Plasma Treated and a-C:H Coated PET Performance in Inhibiting Bacterial Adhesion

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A comparative study of the effects of plasma deposited amorphous carbon (a-C:H) thin film and He/O_2 plasma surface treatment of PET on the physicochemical surface properties and the initial bacterial (*Staphylococcus epidermidis*) adhesion was performed. CH_4/H_2 RF discharges with different substrate bias voltages were used for the deposition of a-C:H thin films while He/O_2 plasmas with different O_2 fractions were used for the surface treatment of PET. All the plasma modifications were shown to significantly reduce the bacterial adhesion in comparison to the untreated PET; however, the reduction was greater for the He/O_2 surface treatment. The increase in the surface free energy of the substrate surfaces due to He/O_2 plasma treatments seems to inhibit bacterial adhesion and aggregation.

Introduction

Infection of biomedical devices is a serious impediment of their long-term use, even though their materials, such as poly(ethylene terephthalate) (PET), are considered biocompatible.[1,2] *Staphylococcus epidermidis* has been identified as a predominant cause of infection in the presence of a medical device, due to its ability to adhere to surfaces (initial-physicochemical adhesion phase), and to form large biofilms with multilayered cell clusters embedded in an amorphous extracellular material more commonly known as slime (molecular-specific phase).[3]

One possibility to reduce the bacterial adhesion to medical devices is to modify the surface of existing biomaterials or devices and therefore to alter the physicochemical interactions between bacteria and substrate.[4] As a result, a significant number of studies on improving the antibacterial properties of materials have focused on surface modification.[5–9] Among the methods used, plasma surface treatment or deposition of different kinds of thin films present some major advantages; they are fast and economical processes performed at room-temperature, capable of treating complex shapes while they modify only the surface of the polymer leaving the bulk properties largely unaffected.[10,11]

The present work investigates the effects of the substrate bias potential, in the case of amorphous carbon (a-C:H) thin film depositions, as well as the effects of oxygen percentage in helium on the physicochemical and morphological surface properties of treated PET and on bacterial adhesion. Since in biological systems, hydrophobic interactions are usually the strongest of all the long-range non-covalent forces,[12] the aim of this study is to investigate the role of surface hydrophobicity on bacterial adhesion and aggregation, by quantitatively comparing *in vitro* adhesion of *S. epidermidis* onto PET, a-C:H-coated PET and He/O_2 plasma treated PET. These modifications allow for a direct evaluation of the effects of discharge conditions and the resulting biomaterial surface energy on bacterial adherence.

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Experimental Part

The plasma treatments were performed in a cylindrical, 160 mm in diameter, stainless steel chamber that has two parallel round stainless steel electrodes with a diameter of 55 mm and an interelectrode distance of 25 mm.[13] PET films with a thickness of 100 μm, supplied by Goodfellow, were mounted on the grounded electrode surface. For both processes the total flow rate was fixed at 20 sccm. Various compositions of the gaseous mixture: between 100% He and 100% O_2, were used in the case of He/O_2 PET treatments. These treatments were performed in constant power conditions (1.2 W) while applying a DC substrate bias voltage (V_d) of -30 V, at a total gas pressure of 0.25 Torr. The values chosen for the total gas pressure and the DC bias are based on the observation that these conditions were found to reduce the aging effect in plasma-treated polymers.[14] On the other hand, a-C:H depositions were done after a 10 min treatment of the PET substrates in helium plasma. The flow ratio and consequently the mole fraction of CH_4 in H_2 were maintained at 50%, at the total gas pressure of 0.5 Torr. The radio frequency (RF) power from a 13.56 MHz generator was fed into the discharge through a wattmeter and a proper impedance matching network, while a 30 kHz AC bias (V_{AC}) was applied to the substrate holder. In the a-C:H depositions, the RF electrode peak-to-peak voltage (V_{RF}) was set to 300 V. The substrate bias (V_{AC}) was varied from 100 to 400 V peak to peak, in order to study all the possible a-C:H growth regimes, from polymeric to diamond-like carbon (DLC) and finally to graphitic thin films, as energetic ions play a significant role in the deposition rate and structure.[15,16] Current and voltage waveform measurements (CVWM) on the RF electrode were used to determine the real power consumed in the discharge.[17] The increase in V_{AC} while maintaining contant the V_{RF} as well as all the other plasma parameters, leads to an enhancement of the total power dissipated in the discharge.[16]

The deposition rate or the PET surface etching rate were measured in situ using laser reflectance interferometry (LRI).[18] The deposition time was thus tuned to produce a-C:H films with a thickness of 200 nm in all the cases. The specific thickness is conveniently achieved at reasonably low deposition times (<75 min).

