Plasma Modification of Biomaterials Controlled by Surface Analysis

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Biomaterials are defined as materials to interact or be in contact with biological systems. This paper describes the fumctionalization of soft polymer surfaces against non-specific protein adsorption. A RF plasma changes the chemical composition of a surface within the nm-range, without changing bulk material properties. The chemical control of the surface is assured by X-ray Photoelectron Spectroscopy (XPS) and contact angle measurements (CAM).

NH_3-H_2 treated Polystyrene (PS) chips are used for enhanced biological-immobilization for sensors. Such biosensors use fluorescence immunoassays in medical diagnostics.

Pseudomonas aeruginosa is one of the most prevalent bacterial strains in a clinical environment. Teflon-like films deposited on native PVC are used for the preparation of non-fouling surfaces through the physisorption of PEO-PPO-PEO Pluronics® co-polymers. Secondly, PEO-like and Ag/PEO-like polymers were prepared by plasma polymerization techniques.

INTRODUCTION

Surface modification is a modern tool that can change the chemical composition of the outermost layers of polymers, rendering a new surface within nm-range for biomedical applications, without changing bulk properties. Biomaterials are defined as materials of either synthetic or natural origin, which are intended to interact or be in contact with biological systems [1]. One of the main requirements of biomaterials used in medical devices is that they are biocompatible, i.e. often implying minimal non-specific protein adsorption properties. In this paper we address the surface properties of biomaterials, such as chemistry and wettability that play an important role in the interaction with the environment and the success of their applications [2-4]. We describe the rf (radio frequency) plasma functionalization of soft polymer surfaces, which is intended to reduce non-specific protein adsorption, and is controlled by X-ray photoelectron spectroscopy (XPS) and water contact angle (WCA) measurements. We present data (1) that are based on the development of a functionalized Polystyrene (PS) sensor chip capable of detecting and differentiating functional groups of human body-fluids, i.e. blood. In the second part (2), data are presented which are part of the doctoral thesis work of D.J. Balazs [5], who investigated the different biomaterial properties that influence bacterial adsorption on native Mallinkrodt™ intubation tubings. Some of the highlights of these results are outlined as follows.

PLASMA REACTOR AND SURFACE NALYSISMETHODS

Plasma processing was carried out in a capacitively coupled, rf reactor operating at 13.56 MHz [5, 6]. The reactor (compare Fig. 1) consisted of two cylindrical electrodes of 13 cm diameter and separated by 4.3 cm apart in a cubic vacuum vessel. The upper electrode and the reactor walls were grounded. XPS analysis was performed using an imaging Kratos Axis Ultra X-ray photoelectron spectrometer equipped with a conventional hemispherical analyser. The X-ray source employed was a monochromatized Al K_α (1486.6 eV) source operated at 150 W. Data acquisition was performed under UHV (10^{-9} mbar) conditions. Analysis area was 0.21 mm^2 (300 μm × 700 μm) using a take-off angle

