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2008 J. Phys.: Conf. Ser. 100 062017

(http://iopscience.iop.org/1742-6596/100/6/062017)

Journal of Physics: Conference Series **100** (2008) 062017 doi:10.1088/1742-6596/100/6/062017

Protein film removal by means of low-pressure microwave plasma – an imaging ellipsometry study

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Abstract. Non-equilibrium plasma discharges have been recently proposed to be an effective tool for the removal of proteinaceous residuals from heat-degradable medical instruments. However, the knowledge regarding plasma-protein interactions is still relatively poor, which is a serious drawback for the validation of this technique as well as for its optimisation. This is, among other reasons, caused by the limitations of currently used techniques for monitoring of the rates of protein removal during plasma treatment. The objective of this article is to present an alternative method of evaluation of protein removal, based on imaging ellipsometry, which allows fast and semiquantitative analysis of the treatment efficiency.

1. Introduction

Application of non-equilibrium discharges for sterilisation and decontamination of surfaces of various objects is a promising alternative to commonly used techniques. This is mainly due to their high efficiency at relatively low cost, the possibility to maintain low temperatures during operation, as required for the treatment of heat-degradable instruments, and the non-toxic operation conditions. These key advantages already triggered off detailed investigations of plasma interaction with micro-organisms as well as with various other substances of biological origin. According to those studies it has been demonstrated that non-equilibrium discharges are capable both to kill bacteria or bacterial spores (e.g. review articles [1-3]), and to deactivate or to remove pathogenic biomolecules such as bacterial endotoxins (e.g. [4, 5]) or infectious prions [6]. In particular, the latter finding is of a great interest, since prions, transmissible agents of severe neurodegenerative diseases, have been found to exhibit extreme resistance to both physical and chemical treatment [7].

However, experimental studies focussed on the destruction of infectious prions by means of plasma discharges are rather problematic. This is primarily because of the necessity of high safety precautions, due to the extreme level of biohazard associated with such proteins. Therefore, in order to understand the mechanisms of plasma-protein interactions as well as to evaluate favourable conditions for their destruction, the effects of non-equilibrium plasma discharges are usually studied on non-pathogenic model proteins, employing a wide spectrum of experimental techniques, including biological assays [8, 9], surface analytical methods [10, 11], surface plasmon resonance (SPR) [8] and quartz crystal microbalance (QCM) experiments [4,12]. Nevertheless, the applicability of these methods is limited. For example in the case of surface analytical methods, such as like X-ray photoelectron spectroscopy

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(XPS) or time-of-flight secondary ion mass spectroscopy (ToF-SIMS) the process cannot be monitored *in-situ*, which also holds true for biological assays. On the other hand, SPR and QCM can be used for on-line observation of protein removal, but the applicability of these methods is limited to a narrow spectrum of substrate materials. In order to overcome these drawbacks, different approaches are to be developed. A possible candidate to overcome some of these drawbacks is imaging ellipsometry, as it is presented in this article.

2. Experimental

2.1. Sample preparation

The efficiency of plasma treatment in terms of protein removal was studied on Bovine Serum Albumin (BSA), selected as a model, non-pathogenic protein. For the preparation of samples, small droplets of 0.5% water solution of BSA were deposited on polished Si wafers by means of sterile syringes. Subsequently, the samples were placed in a common flow hood and allowed to dry at ambient temperature over night. Finally, the samples were treated by O_2/H_2 plasma discharge and examined by imaging ellipsometry.

2.2. Plasma treatment

Treatment of protein samples was carried out using a plasma reactor identical to the one used in our previous studies [4,5,12]. It consists of a stainless steel cylindrical vacuum chamber (200 mm in diameter and 380 mm in length) equipped with several diagnostics windows and one port for the sample introduction. The processing chamber is connected to the gas inlet system, which includes MKS mass flow controllers (*F*) attached to the gas lines and is evacuated by a primary pump and a roots blower pump.

The plasma is sustained by microwaves with an excitation frequency of 2.45 GHz. The microwave circuit includes the microwave supply (*MW*), a circulator (*C*), a three-stub impedance matching system (*3S*), and a rectangular-circular wave-guide transition (*R-C*). Microwaves are introduced into the plasma chamber through a silica window (*SiW*) placed at the extremity of a circular wave-guide.

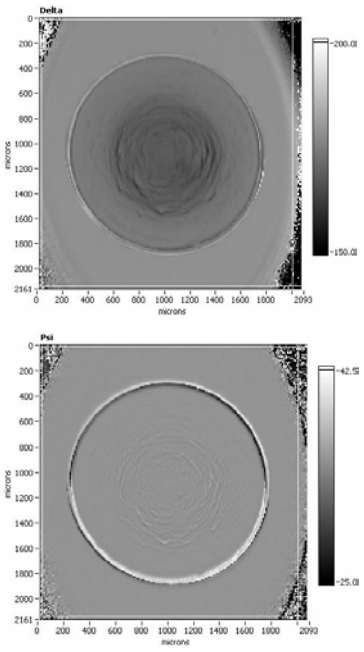
The results presented here were obtained at a pressure of 16 Pa, applied MW power of 1000 W, and at total gas flow of 100 sccm in a discharge mixture of O_2/H_2 (50:50), i.e. under conditions identified earlier to lead to the fastest etching of proteins using this kind of experimental setup [4,12].