Examination of the surface topography of native and plasma-modified PET surfaces as well as the evaluation of the average surface roughness (R_a), for 10 μm^2 area images, were conducted by using a multimode atomic force microscopy (AFM; Nanoscope III, Digital Instruments) in contact mode.[19] The substratum surfaces were characterized by their contact angles using the sessile drop technique with water and methylene iodine as the wetting agents. The contact angles of the various substrates were measured on the day of the bacterial adhesion experiments (within 10 d after surface modification). In the case of the bacterial cells, the measurements were performed on the bacterial layers deposited on membrane filters, according to the method described by Busscher et al.[20] The bacterial strain used in this study was the reference type culture *S. epidermidis* ATCC 35984 that is slime producing. Bacterial adhesion was performed by placing the substrates (1 cm^2) in 1 ml of the bacterial suspension [3×10^8 colony-forming units (CFU) per ml phosphate-buffered saline] for 150 min at 37 °C, under gentle shaking. Quantification of bacterial adhesion was conducted by the CFU counting method and by scanning electron microscopy (SEM).[19] The effect of the various plasma processes on the bacterial adhesion was statistically analyzed using the SPSS 12.0 package for windows. $p<0.001$ or <0.05 was chosen to denote the significance level.

Results and Discussion

The exact experimental conditions used for either He/O_2 plasma treatment or plasma deposition of a-C:H on PET are summarized in Table 1. Figure 1(a) and 1(b) present the electrical conditions of the etching and deposition discharges, respectively. Figure 1(a) (left axis) shows the V_{RF} variation required for maintaining the same power level

Table 1. Gas flow rates (sccm), total gas pressure (Torr), RF electrode voltage, DC and AC substrate bias (V), and total discharge power (W) applied for the deposition of a-C:H thin films (PET1-PET4) and the treatment of PET films (PET5-PET10).

Sample name	CH_4 flow rate	H_2 flow rate	He flow rate	O_2 flow rate	Pressure	RF oltage V_{RF}	AC bias V_{AC}	DC bias V_d	Power
	sccm	sccm	sccm	sccm	Torr	V	V	V	W
PET1	10	10	–	–	0.5	300	100	–	4.5
PET2	10	10	–	–	0.5	300	200	–	5.1
PET3	10	10	–	–	0.5	300	300	–	5.9
PET4	10	10	–	–	0.5	300	400	–	6.1
PET5	–	–	20	0	0.25	305	–	-30	1.2
PET6	–	–	18	2	0.25	306	–	-30	1.2
PET7	–	–	16	4	0.25	278	–	-30	1.2
PET8	–	–	10	10	0.25	242	–	-30	1.2
PET9	–	–	0	20	0.25	200	–	-30	1.2
PET10	–	–	20	0	0.5	300	–	-30	2.0