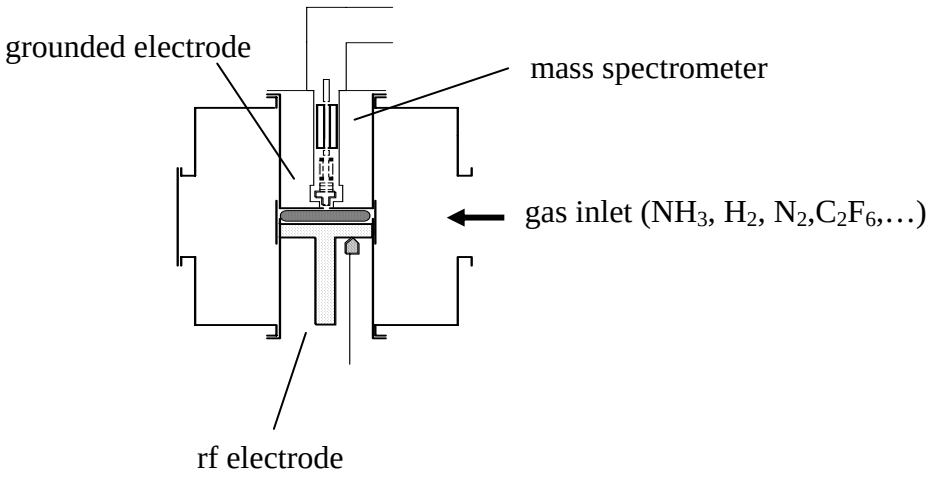


Fig.1 rf plasma chamber used for PS surface modification (NH_3, H_2, N_2 gases) and Teflon-like deposition on PVC (C_2F_6 and H_2 gases).

of 70° relative to the surface. The pass energies were 80 eV and 20 eV for wide scan and high-resolution scans, respectively. The operating software, Vision2, corrects for the transmission function. Charge compensation was performed with a self-compensating device (Kratos patent) using field emitted low energy electrons (0.1 eV) to adjust the main C-C component to 285 eV. The data reduction (atomic concentration, shifting, curve fitting, etc) was performed with CasaXPS Version 2.1.9 software [7].

Contact angle measurements on the mirror like PS surface have been studied by means of a Digidrop (GBX, France) contact angle measurement. Double distilled water with a drop volume of about 1.5 µl was used in the static mode at room temperature. The water droplet is dispensed by a mechanical syringe and the contact angle is recorded by a digital camera and PC controlled by Windrop++ software.

SURFACE MODIFICATION OF POLYSTYRENE BIOCHIPS

PS chips were obtained by injection molding of Polystyrol BASF. The PS chips were activated under a flow rate of gas mixture (N_2/H_2) or NH_3 at 60 sccm and at a gas pressure of 0.1 - 0.4 mbar in the chamber. The plasma was then generated at a power of 60 W for 3 minutes. After venting the plasma chamber with air the PS chip was transferred and analyzed by XPS.

We optimized the plasma parameters by investigating the plasma, using diagnostic techniques. The effect of dilution with H_2 of NH_3, on the ion composition within the plasma is shown in Fig 2a-b. The ion distribution for NH_3 dilution gives mainly NH_x^+ (x=2,3) with NH_4^+ at 18 amu as the dominant ions. H_2O has 18 amu as well. However, for H_2O (18 amu at 100%) in a quadrupole based mass analyzer one would expect a different ion fragmentation pattern: in the presence of H_2O^+ one would expect OH^+ (17 amu) at 21% and O^+ (16 amu) at 2%, respectively, which is not observed in Fig. 2a. We deduce therefore that the main peak at 18 amu corresponds to NH_4^+.

Only very little hydrogen H_x^+ (x=1,2,3) is found. These results lead to the conclusion that low addition of H_2 (up to 8%) is sufficient to produce NH_4^+, which is preferred and useful to be incorporated in the PS matrix for reactions favouring an increase of C-N groups in PS [8].

Integration of the measured intensities leads to fig. 2b showing the integrated intensity as a function of the ion energy. The parameter varied was the pressure in the plasma chamber. Energies below 15 eV are more adapted because they match better the binding energy of 3.6 eV for C-N or 6.3 eV for C=N [9]. This indicates that a total pressure of 0.4 mbar favours less energetic ions N-containing ions, which are preferred for incorporation into the PS matrix for biological coupling. For ions undergoing charge exchange reactions (N_2^+, N_2H^+) a broad energy spectrum is observed. In the absence of such a

