SUPRAMOLECULAR ORGANIZATION OF ADSORBED COLLAGEN LAYERS ON POLYMER SUBSTRATA: EFFECT OF TIME AND SUBSTRATUM SURFACE CHEMISTRY

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Introduction

Protein adsorption is the earliest event after implantation of a biomaterial in a living body; it takes place during first seconds or minutes, while cells need hours to adhere [1]. It has been shown that protein adsorption determines how the surface interacts with cells and tissues and finally influences biocompatibility of the material [2-3] therefore understanding the mechanisms of adsorption process is of crucial importance in designing new high-performance biomaterials.

Collagen is a protein of particular interest because this so-called adhesive protein of the extra-cellular matrix is involved in many important biological functions such as tissue structuring and biorecognition process [4]. Collagen is also used for in vitro cell cultures to promote cell adhesion and spreading [5]. Numerous studies have shown that organization of collagen molecules adsorbed on various substrates influences behaviour of different cells [6,7,8]. For this reason understanding the factors influencing the organization of adsorbed collagen is a fundamental key in controlling cells adhesion.

Atomic force microscopy (AFM) is a recent imaging tool [9], which enables visualizing protein molecules under natural (physiological) conditions at high resolution, without special fixation and dehydration artefacts associated with vacuum methods [10]. It also provides a means of measuring interaction forces associated with material surfaces and/or adsorbed protein molecules [11] and of probing the stability of adsorbed protein films [12,13].

In this study, we use AFM together with XPS and radiolabeling to investigate the influence of time on the adsorption (adsorbed amount, supramolecular organization) of collagen on polymer substrates. Polystyrene (PS) was selected because it is widely used as a substrate for in vitro cell culture. In addition its surface chemical composition and hydrophobic/ hydrophilic can be easily modified using oxidation by oxygen plasma discharge (PSox), while keeping a constant surface roughness [14].

Experimental

PS and PSox substrata were prepared according to the method described previously [15] and characterised by: XPS (SSI X-Probe, Surface Science Instruments), water contact angle and atomic force microscopy (AFM, Nanoscope III, Digital Instrument, Santa Barbara, CA).

For collagen adsorption study: i) collagen solution (40µg/ml) was brought in contact with the substrates for various periods of time (up to 24h), ii) after incubation the aqueous phase was replaced by water and this was repeated to wash the sample without air exposure, iii) then the samples were examined in situ under water, by AFM, to study the topography of the protein film and to assess its stability and nanomechanical properties. The substrates were also examined after drying in nitrogen flow to get the insight into the remodelling of collagen film while dewetting.

Results and discussion

Radiolabeling and XPS indicated that for both substrates, the adsorbed amount increased to reach a plateau after about 2 h. Moreover, larger adsorbed amounts were always detected on PS than on PSox. AFM imaging revealed very different supramolecular organizations of the adsorbed films depending on time and on the substrate nature: PS showed patterns of collagen aggregates at all adsorption times (from 1 min to 24 h) while PSox was covered with homogeneous layers (FIG.1). The mechanical stability of the adsorbed films was assessed by scanning the AFM probe at high force. While no perturbations were created on PSox, the layer adsorbed on PS was reorganized by the scanning probe; the minimum force required to create perturbations on PS increased with time indicating strengthening of the film cohesion with time.

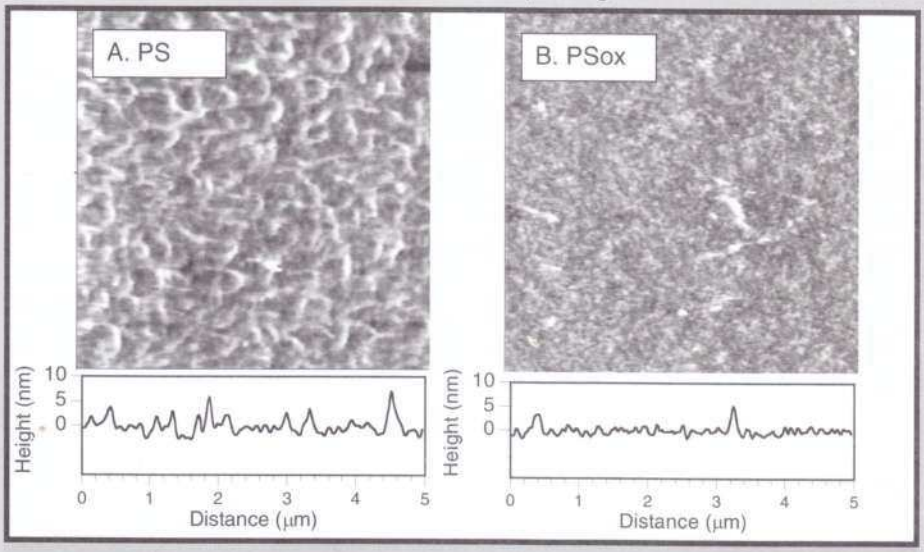

FIG.1. AFM topographic images (5 µm x 5 µm, z-range: 10 nm) recorded under water of collagen adsorbed on (A) PS, (B) PSox (30 min, 40 mg/ml). Cross-sections taken along horizontal lines in the center of the images are also shown.

These results show that adsorption time and substrate chemistry play a key role in controlling collagen adsorption in terms of adsorbed amount, supramolecular organization and stability of adsorbed film. The time dependence of the

collagen organization is modulated by the substrate nature, an effect that may be related to the mobility of the collagen molecules within the adsorbed phase.

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