

PROTEIN AND CELL ADSORPTION KINETICS

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INTRODUCTION: The adsorption kinetic of proteins or cells and their viscoelastic properties during their interaction with a surface as function of different environments or different surface states are determinant for biocompatibility and biosensors applications. In this view we built a new Quartz Crystal Microbalance (QCM), which measures simultaneously the frequency shift and the Dissipation factor (D). We combine these measurements with different microscopy methods (optical, AFM), in order to better characterise the adsorbed material.

METHODS: We used 3 different proteins: the globular Protein A, that is well-known in immunology, and their antibodies, the rabbit immunoglobulin IgG. Secondly we chose a filamentous protein, the Fibronectin. We also adsorbed the A549 human lung carcinoma cells. The Quartz Crystal Microbalance (QCM) enables us to detect the deposition of some nanograms per cm² by measuring the resonant frequency of the crystal, which decreases linearly with increasing adsorbed mass ($\Delta m \sim \Delta f$). The dissipation factor, which is the inverse of the Q factor, can be obtained by fitting the exponential decrease of the amplitude after the stopping of the crystal excitation. The quartz crystals have gold electrodes that we have also coated with Ti.

RESULTS: The QCM allows to follow the adsorbed mass as a function of time, as well as the energy dissipated in the system, which gives information about the adhesion, water content or thickness of the adsorbed film. Fig. 1 presents the frequency shift Δf vs. time and the dissipation factor D vs. Δf for different concentrations of Fibronectin on Ti. On the upper graph, it can be seen that the time to reach a saturation state is proportional to the protein concentration. Furthermore at least 2 different kinetics occur during the protein adsorption. At high concentrations, it is visible that a rapid adsorption occurs, followed by a slower process. This phenomenon is also observable in the D vs. Δf graph. The measurements can at least be fitted by 2 lines of different slopes, which means that the viscoelastic properties of the fibronectin film change during the adsorption process.

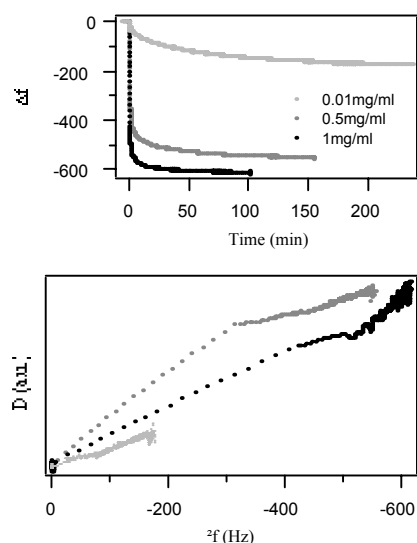


Fig. 1: Adsorption of Fibronectin on Ti at different concentrations.

DISCUSSION & CONCLUSIONS: The QCM is a powerful technique which permits to follow in time the adsorbed mass and the viscoelastic changes of the adsorbed film, as well as to evaluate the arrangement, packing or crystallisation degree or to determine the cell growth in different environments.

In this example, 3 different adsorption processes take place. A tentative explanation is that at low concentration, the filaments adsorb slowly to the surface, forming a non-complete thin film. At the concentration of 0.5 mg/ml, the firstly adsorbed filaments must have a high content of entrapped water because the energy dissipation is the highest, in contrast to the frequency shift. Afterwards the filaments are rearranged so that the film becomes more rigid. Finally at high concentration packed filaments adsorb on the surface to form a film containing less entrapped water. At the end, the dissipated energy increases, probably due to the increases of contact area of the upper adsorbed filaments with water.

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