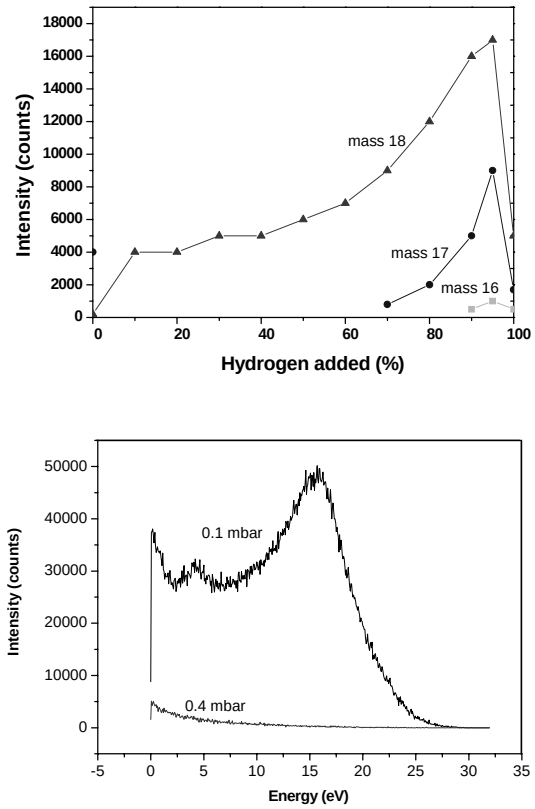


Fig.2 Plasma optimization for NH3.-H2 gas: (a) influence of the hydrogen dilution showing the most prominent ions determined by quadrupole mass analysis (b) energy of the integrated intensity at total pressures between 0.1 – 0.4 mbar

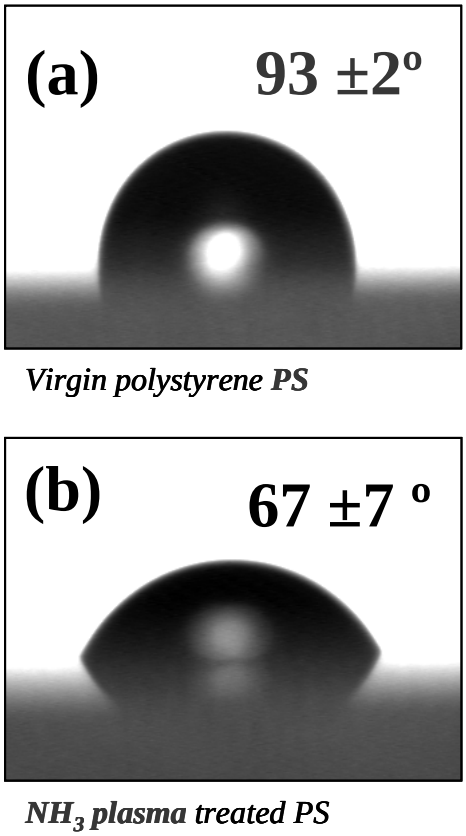


Fig. 3 Contact angle measurements for (a) virgin polystyrene (PS) and (b) NH3 treated PS

reaction (NH_4^+, NH_3^+) the ion energy was found at a maximum of 11-12 eV. One observes that the pressure influences strongly the NH_4^+ energy distribution. At 0.4 mbar and 0.2 mbar the ion energy decreases continuously to an energy between 15-20 eV. As for 0.1 mbar the energy distribution displays a strong maximum around 15 eV.

The ion flux and dose (fluence) was also determined for various plasmas. Tab. 1 presents examples for selected gas mixtures such as pure NH_3 plasma compared to NH_3-H_2 (92-8%) and N_2-H_2 mixtures (95-5, 90-10, and 70-30%, respectively). Inspection of Tab. 1 indicates that H_2 addition increases the total ion flux and ion dose. The measured ion flux for the PS surface activation is 10^{16} - 10^{17} ions/cm^2. One notices in Tab. 1 that the admixture of H_2 to NH_3 or N_2 respectively, has only little influence on the ion composition and nitrogen concentrations in agreement. It was therefore decided to continue with NH_3 gases which in return yield higher nitrogen activity in PS.