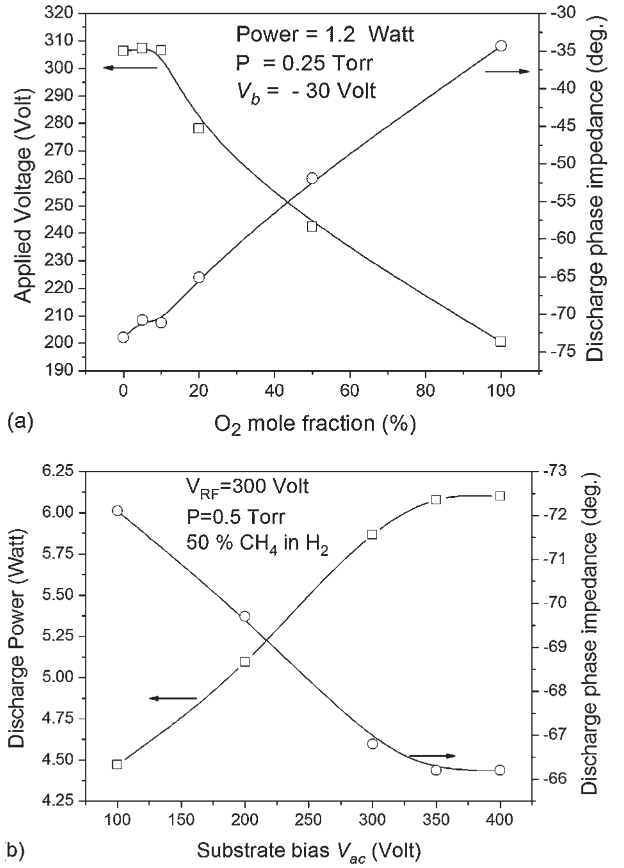

Figure 1. (a) RF electrode peak-to-peak voltage (left axis) and discharge phase impedance (right axis) as a function of O_2 mole fraction in O_2/He 0.25 Torr discharges and (b) total discharge power (left axis) and discharge phase impedance (right axis) as a function of the substrate bias peak-to-peak voltage in 50% mole fraction of CH_4 in H_2 0.5 Torr discharges.

(1.2 W), as a function of the %O_2 mole fraction. The drop of V_{RF} with an increase in %O_2 is due to the fact that O_2 has quite larger electron–molecule collision cross-sections compared to He. In addition, a large number of low-energy electron–molecule collision processes (rotational–vibrational, excitation, dissociation) take place in O_2-containing discharges. This is also supported by the results concerning the discharge phase impedance, included in Figure 1(a) (right axis). The higher %O_2 results in less negative discharge phases, indicating an enhancement of the ohmic (collisional) character of the discharge.

Figure 1(b) (left axis) shows the variation of discharge power as a function of the substrate bias voltage in a-C:H deposition for 50% CH_4/H_2 discharges. The power consumption increases with V_{AC} up to 300 V, while for higher values the effect is negligible. A similar increase in the power consumption with substrate bias has been previously reported[16] for highly diluted CH_4/H_2 discharges and was mainly attributed to the enhancement of the energy supplied to the electrons rather than to the energy spent for the acceleration of ions in the substrate holder sheath. This also seems to be valid in this case where the fraction of CH_4 is much higher. In addition, the discharge phase impedance, plotted on the right axis of Figure 1(b) supports this interpretation since the increase in V_{AC} leads to less negative phase values.

By comparing the experimental conditions of the two processes, one can say that a-C:H deposition has much higher energetic demands compared to He/O_2 treatment, since for about the same RF electrode voltage, the consumed power is four times higher. However, despite the much higher power dissipation, a-C:H deposition is quite slower compared to the PET etch process, due to the more complicated plasma–surface interaction in CH_4/H_2 discharges. Therefore, the actual treatment and deposition times were quite different. For He/O_2 plasma the treatment time was 15 min and the achieved etch rates were up to 2.7 $Å \cdot s^{-1}$, while for the CH_4/H_2 discharges the deposition time was between 75 min for the sample PET1 (Table 1) and 40 min for the sample PET4 as the increase in V_{AC} increased the film growth rate. In all the cases, the deposition rate was below 0.1 $Å \cdot s^{-1}$.

Furthermore, a change of the PET surface morphology is expected as a consequence of either the a-C:H deposition or the etching by He/O_2 plasmas. The AFM images show that the unmodified PET surfaces are relatively smooth with granular structures of conical shape and moderate roughness.[19] Amorphous carbon thin films present smoother surfaces than the native PET substrate, while He/O_2 treated PET films show an enhancement of the already existing granular structures, depending on %O_2 content. Table 2 summarizes the calculated R_a for PET films modified at different discharge conditions. The increase in V_{AC}, in the case of a-C:H depositions, does not significantly influence the surface roughness; however, there is a trend of decrease in R_a with higher substrate bias. On the other hand, R_a increases as a function of %O_2. It is remarkable that, overall, R_a remains quite small (<10 nm), indicating the high stability of PET films against plasma treatment, as the phenyl ring is quite stable contributing to a global protection of the ester group from the attack of ions and other reactive species. Moreover, Table 2 presents the total free energy of the surface-bacterial cells (γ_s^{TOT}) and its dispersion (γ_s^d) and polar (γ_s^p) component, calculated from the linear fitting curves of the equation:

$$(1 + \cos\theta)\gamma_{lv} = 2(\gamma_s^d \gamma_{lv}^d)^{1/2} + 2(\gamma_s^p \gamma_{lv}^p)^{1/2} \qquad (1)$$

where θ is the water or the methylene iodide contact angle (data not shown), according to the Owens and Wendt approach.[21] The influence of R_a on the measured contact angles is considered as negligible since R_a is quite small

