ATMOSPHERIC PRESSURE PLASMA PREPARATION OF MICROPATTERNED TEFLON SURFACES FOR INTERACTION WITH CELLS*

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We report on the micropatterning of teflon foils by a cold atmospheric pressure plasma source, and on the effects of plasma treatment on epithelial cells attachment to surface. The plasma treatment causes wettability improvement and surface roughening. The cell attachment and biocompatibility of foils are both increased by the treatment.

Key words: plasma treatment of polymers, cells surface interactions, atmospheric plasma, PTFE surface modification.

1. INTRODUCTION

Among others materials, teflon (PTFE - polytetrafluoroethylene) is extensively used in biomedical applications like construction of heart stents, vascular grafting, etc., because of its special properties as high thermal stability, chemical inertness, low surface tension and low coefficient of friction [1]. For some particular applications it is necessary to improve the adhesion and wettability of the PTFE surface. As example, it is well known that the wettability, topography and surface chemistry can affect cell response on the surface [2]. Due to the ability to modify the surface characteristics without affecting the bulk material properties, radiofrequency plasma sources are widely used for treatments of polymers [3]. Most of polymers surface treatments are performed using low pressure plasma systems [4], but in recent years atmospheric pressure plasma techniques have appeared as an interesting alternative [5, 6, 7].

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In this paper we report results concerning the interaction of cells with PTFE surfaces, modified by a cold plasma source jet. Prior to the biologic tests PTFE foils were plasma treated through a metallic mask with circular openings, leading to micropatterned samples which posses alternating plasma treated and untreated zones. The modifications of wettability and topography of the surface after atmospheric plasma treatment were investigated by contact angle measurements and atomic force microscopy. The cell interaction with the plasma micropatterned PTFE samples was investigated in vitro, in epithelial cell culture systems, in order to evaluate the samples properties in relation with cell adhesion. The biocompatibility of untreated and plasma micropatterned surface was assessed by qualitative methods (optical microscopy investigations) and by quantitative methods [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay].

2. EXPERIMENTAL PROCEDURE

2.1. MATERIALS

Commercial flat sheets of PTFE 127 μm in thickness were purchased from DuPont (USA). The gas used for plasma treatments was Argon (99.999% purity).

2.2. PLASMA TREATMENT

Prior to plasma treatment, samples of PTFE were cut from PTFE sheets, washed ultrasonically for 15 minutes in ethyl alcohol and then air-dried.

Details on the radiofrequency atmospheric plasma source operation have been described in Ref [8]. It has small size: a total length of less than 100 mm and a diameter of 8 mm at the tip. To decrease the plasma source outer dimensions and considering the fact that the operating radiofrequency power values are low, the cooling of the electrodes was discarded. The atmospheric pressure plasma jet parameters working in argon were set as follows: RF power at 14 W, argon gas flow rate at 4500 sccm, substrate–nozzle distance at 2 mm. The gas temperature, as measured by a small thermocouple inserted in the plasma jet was 51^0C.

The plasma source was fixed above a motorized scanning stage with computer control movement in x–y coordinates (Figure 1). The PTFE foils placed on stage were scanned, by the Argon plasma expanding in the open atmosphere, over a 10 mm×10 mm area. The scanning route, consisting of parallel paths spaced at 1 mm distance, was designed to assure a quasi-uniform treatment of the surface with the plasma jet of 1 mm diameter. The scanning speed along the scanning path was 5 mm/s, which corresponds to a surface exposure of 0.2 s/mm^2.

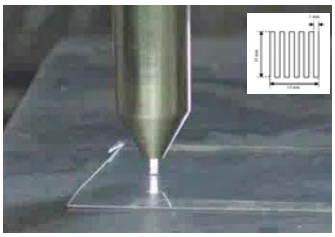


Fig. 1 – Image of the plasma source during surface scanning (inset: the scanning path on the probe).

Fig. 2 – Image of metallic mask, which is placed between plasma and PTFE foils (holes: 120 μm).

Both continuous and micropatterned PTFE surfaces were obtained. For patterned surface the plasma treatments were performed through a metallic mask with regular circular openings (diameters 120 μm). An image of the mask is shown Figure 2. By scanning through the mask age alternating plasma treated and untreated zones were realized.

2.3. SURFACE ANALYSIS

The effects of plasma on PTFE untreated and treated surfaces were analyzed by contact angle measurements and atomic force microscopy (AFM) investigations.