Contact angles were measured before and after plasma activations as shown in Fig.3a-b. Virgin PS shows contact angle of 93 ±2°, while after plasma treatment, it decreased to 67±7°, indicating an increase of hydrophilicity. The contact angle θ is related to the surface tensions at the interfaces between solid (S), liquid (L) and vapour (V) by:

$$\cos\theta = \frac{\sigma_{SV} - \sigma_{SL}}{\sigma_{LV}} \qquad (1)$$

Such a change is determined by a change in surface chemistry as evidenced by XPS measurements [10]. Fig. 4 illustrates the situation after a NH_3 plasma treatment showing the binding energy spectra of the wide and high-resolution scans illustrating, after fitting, the many functional groups created by the plasma [8]. One observes the presence of nitrogen, which has been quantified to give 19 at% for a NH_3 plasma (compare Table. 1).

Table. 1 Plasma characterization of NH3-H2 plasma

gas	ion mass	flux(μA/cm²)	dose (ions/cm²)	at% N_2
100% NH_3	18,17	26	2.9×10^{16}	19
NH_3-8 % H_2	18,17	26	3.0×10^{16}	19

The resulting PS surfaces carrying primary amino groups were chemically activated with Glutaraldehyde and then with Neutravidin [6]. Fluorescence immunoassay tests showed that the modified PS surfaces can be used in binding biotin-binding proteins on the basis of high affinity of avidin for biotin.

In conclusion (I), it was demonstrated (1) that quantitative chemical control of plasma modification was obtained illustrated by CAM and XPS and (2) that a tailored NH_3 plasma gives an increased number of hydrophilic groups including C-N coupling allowing binding of Neutravidin.

NON-FOULING SURFACES

Pseudomonas aeruginosa (P. aeruginosa) is considered to be the ideal opportunistic pathogen, which is unable to cause disease in healthy immune-competent individuals, but can infect those with weakened immune systems [11]. This gram-negative, rod-shaped bacterium can migrate and colonize surfaces, like the poly(vinyl chloride) PVC intubation tubes. Furthermore, *P. aeruginosa* is one of the most prevalent bacterial strains found in a clinical environment, responsible for 30% of hospital-acquired infections, 47% of which are pneumonia [12]. It has been demonstrated [13] that bacterial colonization of medical devices occurs over several steps, including the transport of the bacterial to the surface, an initial adhesion phase, which is hence followed by the molecular process of firm attachment, and colonization. Surface properties, such as chemistry, wettability and morphology will strongly influence how bacteria adhere to a biomaterial surface [14].

Polytetrafluoroethylene (PTFE) is widely employed in many biomedical applications due to its excellent blood biocompatibility that results from its protein adsorption properties. Teflon-like coatings can be plasma-deposited on PVC providing a pinhole

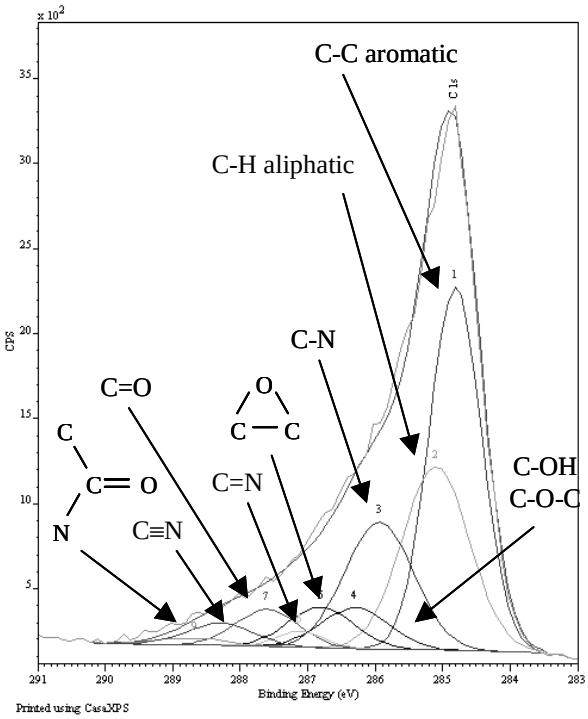
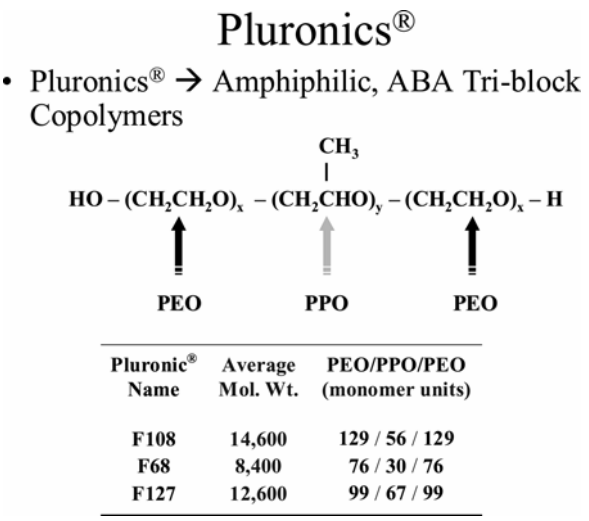