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Table 2. Average surface roughness (R_a) in nanometers, total free energy (γ_s^{TOT}) and its dispersion (γ_s^d) and polar (γ_s^p) components of the various materials and bacterial cells in $mJ \cdot m^{-2}$, γ_s^p/γ_s^d ratio, total free energy of bacterial adhesion (ΔG_{adh}^{TOT}) and its dispersion (ΔG_{adh}^d) and polar (ΔG_{adh}^p) components in $mJ \cdot m^{-2}$, and number of adherent bacteria per cm² (N) to the various materials.

Sample	R_a	γ_s^d	γ_s^p	γ_s^p/γ_s^d	γ_s^{TOT}	ΔG_{adh}^d	ΔG_{adh}^p	ΔG_{adh}^{TOT}	N^* **E6**
PET	3.08 ± 0.99	41.8	3.8	0.09	45.6	−1.6	−15.2	−16.8	9.58 ± 0.99
PET1	2.68 ± 0.19	37.4	2.4	0.06	39.8	−1.3	−16.4	−17.7	7.25 ± 0.36*
PET2	2.52 ± 0.30	35.3	1.9	0.05	37.2	−1.1	−16.9	−18.0	7.68 ± 0.63*
PET3	2.37 ± 0.32	38.6	1.8	0.05	40.4	−1.3	−17.1	−18.4	8.01 ± 0.54*
PET4	2.11 ± 0.15	36.2	0.9	0.03	37.1	−1.2	−18.2	−19.4	8.16 ± 0.37*
PET5	3.68 ± 0.34	37.4	26.0	0.70	63.4	−1.2	−6.0	−7.2	2.34 ± 0.18*,**
PET6	3.07 ± 0.54	32.2	24.2	0.75	56.4	−0.9	−6.5	−7.4	2.48 ± 0.26*,**
PET7	2.99 ± 0.32	38.1	23.4	0.61	61.5	−1.3	−6.8	−8.1	2.45 ± 0.13*,**
PET8	4.56 ± 0.62	35.7	28.8	0.81	64.5	−1.1	−5.2	−6.3	3.20 ± 0.18*,**
PET9	5.21 ± 1.18	34.3	29.9	0.87	64.2	−1.0	−4.9	−5.9	2.76 ± 0.28*,**
PET10	4.82 ± 0.37	35.3	31.6	0.90	66.9	−1.1	−4.5	−5.6	1.44 ± 0.21*,**
S. epidermidis	–	26.0	32.2	1.24	58.2	–	–	–	–

$^*p<0.05$, where all the values of the modified surfaces were compared with the value of the unmodified PET; $^{**}p<0.05$, where all the values of the He/O_2 plasma treated samples were compared with those of the amorphous carbon thin films.

and therefore the real area of the surface is not significantly different than the geometric area.[22] The results show that PET and a-C:H films have a hydrophobic behavior with much higher γ_s^d than γ_s^p. The increase in the substrate bias potential results in a drop of γ_s^p and γ_s^p/γ_s^d ratio. In the case of He/O_2 discharges, the increase in O_2 mole fraction in He is found to favor the γ_s^p/γ_s^d ratio, while in pure He discharges the increase in pressure from 0.25 to 0.5 Torr has the same effect.

Table 2 summarizes the results of the bacterial adhesion experiments onto the different samples, showing that the highest number adhered to the unmodified PET, compared to all the modified samples ($p<0.05$). The He/O_2 plasma treated PET significantly reduced the bacterial adhesion not only in comparison to the untreated PET but also to all the a-C:H films. In addition, clusters of bacteria appeared on the unmodified PET [Figure 2(a)] and the a-C:H films [Figure 2(b)], the most hydrophobic materials, while on all the He/O_2 plasma-treated samples the bacteria were isolated [Figure 2(c)]. Due to the high ionic strength of the buffer used for the bacterial suspension, changes in the chemical structure that took place during the plasma modification of PET and concern the wettability, and therefore the surface energy of the substrate must be the important parameter that influenced bacterial adhesion and accumulation. In particular, the number of adherent bacteria per cm^2 (N) was observed to be negatively correlated with the total free energy of the substratum surfaces (γ_s^{TOT}) (linear correlation, $N = -240\,644\gamma_s^{TOT} + 2E7$, $p<0.001$, $R^2 = 0.81$; non-linear correlation of form $N = 7E7e^{-0.0528\gamma_s^{TOT}}$, $p<0.001$, $R^2 = 0.83$) and its polar (γ_s^p) component (linear correlation, $N = -226\,434\gamma_s^p + 9E6$, $p<0.001$, $R^2 = 0.92$; non-linear correlation, $N = 9E6\exp(-0.0488\ \gamma_s^p)$, $p<0.001$, $R^2 = 0.91$), whereas it was not significantly correlated with its dispersive (γ_s^d) component (linear correlation $p = 0.037$, $R^2 = 0.34$; non-linear correlation, $p = 0.062$, $R^2 = 0.28$).

