Non-destructive techniques to investigate cell-protein-biomaterials interactions

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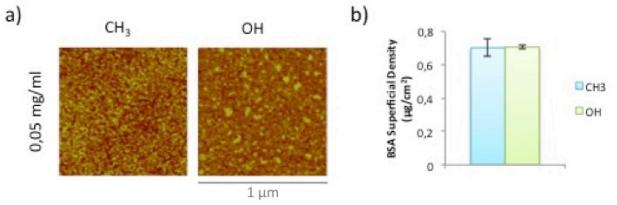
Statement of Purpose: The aim of the study is to compare electrochemical techniques with conventional techniques to characterize protein adsorption on biomaterials surface seeking to understand the cell-materials interface. To do so, we employed a family of self-assembled monolayers (SAMs) with well-defined surface chemistry - CH_3 and OH - and investigated the adsorption of bovine serum albumin, BSA. Quantification of the total amount of adsorbed protein and its conformation was measured by different techniques (bicinchoninic acid assay, BCA and atomic force microscopy, AFM) and then compared with results of the same experiments attained using electrochemical techniques. In addition, electrochemistry allowed us to characterize the kinetics of the adsorption phenomenon, as well as to perform measurements at different temperatures to obtain the thermodynamic parameters of the process. Capacitance measurements of the surface/electrolyte interface indicated conformational changes of the protein upon adsorption on the material surfaces. These results were in accordance to the quantification carried out with classical techniques (BCA) and with the AFM studies.

Methods: Glass coverslips were coated with Ti (150A°) followed by Au (150 A°) using a high vacuum evaporator (Polaron E6100). SAM surfaces were prepared from alkanethiols 1-dodecanethiol (HS-(CH2)11-CH3), 11-mercapto-1-undecanol (HS-(CH2)11-OH) (Sigma). Surfaces were validated by water contact angle measurements (Dataphysics OCA). Solutions of BSA (from Merck Fraction V) were prepared in PBS. In order to analyze the adsorption mechanism of the protein 50, 250 and 500 mg/L BSA were used. Different electrochemical tests were conducted, potentiodynamic curves and electrochemical impedance spectroscopy (EIS) were measured at 3 different temperatures (20, 35 and 50 ºC) (Solartron 1287). Micro BCA (Thermo Scientific) and plate reader VICTOR III (Perkin Elmer) was used to measured the protein adsorption (from a 4,5 μg/ml BSA solution) on the different samples. AFM (tapping mode) experiments (2,5 μg/ml 4,5 μg/ml and 0,05 mg/ml BSA) were performed using a NanoScope IIIa instrument

Results: Electrochemical Impedance Spectroscopy (EIS) was used to characterize the adsorption of BSA on OH and CH_3 SAMs. The amount of adsorbed BSA was higher on the hydrophobic CH_3 surface, in agreement with BCA results. In addition, electrochemistry demonstrates that BSA is adsorbed with faster kinetics on CH_3 than OH surfaces. Moreover, EIS measured at different temperatures allowed us to calculate the energetic of the protein adsorption process, including the enthalpy (ΔH_{ADS}), entropy (ΔS_{ADS}) and Gibbs free energy (ΔG_{ADS}) of the adsorption process, which is difficult to obtained using other techniques (Figure 1, c). Calculated values displayed in table 1 and evolved differently for both surfaces. Results obtained by AFM (Figure 1, a - direct visualisation of BSA distribution on surfaces) and BCA (Figure 1, b - surface density of adsorbed) supported those obtained using less conventional electrochemical measurements. AFM experiments were performed using different BSA concentration (2,5; 4,5 and 50 μg/ml). BSA adsorption ended up in a monolayer of molecules that cover the material surfaces, by both increasing the adsorption time for a fixed concentration of the protein solution or, on the contrary, by increasing the concentration of the solution for a settled adsorption time.. BCA results (BSA adsorption performed at room temperature) were in concordance with electrochemical results showing that due to the spontaneity of the adsorption process ($\Delta G_{ADS}<0$, Figure 1, c), almost of the BSA present in the solution is afterwards adsorbed on the different surfaces.



c)

T (ºC)	Γ_{max} (M·Ω⁻¹)	$B_{ADS} \cdot 10^{-7}$ (dm³·mol⁻¹)	ΔG_{ADS} (kJ·mol⁻¹)	
20	0,07	0,16	-44,63	
	0,06	509	-64,23	
35	0,16	0,13	-46,43	CH_3
	0,11	0,35	-48,88	OH
50	0,44	0,06	-46,61	
	0,23	0,14	-48,86	

Figure 1. a) Height AFM images after adsorption of BSA (50 mg/ml) on the different surfaces. b) Surface density of adsorbed BSA on CH_3 and OH SAMs using a solution of concentration 4.5 μg/ml. c) Electrochemical results: Saturated surface concentrations and affinity constants obtained from EIS measurements at different temperatures.

Conclusions: We characterized protein adsorption mechanisms on surfaces with defined chemistry (CH_3 and OH SAMs) using Electrochemical Impedance Spectroscopy and results were compared with more conventional techniques (BCA and AFM). Elucidation of the underlying mechanisms is included in the framework of a bottom-up approach to design novel biomaterial surfaces able to direct protein adsorption and cell response. In the future, we intend to extend this methodology to evaluate the state of the cell population adhered on material surfaces and assess the state of the cell population during culture in a non-destructive way.

References:

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