Fig. 4 High resolution C1s XPS spectrum of NH3 plasma treated PS



Pluronic® Name	Average Mol. Wt.	PEO/PPO/PEO (monomer units)
F108	14,600	129 / 56 / 129
F68	8,400	76 / 30 / 76
F127	12,600	99 / 67 / 99

Fig. 5 Chemical structure of Pluronic® block copolymer surfactants, where x and y represent the number of PEO and PPO chains, respectively.

free, reproducible coating with excellent adhesion to the biomaterial surface [2]. Furthermore, plasma polymerization techniques allow the possibility to create coatings with tailored chemical composition. Plasma-deposited Teflon-like surfaces exhibit a high chemical stability with low surface energy and are a stable platform for further surface modifications [15]. Pluronics® (BASF) are non-ionic, triblock copolymer surfactants containing a hydrophobic polypropylene oxide (PPO) segment, and two hydrophilic polyethylene oxide (PEO) segments, organized as PEO / PPO / PEO [16] as illustrated in Fig. 5. The Teflon-like coated surfaces are treated with Pluronics® to create non-fouling brushes, as protein adhesion is believed to be a key event responsible for specific adhesion of bacteria to a surface [5, 17]. The substrate used for the investigation of the Teflon-like film and Pluronic® modification was PVC, obtained from Mallinckrodt Hi-LoTM endotracheal tubes. The tubes were flattened to allow microscopic counting of bacteria as intended for future investigations. The custom-made plasma system, described above (see fig. 1), was used for the Teflon-like film deposition [15]. In order to deposit the Teflon-like coatings C_2F_6 and H_2 were then introduced into the chamber with varying gas feeds (total flow rate 60 sccm) to yield various C_2F_6 -H_2 ratios. The effects of each variable including, C_2F_6 %, power, and pressure, was investigated separately, and unless varied, the parameters were fixed to 80-20 % of C_2F_6 -H_2, 0.4 mbar, and 30 W, respectively [15].

The surface composition and the O/C elemental ratios for the Pluronic®-treated substrates, as compared to native PVC and the Teflon-like films were measured [17] - data are not represented here. There is clear evidence for Pluronic® adsorption to the Teflon-like substrates as the there is significant incorporation of ether carbon. The O/C ratio increases by ~5.5 fold following Pluronic® adsorption, as compared to the Teflon-like films. The curve fittings of the O 1s high-resolution scans for F108-treated native PVC and Teflon-like are illustrated in Fig. 6 where the non-treated Teflon-like surface are shown as references.

The next step was to explore the ability of the Pluronic® molecules to reduce the adsorption of the proteins, albumin and fibrinogen. Pluronics® F108 and F127 were chosen for the protein adsorption studies, because of their high adsorption to the Teflon-like surface [5, 17] and moreover, both have been shown to be very efficient at preventing protein adsorption [18].