The pronounced effect of the total free energy of the substratum surfaces and its polar component on bacterial adhesion may be explained by thermodynamic models.[23–25] In such models the total free energy of bacterial adhesion (ΔG_{adh}^{TOT}) is determined by the equation:

$$\Delta G_{adh}^{TOT} = \gamma_{BS} - \gamma_{BL} - \gamma_{SL} \qquad (2)$$

where γ_{BS}, γ_{BL}, and γ_{SL} are bacterium–substratum, bacterium–liquid, and substratum–liquid interfacial free energies, respectively, and lowering of the ΔG_{adh}^{TOT} favors bacterial adhesion. In this study, the dispersive-polar approach[24] was used to calculate the interfacial energies, the ΔG_{adh}^{TOT} and its polar (ΔG_{adh}^p) and dispersive (ΔG_{adh}^d) components (Table 2). This approach, although it could not predict the decrease in bacterial adhesion to a-C:H in comparison to the unmodified PET, probably due to the small change in the surface wettability, it predicts well the decrease of adhesion to all the He/O_2 plasma treated samples. In particular, adhesion was observed to be negatively correlated with the ΔG_{adh}^{TOT} [linear correlation,

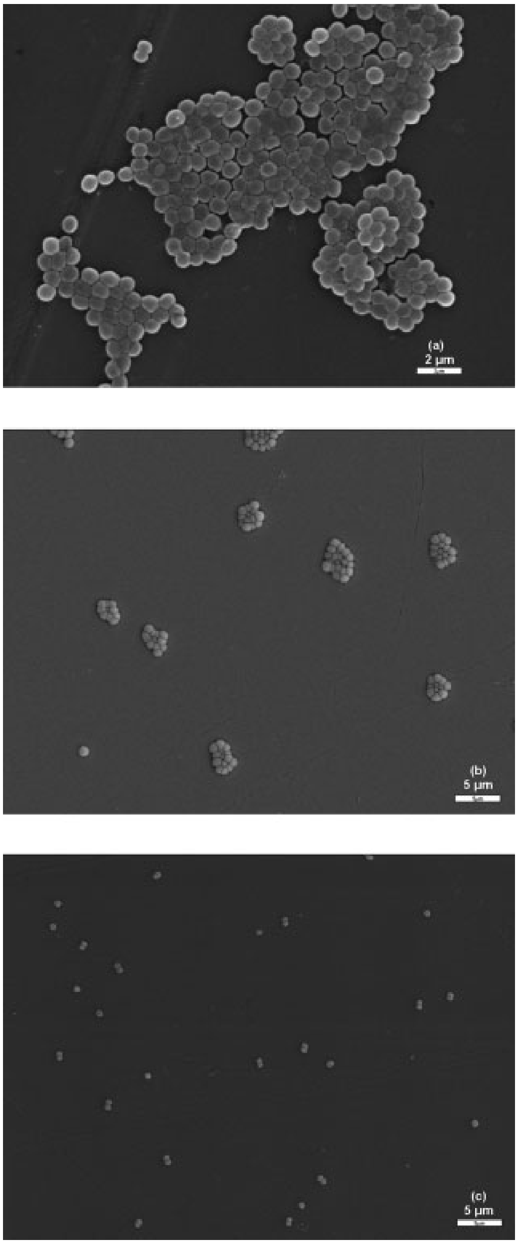

Figure 2. SEM images of *S. epidermidis* cells adhered to (a) untreated PET ×5 000, (b) amorphous carbon thin film deposited under $P = 0.5$ Torr, $V_{AC} = 400$, $V = 300$ ×2 000 and (c) biased PET film treated with 0.25 Torr, 20% O_2 in He discharges ×2 000.

