these two instruments. The AFM images show the surface changes with molecular resolution while the corresponding resonance frequency shift of the QCM provides quantitative adsorbed mass estimates over the whole sensing area. The combination of AFM with QCM allowing the simultaneous measurements with two techniques working at very different scales and probing different properties of the adsorbed layer provides quantitative and qualitative information that can distinguish different protein adsorption mechanisms. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1500777]

The adsorption and deposition of biomolecules on inorganic surfaces is an important topic in biomaterials research and for the development of biosensors. The quartz crystal microbalance (QCM) has been extensively used to investigate the adsorption of proteins on metal surfaces.[1,2] Especially, the QCM technique has a potential application for the developments of biosensors.[3,4] A number of contributions have been published on the resonance vibration behavior of the QCM when a contact probe approaches its sensing electrode.[5–8] It displays a decrease of its resonance frequency as a function of the change of deposited mass.[9–11] However, the detailed physical adsorption mechanisms cannot be identified with such a crude technique whose sensing area is in the cm^2 range.

Qualitatively, atomic force microscopy (AFM) is one of the commonly used techniques to provide information about these adsorbed proteins with molecular resolution.[12] The observation of biomolecules adsorbed on various kinds of surfaces by AFM has been widely studied and reviewed.[13–16] It is therefore of interest to directly combine tapping-mode AFM with QCM and to observe the adsorption of biomolecules in liquid phase with these two complementary techniques at once.

The merits of this combination include simplicity, simultaneous observation of the adsorption of biomolecules at different scales, and most importantly the possibility to understand the adsorption kinetics of biomolecules through the real time comparison of the resonance frequency shifts of QCM with the obtained molecular resolution images of AFM. Fibrinogen was chosen as a model biomolecule since it is often used as a reference for biocompatibility analysis and is large enough to be resolved by AFM imaging.[15–17] The experimental setup including the tapping-mode AFM (Molecular Imaging, Phoenix, AZ) and QCM (*Q*-Sense *AB*, Göteborg, Sweden) combination is represented in Fig. 1. The QCM resonator is made of an AT-cut quartz wafer coated on both sides with conductive electrodes obtained by evaporating 10–50 nm Ti and 50 nm Au. The typical rms surface roughness of the sensing electrode is 0.8 nm and peak to valley height is 1.9 nm. This surface is thus flat enough to achieve the molecular resolution.

The gold surface is modified with 10 mM CH_3 terminated dodecanethiol (Fluka Chem. Co.) in ethanol in order to enhance the hydrophobic interactions between the proteins and its surface. Other details of AFM and QCM combination are described elsewhere.[18] Various concentrations ranging from 250 ng/ml to 100 μg/ml of human plasma fibrinogen (HPF) (Sigma, F3897) were prepared by dissolving HPF in phosphate buffer saline solution (PBS solution, 10 mM KH_2PO_4/Na_2HPO_4, 150 mM NaCl, *p*H 7.4). For the desorption of proteins from Au/QCM surface, 10 mM glycine·HCl(*p*H 2.2) solution was used. All solutions were prepared with sterile, autoclaved materials and filtered with 0.2 μm pores filter (cellulose membrane, Schleicher & Schuell, Belgium) right before use. The resonance frequency of QCM in its fundamental mode is 4.7 MHz, and our instrumentation allows simultaneous tracking of the resonance frequencies of the first, third, and fifth overtones.

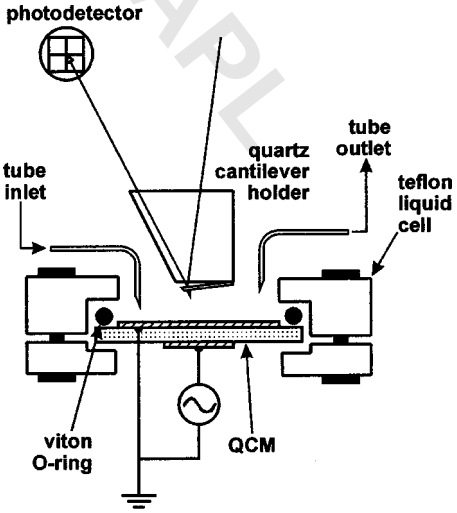

FIG. 1. Schematic diagram of the apparatus combining AFM and QCM.

[a]Electronic mail: choik@imec.be

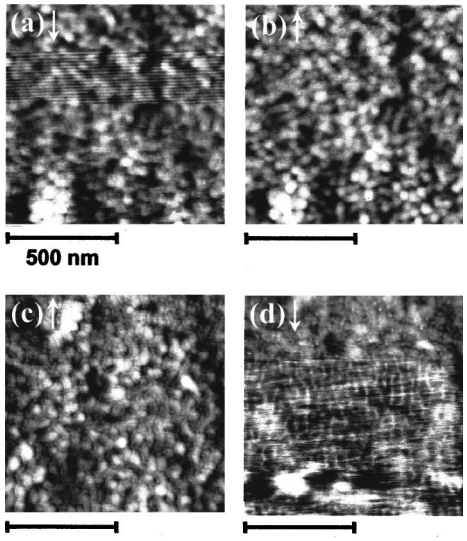

FIG. 2. The AFM topographic images of the Au/QCM surface and their scan directions with the scan size of 1×1 μm^2 in (a) PBS solution and introduction of fibrinogen solution with the concentration of 250, (b) 250 ng/ml, (c) 25, and (d) 100 μg/ml fibrinogen solution.