The surface composition and the N/C elemental ratio for the Teflon-like (T), Pluronics® F108 and F127 physisorption to Teflon-like (T+F108 and T+F127), and all of these substrates following protein exposure are represented in Table 2. Evidence of protein adsorption to Teflon-like coatings was seen as the creation of nitrogen-containing functional groups, as the nitrogen content increases from 0%, prior to exposure, to 8.6% and 4.9% following fibrinogen and BSA exposure, respectively.

Pluronics F127 was found to be very effective at reducing both fibrinogen and albumin adsorption, as the values of the N/C ratios were 0, as nitrogen was not detected, whereas the N/C ratio determined for the Teflon-like without Pluronic modification were 0.15 and 0.9, following fibrinogen and BSA exposure, respectively. Pluronic® F108 was found to be quite efficient at reducing protein adsorption, as well. However, fibrinogen and BSA adsorption were not completely prevented as nitrogen was identified at an atomic percentage of ~0.4% following exposure to the F108 treated Teflon-like substrates. Adhesion was

Table 2 Atomic percentages for the Teflon-like films (T), following incubation in Pluronics® F108 and F127, and subsequent incubation in the proteins, fibrinogen (F) and bovine serum albumin (BSA), where T correspond to the Teflon-like film substrates

Pluronics® and Protein Fouling

	C 1s (%)	F 1s (%)	O 1s (%)	N 1s (%)	N/C
T	37.6	61.8	0.6	-	-
T + F	56.2	23.5	11.7	8.6	0.15
T + BSA	52.8	35.0	7.3	4.9	0.09
T + F108	44.0	52.1	4.0	-	-
T + F108 + F	44.3	49.6	5.7	0.4	0.01
T + F108 + BSA	44.7	48.3	6.7	0.3	0.01
T + F127	45.1	50.9	4.1	-	-
T + F127 + F	47.1	47.2	5.6	-	-
T + F127 + BSA	49.5	45.3	5.2	-	-

evaluated the Teflon-like + Pluronic surfaces using for four different *P. aeruginosa* strains including a wild-type PAO1, one mutant (AK44) lacking various surface structures and two different clinical isolates (1.1.A1 and 19G12). Despite their non-fouling behaviour, the Pluronic surfaces did not prevent bacterial adhesion.

Plasma polymerized PEO-like and Ag/PEO-like were also tested for their ability to repel fibrinogen (F) and bovine serum albumin (BSA) [19]. The Ag-free films with the highest possible PEO-character (65%) produced were completely non-fouling and prevented adsorption of both albumin and fibrinogen. However, like the Pluronics the non-fouling PEO-like films did not reduce bacterial adhesion. On the contrary Ag/PEO-like films, which showed significant protein adsorption [19], reduced bacterial adhesion by 100% (see Fig.7) [19].