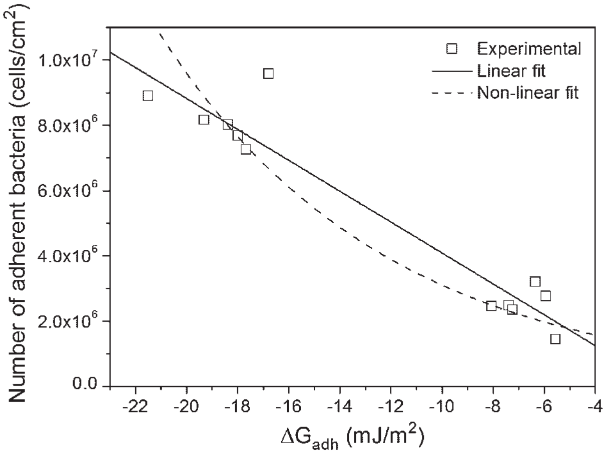

Figure 3. Number of adherent bacteria per cm² (*N*) correlated with total adhesion free energy (ΔG_{adh}^{TOT}). Linear correlation ($N = -482\,436\Delta G_{adh}^{TOT} - 839\,209$, $p < 0.001$, $R^2 = 0.92$) and non-linear correlation ($N = 1E6\exp(-0.1033\ \Delta G_{adh}^{TOT})$, $p < 0.001$, $R^2 = 0.90$) are also included.

$N = -482\,436\Delta G_{adh}^{TOT} - 839\,209$, $p < 0.001$, $R^2 = 0.92$; non-linear correlation, $N = 1E6\exp(-0.1033\ \Delta G_{adh}^{TOT})$, $p < 0.001$, $R^2 = 0.90$); Figure 3] and its polar component, whereas it was not significantly correlated with its dispersive component. Therefore, since the γ_s^d does not vary significantly among the various materials and $\gamma_b^p < \gamma_l^p$, bacterial adhesion is not energetically favorable as γ_s^p increases. This could be explained by the presence of hydrated layers at the He/O_2 treated surfaces and around bacteria, due to their hydrophilic-polar nature, which, during bacterial adhesion, overlap giving rise to repulsions which are commonly known as "hydrophilic repulsions" or "hydration forces".[25]

In an attempt to compare our results with other literature data, we observed that there are controversies concerning the effect of γ_s^p on bacterial adhesion. For example, *S. epidermidis* adhesion was reduced by argon plasma treated polyethylene[8] and by carbon films deposited on PET,[5] whereas it was increased by O_2 plasma treated polystyrene.[26] These controversies may appear due to differences in the bacterial strains used or in the experimental conditions.

Another parameter that may also influence bacterial adhesion and is not considered in the thermodynamic theory is the surface roughness. However, in this study there were no observable ($p = 0.110$, $R^2 = 0.22$) effects of R_a on bacterial adhesion, meaning that the total free energy of the substratum surfaces and their polar components are the key factors affecting adhesion. If we try to correlate adhesion to surfaces with similar surface energy but different R_a, including the results from our previous work,[19] we can observe that adhesion to He/O_2 plasma treated samples was higher to surfaces that were rougher ($p < 0.01$, $R^2 = 0.52$).

Conclusion

He/O_2 plasma treatment of PET and plasma deposition of a-C:H thin films on PET, were compared in terms of the power consumption, the process speed, the effect on the PET surface morphology, and finally its effectiveness on the *S. epidermidis* repellence. The a-C:H deposition process

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was found to be much slower and with quite higher energy demands.

All the modified samples significantly reduced the bacterial adhesion. However, He/O_2 plasma treated samples caused isolated bacterial adherence, preventing or delaying the biofilm formation as well. Such an isolation was not observed in the unmodified PET and the various a-C:H films. The changes in the total surface free energy and its polar component due to He/O_2 plasma treatments seem to be the main reason for this behavior.

Moreover, no simple relation was found between the surface roughness and bacterial adhesion, probably due to the large differences in the polar component of the free energy of the surfaces produced by deposition and treatment processes and the relatively small changes in the surface roughness.

Finally, we can conclude that the treatment of PET films with He/O_2 discharges is a much more suitable technique for the inhibition of *S. epidermidis* adhesion to PET thin films compared to the a-C:H thin films deposition. However, further experimental work is required to prove the applicability of the method, under *in vivo* conditions.

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