Static contact angle measurements were recorded using a KSV CAM101 microscope consisting of a single compact unit equipped with Fire Wire video camera with resolution of 640×480 pixels, a test stand, a standard syringe and a LED source. Distilled water drops of a 1μl liquid were deposited on the surface at room temperature.

The changes of surface topography were investigated by AFM using a Park Systems XE-100, in the non-contact mode. Surface topography was evaluated for areas of 5μm×5μm, and the surface roughness was evaluated using the root mean square roughness parameter.

2.4. CELLS AND CULTURE CONDITIONS

The specific assays were carried out, in vitro, on epithelial cells, Vero cell line, isolated from normal kidney of an African green monkey. To cultivate the cells, the untreated and micropatterned samples were sterilized by UV for 6 hours each side, inserted into 24-well polystyrene multidishes and seeded with Vero epithelial cells ($5x10^4$ cells/mL) in Minimum Essential Medium (MEM) supplemented with 10% Fetal Bovine Serum (FBS) and antibiotic mixture. The cells were cultivated for 24 h at 37^0 C in a humidified atmosphere containing 5% of CO_2.

2.5. EVALUATION OF THE CELL SPATIAL DISTRIBUTION ON THE SURFACE

Prior to microscopy imaging the cells were fixed with cacodylate-buffer and glutaraldehyde, desiccated four times at different ethylic alchol concentrations (50%; 70%; 96% and 100%) and left to dry. The distribution of the cells on untreated and micropatterned surface was evaluated on optical images taken under a Carl Zeiss Axioskop 40 FL optical microscope.

2.6. EVALUATION OF CELL VIABILITY

Cell viability was measured by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After 24 h, the culture medium was discarded, 50 µl of MTT solution (5 mg/ml) dissolved in the culture medium were added in each well and then, cells were incubated at 37^0C for 3 h. Water-insoluble dark blue formazan crystals formed in viable cells were solubilized with isopropanol and the absorbance of every well was measured at 570 nm using a Tecan spectrophotometer. Concentration of converted dye directly correlates to the number of metabolically active cells in the culture. Cell viability was calculated by comparison with control sample (epithelial cells control culture in contact with a full biocompatible polystyrene positive test plate).

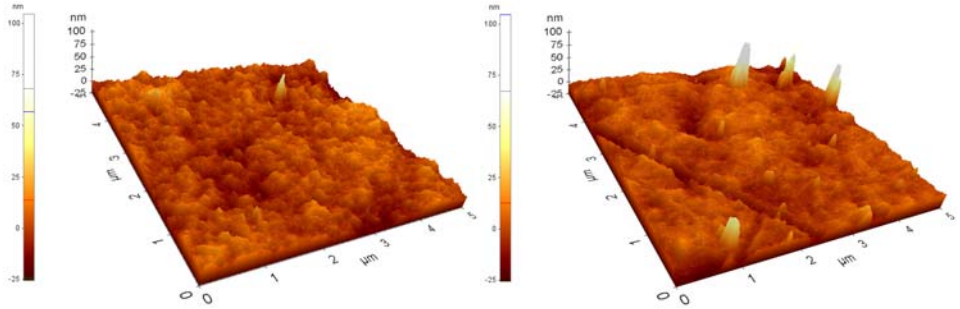
3. RESULTS AND DISCUSSION

3.1. PTFE SURFACE WETTABILITY

By treating in the atmospheric ambient the PTFE with the Ar plasma jet source, for 10 scans, the contact angle decreased from 104 to 85 degrees. In spite of the very short treatment time (total exposure 2 sec/mm^2) the water contact angle decreased by 19 degrees.

3.2. PTFE SURFACE TOPOGRAPHY

The images of untreated and plasma treated surfaces over a AFM scanned area of 5 µm×5 µm are shown in Figure 3. The average roughness value increased from 6.6 nm on the untreated surface to 7.5 nm, after 10 scans plasma treatment, showing that the roughening process is slow. Nevertheless, it has previously shown that the surface roughness can be modified in a larger measure, by increasing the number of scans [5].



a) b)

Fig. 3 – AFM images (vertical scale magnified 10 times) of a) untreated PTFE, RMS: 6.6 nm; b) PTFE plasma treated (10 scans), RMS: 7.5 nm.

