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Studies on antibacterial dressings obtained by fluorinated post-discharge plasma

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ABSTRACT

Surface modification of wool, polyamide 6 and cotton fabrics was investigated with an Ar–CF_4 post-discharge plasma. The radical F, as determined by optical emission spectroscopy, is considered to be the main active species acting on the fabrics and producing different effects as a function of the textile substrate. Fluorination of the surface is achieved on the three materials studied, but only wool and polyamide 6 fluorinated surfaces become hydrophobic at long treatment times, and show antibacterial properties. The treatment conditions used are mild enough so as not to alter surface topography, as confirmed by scanning electron and atomic force microscopy.

1. Introduction

Wounds often provide a favourable environment for the colonization of microorganisms (Chambers et al., 1962; Adams et al., 1999; Brook, 1996; Bowler and Davies, 1999). In order to improve the opportunity for wound healing, it is important to create conditions that are unfavourable to microorganisms and favourable for the host repair mechanisms, and topical antimicrobial agents are believed to facilitate this process.

Wound dressings and devices form an important segment of the medical and pharmaceutical wound care market worldwide. Although antibacterial agents do not necessarily take an active physiological part in the wound healing process, they prevent or treat infections, and can aid in wound healing (Boateng et al., 2008).

Antimicrobial agents, such as silver, povidone–iodine, polyhexamethylene biguanide or chitosan are sometimes incorporated into dressings to control infection. Traditional dressings include cotton, wool, natural or synthetic bandages and gauzes. They may be used as primary or secondary dressings, or form part of a composite of several dressings with each performing a specific function. Silver exhibits good antibacterial properties and in recent years has been used on medical devices and healthcare products ranging from wound dressings to urinary catheters (Dowlinga et al., 2001). In the field of wound care, and a wide variety of silver-containing dressings are now commonplace (e.g. hydrofibre dressing, polyurethane foams and gauzes). However, concerns associated with the overuse of silver and the consequent emergence of bacterial resistance are being raised (Dowlinga et al., 2001).

Low-temperature plasma (plasma) is generated when a gas at low pressure and near ambient temperature is exposed to an electric field and contains radicals, ions, electrons, photons and other excited species (Grill, 1993). These species can interact either physically or chemically with the substrate surface to a depth of a few tenths of nanometers (Mittal, 1999) due to their high reactivity. The advantages of plasma technology for making suitable implants or medical devices are both technological and administrative. The techniques are easy to implement, reproducible, clean and can be set up in any type of clean room. The techniques are non-pollutant, there are no organic residuals. Plasma treatment can be used to create a functionalised surface through attachment of new chemical groups. Both hydrophobic and hydrophilic surfaces can be obtained. CF_4 plasma has been used to obtain hydrophobic surfaces for biomaterials (Poncin-Epaillard and Legeay, 2003). The modified surface presents a non-adherent PTFE-like structure ($-(CF_2)_n-$) with low surface energy that varies from a few to 20 mJ/m^2. In the biomedical domain, CF_4 plasmas have been applied to produce hydrophobic surfaces, using this anti-adhesion property to prevent biofilm formation, for instance in cellulose hemodialysis membranes (Man et al., 1990). In the case of poly(methyl methacrylate) intra-ocular lenses (Eloy et al., 1993), adhesion of proteins and con-

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sequently the development of inflammatory cells were prevented when the substrate was treated in CF_4 plasma.

However, in practice, the density of fluorine atoms has to be low in order to avoid surface degradation, so plasma conditions have to be carefully controlled. In plasma post-discharges used for treatments in this paper only the stable and metastable non-ionic species are present, so surface degradation is minimized.

The research on obtaining fabrics with antibacterial properties has focused attention lately, and the conventional approach has been through chemical treatments, or, in more recent papers (Virk et al., 2004; Gawish et al., 2007; Yen et al., 2006), the action of plasma has been evaluated, but always in combination with chemical processes.

In the present paper, we have investigated the possibility of obtaining antibacterial fabric with views on their application on dressings by using a single process, fluorinated post-discharge plasmas. Different natural (wool and cotton) and synthetic (polyamide 6) fabrics have been treated, their surface properties have been studied by different techniques, and correlated to their antibacterial action.