2.3. Imaging ellipsometry

The protein deposits before and after plasma treatment were analyzed by ellipsometric measurements using a variable angle multi-wavelength imaging ellipsometer (model EP^3 by Nanofilm Surface Analysis GmbH, Germany). All measurements were performed in air at room temperature at an angle of incidence of 42° and a field of view of 2000 μm × 2000 μm. A monochromatized Xe arc lamp (λ=554.3nm) was used as light source. Conventional PCSA (Polarizer-Compensator- Sample-Analyzer) null-ellipsometric procedure is used to obtain 2D maps of the Δ and Ψ angles.

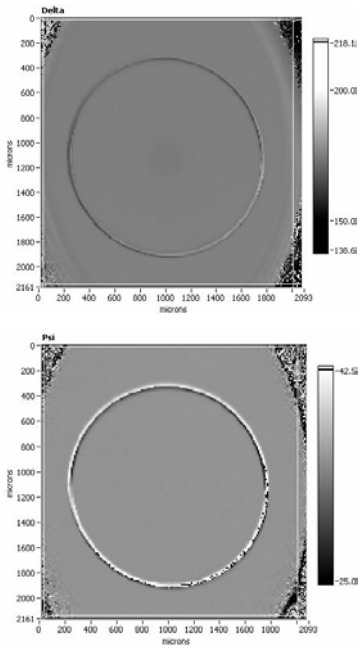
3. Results

On the as-deposited samples a well organized structure of protein was observed on the surface. As can be seen in figure 1, two distinctly different regions can be easily distinguished. At the border of the dried droplet a relatively thick ring is formed, whereas in the central region the coating is much thinner. Stylus profilometry showed that the ring has height of approximately 1 μm, whereas the centre region is less than 100 nm thick. After the plasma treatment the ellipsometric images are markedly altered, which is especially true for the Δ values, as demonstrated in figure 2. These modifications are clear indications that the thickness of deposit decreases during the plasma treatment.



(a) (b)

Figure 1. (a) Δ and (b) Ψ maps of the untreated sample.



(a) (b)

Figure 2. (a) Δ and (b) Ψ maps of a sample treated for 20 min in O_2/H_2 plasma discharge.

However, such observations are only a qualitative way of characterizing the samples. In order to perform a semiquantitative analysis of the central part of the BSA coating, the following procedure for data processing was applied. First, after each treatment step the central parts (150 pixels by 150 pixels) of Ψ- and Δ-maps are replotted point by point in a Δ-Ψ graph. As can be seen in figure 3, each sample is represented as a cloud of values in the Δ-Ψ plot. It is obvious that modifications of protein samples which occur during plasma treatment lead to pronounced variations of the datapoint distributions in the Δ-Ψ plot. These distributions can now be quantified using statistical methods to assign a unique value to each sample after each treatment step. For the evaluation of the removal efficiency of protein films on a large scale a suitable parameter that can be used for a quick estimation of the treatment progress is the area of the cloud of data points in Δ-Ψ space which corresponds to a certain treatment stage. It is evident that removal of the deposit leads to an equalization of the Δ and Ψ values over the analyzed surface portion and consequently to the reduction of the cloud area down to a size which corresponds to the uncontaminated substrate as demonstrated in figure 4. Furthermore, the observed trend is similar to the one reported recently employing quartz crystal microbalance for on-line measurement of BSA etching in O_2/H_2 plasma discharge [12]. This similarity allows us to safely assume that the ellipsometric results processed in the way described above can be used as a measure of the treatment efficiency as well as to analyze the kinetics of the removal process.

4. Conclusions

An application of imaging ellipsometry for monitoring of the removal of protein coatings from surfaces by plasma discharges is presented. This technique not only provides a qualitative description of the process, but it can be also used for the estimation of removal kinetics. Advantageous for possible applications, the method offers simple and fast estimation of the extent of protein removal especially for the highly inhomogeneous thin coatings, it is independent on the substrate and can be in principle used also for *in-situ* monitoring of plasma treatment. Particularly the last point is of high significance, since this approach can be used as process end-point detection.

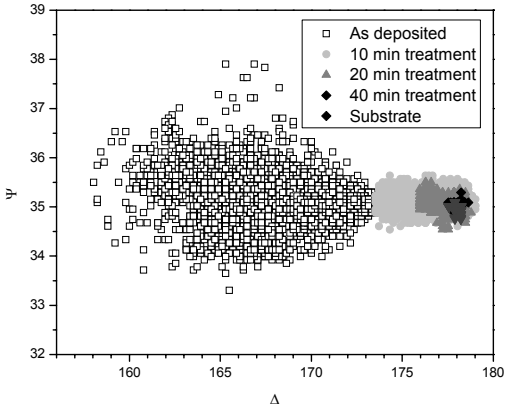


Figure 3. Δ-Ψ plots of the central part of the BSA deposit after sequential plasma treatment.

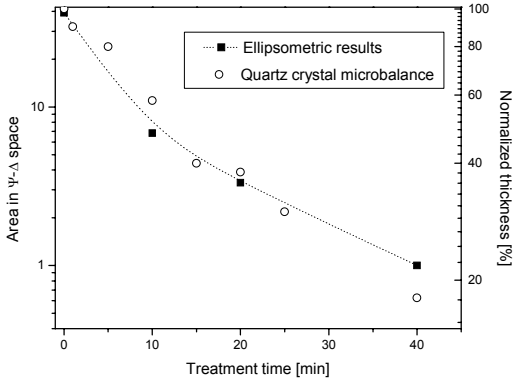


Figure 4. Temporal evolution of area in Δ and Ψ space representing different treatment stages and its comparison with the results obtained by QCM [12].

Acknowledgements
This work has been supported by the FP6 2005 NEST project "Biodecon" and the JRC Action 15008: NanoBiotechnology for Health.

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