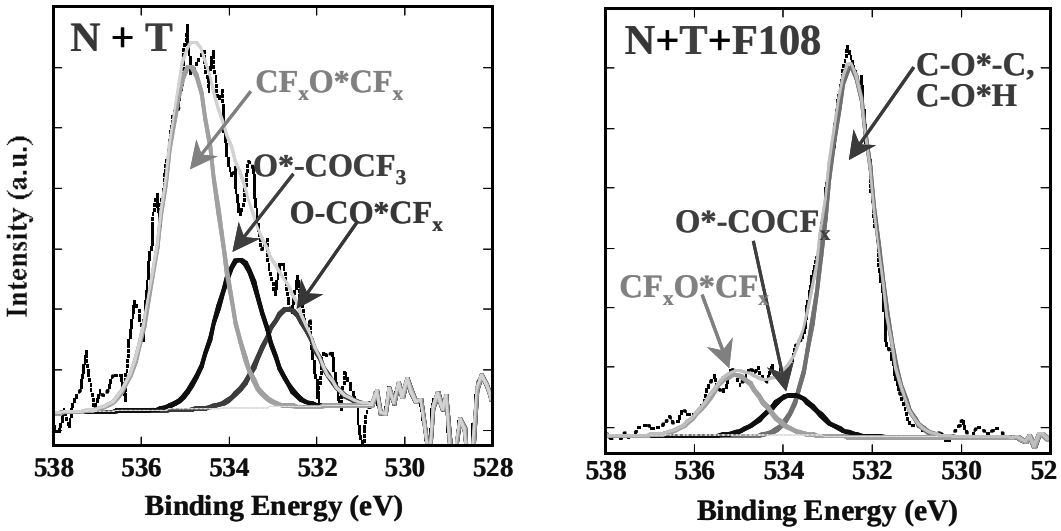


Fig. 6 Fitted O 1s high-resolution scans of native and fluoropolymer films following incubation with Pluronic F108. N and T correspond to the native and teflon-like film substrates, respectively.

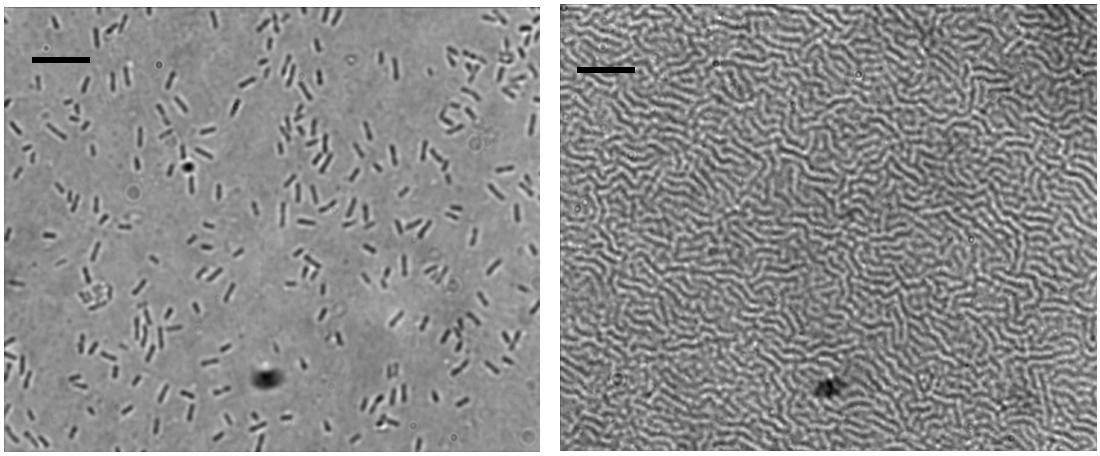


Fig. 7 Bacterial adhesion assays of (a) native PVC and (b) native PC treated with Ag/PEO-like coating.

In conclusion (II), it has been shown that both PEO-based Pluronics and plasma polymer PEO-like coatings produce non-fouling surfaces, but do not guarantee a reduction in bacterial adhesion. On the contrary, Ag/PEO-like plasma polymers reduce colonization of *P. aeruginosa* considerably, despite their protein-adsorptive nature. This indicates that it is the germicidal property of Ag that is responsible for the reduction of bacterial adhesion and not the non-fouling ability of a coating [5, 17, 19].

GENERAL CONCLUSIONS

Modification of materials for biomedical applications by plasma processing is an efficient way to create surfaces with tailored chemical functionality, without changing the bulk properties of the native biomaterial. Surface chemical composition and wettability changes can be determined in the nanometer range by X-ray induced photoelectron spectroscopy (XPS) and contact angle measurements, respectively. By the choice of appropriate gases and optimization of the plasma processing parameters one can create new surfaces, having improved biological interactions. Both non-fouling and anti-bacterial properties can be established using these techniques as proven by biological tests.

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