2. Materials and methods

2.1. Materials

Worsted wool fabric (0.028 g/cm^2, Dimtex S.A., Spain), cotton fabric (0.023 g/cm^2, Mas Molas S.A., Spain) and knitted polyamide 6 microfibre fabric (0.030 g/cm^2, Flotats S.A., Spain) were used throughout the work.

2.2. Post-discharge treatments

The microwave flowing post-discharge reactor is composed of a pyrex cylinder of 15 cm of internal diameter (i.d.) and 20 cm height separated 30 cm from the plasma source by a 5 mm (i.d.) quartz tube (Fig. 1). The discharge is generated by a 2.45-GHz microwave source. The discharge tube is sealed to a bent quartz tube of 15 mm (i.d.) which is connected to the post-discharge reactor. The gas flow rate was regulated through a mass flowmeter and the pressure in the reactor was 667 Pa. The samples were placed horizontally in a quartz support. The treatments were carried out at treatment times between 2 and 80 min by using Ar–2%CF_4 as plasma gas. Remark that the Ar–2%CF_4 mixture is employed to enhance CF_4 dissociation and to consume less CF_4 gas, the Ar gas being not reactive with fabrics in the post-discharge.

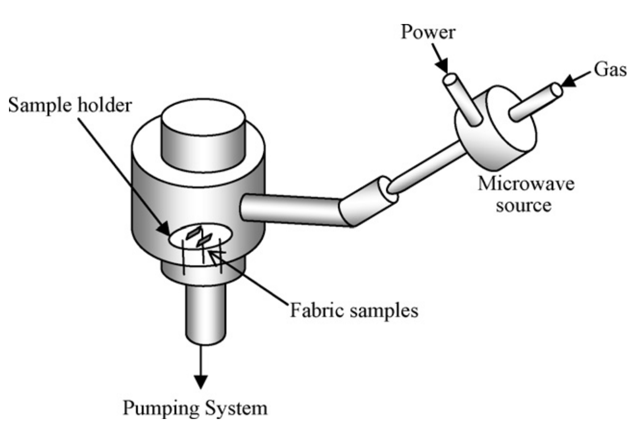

Fig. 1. Configuration of the microwave flowing post-discharge reactor, with detail of the pyrex sample holder and horizontal location of the samples during treatments.

2.3. Optical emission spectroscopy (OES)

The plasma emissions were collected by means of an optical fibre, connected to a Jobin-Yvon 270 M spectrometer equipped with a coupled camera device (CCD) detector. Evolution of the intensity of F atoms, and CF molecules has been obtained by measurements along the discharge tube. OES could not be carried out in the post-discharge chamber due to the lack of emitting species.

2.4. Contact angle

Static contact angles were obtained with a Digidrop Contact Angle Meter (GBX Scientific Instruments) by depositing a 5-µl milli-q water droplet on the surface of the fabrics.

2.5. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was used to monitor the chemical modifications produced in the outermost (5–10 nm) surface of the fabrics. The fabric samples were analysed using an ESCA-LAB MKII (VG scientific LTD) spectrometer with a Mg monochromatic X-ray source (1253.6 kV). Spectra were obtained with monochromator and detection angle of 0°, working at a residual vacuum of 10^{-6} Pa. Curve fitting was carried out with the software Spectrum NT. As fabrics may be relatively insulating materials, in the XPS spectra the shift of the peaks due to the superficial charge effect was taken into account. As the C_{1s} peak was found to be strongly affected by the new groups generated with the post-discharge treatments, the spectra were referred to the O_{1s} binding energy of 532.0 eV. Surface composition was estimated from the area of the different photoemission peaks modified by their corresponding sensitivity factors (Briggs et al., 1980).

2.6. Atomic force microscopy

Atomic force microscopy (AFM) was carried out on single fibres obtained from the treated fabrics. The microscope used was a NT-MDT Solver Pro with a Silicium Nitride Tip. Images were obtained in the tapping mode, and to obtain the 3D topographic images figures were previously levelled by a second-order polynomial fit.

2.7. Scanning electron microscopy

Biological scanning electron microscopy (SEM) images were obtained in a Fe-SEM Supra 35VP microscope at 2.5×10^{-4} mbar using a mixture of primary and secondary electrons. Samples were observed straight as no previous coating was necessary.