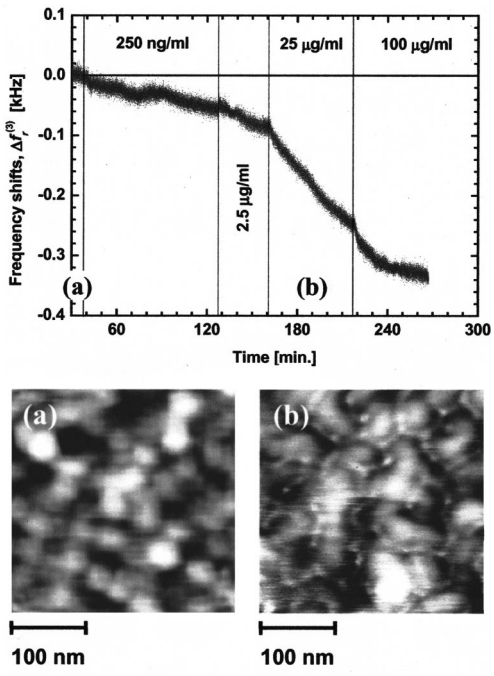

FIG. 3. The resonance frequency shift of the Au/QCM depending on the different concentrations of fibrinogen in PBS solution and the simultaneously obtained AFM images, (a) in PBS solution and (b) in 25μg/ml fibrinogen solution, respectively.

Since the fundamental mode has been shown to be too sensitive to environmental fluctuations,[17] such as flow and viscosity of the liquid, we have monitored the frequency shifts of the third overtone of the QCM (at 14.1 MHz) during the adsorption of proteins on its surface. After stabilization of the resonance frequency of the QCM, the fibrinogen PBS solutions were introduced from lowest to highest concentration by running a flow for 3 min (flow rate around 1.5 ml/min) and then stopping the peristatic pump for low noise measurements. In order to guarantee that the same area of the electrode was always observed by AFM, we kept on scanning the surface and simultaneously monitoring the frequency shift of the QCM during introduction of a fibrinogen solution. However, the liquid flow induced by the pump degraded the data quality during the solution exchange.

Figure 2 displays the obtained *in situ* AFM images and their scan directions with an scan size of 1×1 μm^2. One can recognize the granular structure of a gold surface in upper part of Fig. 2(a). When the fibrinogen with concentration of 250 ng/ml was introduced, lower part of Figs. 2(a) and 2(b), the gold surface was smoothened since the fibrinogen filled the intergrain granular spaces. The adsorbate coverage and smoothening of surface in the AFM image were increased in Fig. 2(c) when increasing the concentration to 25 μg/ml HPF. The entire surface was completely covered with proteins and the granular surface of Au/QCM disappeared in the AFM image of Fig. 2(d) when the fibrinogen concentration of 100 μg/ml was introduced. In this state, the QCM shows a distinctive resonance frequency shift of 330 Hz on its third overtone. This fibrinogen adsorption was mostly reversible with glycine solution.

Figure 3 displays the resonance frequency shifts of the QCM and the AFM images obtained at the same location while increasing the fibrinogen concentration. The shift of the resonance frequency divided by the overtone number was more than 15 Hz when the 250 ng/ml fibrinogen solution was introduced into the liquid cell and 80 Hz when inserting 25 μg/ml fibrinogen solution. Figure 3(a) shows the AFM image of the Au/QCM surface in PBS solution. After introduction of the 25 μg/ml fibrinogen solution, we can clearly observe the adsorbed fibrinogen on the Au/QCM surface [Fig. 3(b)]. Concerning the convolution effect of the AFM tip, the observed protein shape in Fig. 3(b) is in agreement with the model of HPF.[19] At this concentration, it can be observed that the fibrinogen molecules have adsorbed preferentially between each granule of the Au.