3.3. CELLS ADHESION ON THE PTFE SURFACE

Images describing the distribution of cells on untreated and plasma micropatterned PTFE foils, after an incubation period of 24 h, are presented in Figure 4 and Figure 5, respectively. In Figure 5, the plasma treated zones are marked with red circles. It is observed that more cells adhered on the treated zones, peculiar to a selective growth process. In order to obtain a quantitative description of this selective growth, the surface densities of cells grown on the untreated zones and plasma treated zones were calculated. The existing cells were numbered in the two regions and divided by the total number of cells on the unit surface. These values were 497 cells/mm^2 for the untreated zones, respectively 1382 cells/mm^2 for plasma treated zones. These values correspond to an attachment preference of 74.2% for the plasma treated zones, and only 25.8% for the untreated zones.

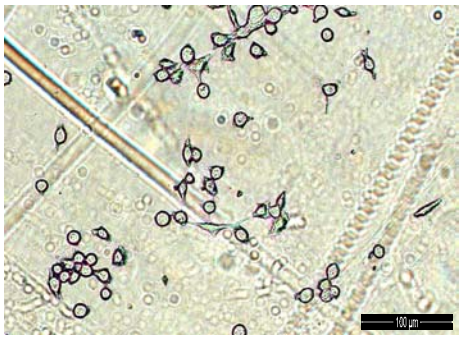


Fig. 4 – Distribution of Vero epithelial cells on untreated PTFE after 24 h of seeding.

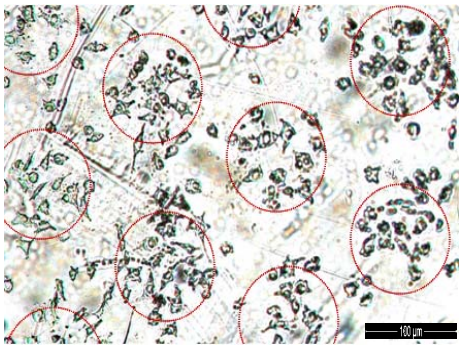


Fig. 5 – Distribution of Vero epithelial cells on plasma micropatterned PTFE surface after 24 h of seeding (red circles indicate the plasma treated zones).

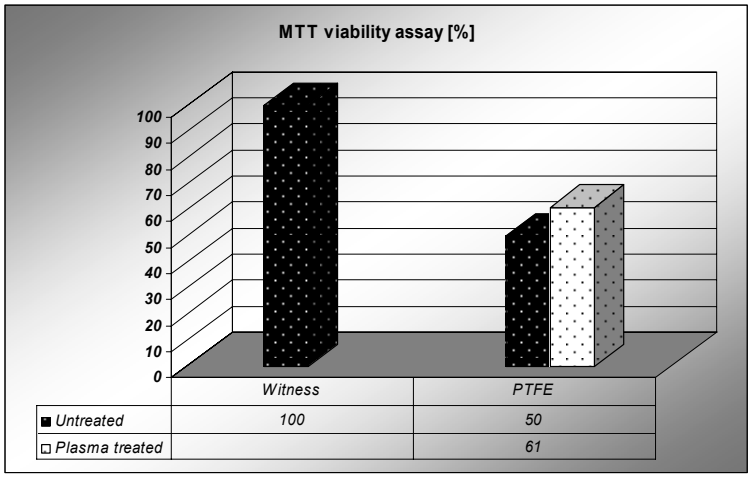


Fig. 6 – Quantitative epithelial cells viability after 24h, expressed as the percentage of viable cells in comparison with the plastic-positive control.

3.4. CELLS VIABILITY ON THE PTFE SURFACE

Cell viability tests (MTT) have been performed in order to evaluate cytotoxicity of the untreated and micropatterned PTFE surfaces, as compared with the control sample. The results of MTT tests are presented on Figure 6. The percent of viable cells in the culture with untreated PTFE foil was 50%. The percentage of viable cells from the culture containing plasma patterned samples increased from 50 to 61%.

4. CONCLUSIONS

We have performed PTFE surface modification and patterning by cold atmospheric plasma jet treatment, realized through a metallic mask. The hydrophilic character has been improved, and also the surface became slightly rougher, even at short treatment times as 2 s/mm^2. The experiments showed that the surface properties of PTFE modified by plasma jet are suitable for cell adherence and growth. Optical images show the selective adhesion of epithelial cells on plasma treated zones. Also, the MTT results indicate an improvement of cell viability in the presence of plasma treated samples.

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