The interesting finding in the combination of AFM and QCM is the relationship between the resonance frequency shift of the QCM and the obtained AFM images from the adsorption scheme point of view. Assuming the adsorbed proteins from a rigidly bound layer on the QCM sensing electrode, we can use the Sauerbrey proportionality relationship between the resonance frequency divided by the overtone number and the mass bound to the surface:[11] the sensitivity of a 4.7 MHz resonator is theoretically 20.1 ng/Hz and an 80 Hz frequency shift corresponds to an adsorbed mass of 1.6 μg over the 1 cm^2 sensing area of the QCM. HPF being a large molecule with a molar weight of 340 kD,[16] we expect to observe 280 molecules in a 100×100 nm^2 area. This value being obviously much greater than that observed on the AFM images, we conclude that the tapping-mode AFM actually monitors only the top-most layer and the surface is totally covered by proteins when the solution concentration is 25 μg/ml. However, one should be cautious in applying the proportionality relationship when using the QCM in a liquid medium and adsorbing biomolecules: the normalization of the overtone frequency shift is no longer by the mode number as in a rigid layer but by its square root in the case of a viscous layer. Unexpectedly high frequency shifts have previously been mentioned when using QCMs in viscous liquid media.[20]

Since the AFM images we obtained show that the fi-

brinogen does not cover the whole surface of the QCM, we conclude that either the fibrinogen is preferentially adsorbed in the valley region of the Au granular structure and the QCM frequency shift is exaggerated because of viscous interactions with the surrounding fluid, or the surface is totally covered but the very low force tapping-mode AFM imaging only displays distinctive features of the layer in the intergranular areas. The entire Au/QCM surface was visibly covered with a much higher concentration, 100 μg/ml, of fibrinogen. Under this concentration, the Au granular structures disappeared and only the thick biopolymer layer surface was observed in the AFM images.

In conclusion, we have demonstrated the useful combination of tapping-mode AFM and QCM by observing the adsorption of human plasma fibrinogen on the metallic surface of the QCM sensing electrode with both techniques at the same time. The complementarity of the information acquired using the combined AFM and QCM shows that this technique is a useful probe for the identification of the binding mechanisms of biomolecules to a surface. The information gathered informs us both on the adsorbed mass of the proteins on the surfaces and the adsorption schemes.

K.-H. Choi is pleased to acknowledge the valuable discussions with J.-P. Bourgoin, S. Palacin and D. Esteve in CEA Saclay, France.

[1] *Surface Characterization: A User's Sourcebook*, edited by H. Brune (Wiley, Weinheim, 1997).
[2] J. Homola, S. S. Yee, and G. Gauglitz, Sens. Actuators B **54**, 3 (1999).
[3] *Application of Piezoelectric Quartz Crystal Microbalances*, edited by C. Lu and A. W. Czanderna (Elsevier, Amsterdam, 1984).
[4] M. Murasugu, F. Ohta, Y. Miya, T. Hosokawa, S. Kurosawa, N. Kamo, and H. Ikeda, Anal. Chem. **65**, 2933 (1993).
[5] Z. Lin and M. D. Ward, Anal. Chem. **67**, 685 (1995).
[6] J. M. Kim, S. M. Chang, and H. Muramatsu, Appl. Phys. Lett. **74**, 466 (1999).
[7] A. Laschitsch and D. Johannsmann, J. Appl. Phys. **85**, 3759 (1999).
[8] C. M. Flanigan, M. Desai, and K. R. Shull, Langmuir **16**, 9825 (2000).
[9] M. Rodahl, F. Höök, A. Krozer, P. Brzezinski, and B. Kasemo, Rev. Sci. Instrum. **66**, 3924 (1995).
[10] F. Höök, M. Rodahl-M, C. Keller, K. Glasmästar, C. Fredriksson, P. Dahiqvist, and B. Kasemo, in *Proceedings of the 1999 Joint Meeting of the European Frequency and Time Forum and the IEEE International Frequency Conference* (IEEE, Piscataway, NJ, 1999), pp. 966–72.
[11] A. Janshoff, H. J. Galla, and C. Steinem, Angew. Chem. Int. Ed. Engl. **39**, 4004 (2000).
[12] B. Drake, C. B. Prater, A. L. Weisenhorn, S. A. C. Gould, T. R. Albrecht, C. F. Quate, D. S. Cannell, H. G. Hansma, and P. K. Hansma, Science **243**, 1586 (1989).
[13] Y. F. Dufrêne, T. G. Marchal, and P. G. Rouxhet, Langmuir **15**, 2871 (1999).
[14] K. D. Jandt, Surf. Sci. **491**, 303 (2001).
[15] P. Cacciafesta, K. R. Hallam, A. C. Watkinson, G. C. Allen, M. J. Miles, and K. D. Jandt, Surf. Sci. **491**, 405 (2001).
[16] P. S. Sit and R. E. Marchant, Surf. Sci. **491**, 421 (2001).
[17] C. F. Wertz and M. M. Santore, Langmuir **18**, 706 (2002).
[18] J.-M. Friedt, K.-H. Choi, L. Francis, and A. Campitelli, Jpn. J. Appl. Phys. (to be published).
[19] P. Cacciafesta, A. D. L. Humphris, K. D. Jandt, and M. J. Miles, Langmuir **16**, 8167 (2000).
[20] R. Schumacher, G. Borges, and K. K. Kanazawa, Surf. Sci. **163**, L621 (1985).