2.8. Microbial analysis

The antibacterial activity was studied on wool, cotton and polyamide 6 fabrics treated for 20, 60 and 80 min with the Ar–2%CF_4 post-discharge described. The activity of the treated fabrics was tested against *Escherichia coli* (gram negative bacterium), *Staphylococcus aureus* (gram positive bacterium), *Bacillus subtilis* (gram positive sporulating bacterium) and *Candida albicans* (fungus).

To qualitatively determine the antibacterial activity on the treated textile materials the Parallel Streak Method AATCC Test Method 147-2004 was used. This method allows a relatively quick and easy qualitative method to determine antibacterial activity of diffusible antimicrobial agents on treated textile materials. It consists in placing several parallel streaks in a standard Petri dish with the appropriate culture media for each bacteria tested. The treated side of the samples was put in contact with the culture media and incubated at 37 °C for 24 h.

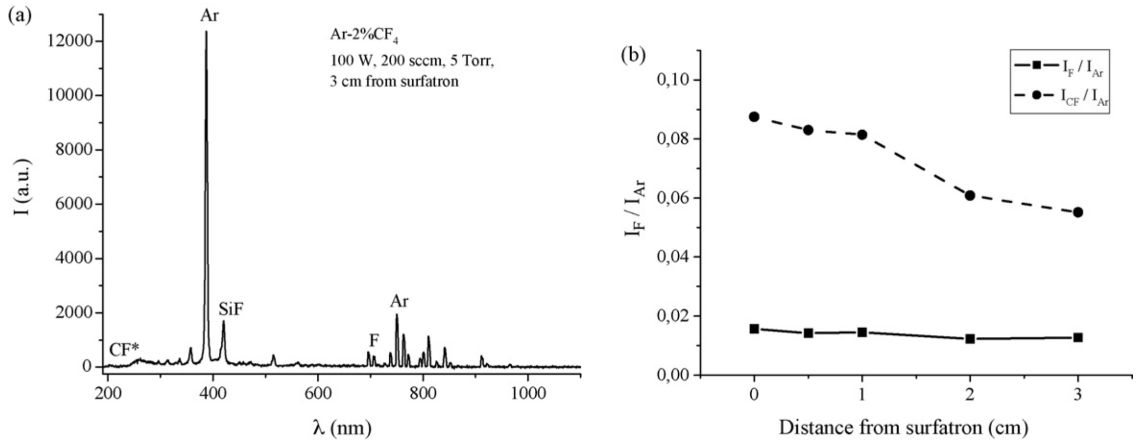

Fig. 2. (a) Complete optical emission spectrum of the Ar–CF_4 near-post-discharge (3 cm from plasma source) and (b) evolution of the intensity of F (703.7 nm) and CF (203.4 nm) with respect to the argon peak, as a function of distance with respect to the plasma gap.

To obtain quantitative results on the degree of antibacterial activity the AATCC Test Method 100-1994 was carried out on the fabrics showing activity by the previous test method. 50 μl of inoculum containing 10^5 cfu of *E. coli* were inoculated to the fabric samples, which, after 2 min were stirred in 1 ml of LB medium. The supernatant obtained was incubated in a Petri dish for 24 h at 37 °C.

The percent reduction (R) of bacteria by the treatments was calculated according to the following formula:$R = 100\frac{(B-A)}{B}$where *A* is the number of bacteria recovered from the inoculated Ar–CF_4-treated fabrics, and *B* is the number of bacteria recovered from the inoculated untreated control fabric sample.

3. Results and discussion

3.1. Analysis of the discharge

Optical emission spectroscopy is a useful tool for the detection of the active species during different plasma and post-discharge plasma processes (Canal et al., 2006, 2007). Given that the long-life species of the Ar–CF_4 discharge are non-emitting in the post-discharge, OES was carried out near the microwave plasma source (Fig. 2a), and the following emitting species were detected; Ar*, F*, CF* and SiF*. SiF* can be attributed to the etching of the quartz tube in which the discharge was made. Ar will not interfere with the sample treatment in the post-discharge as the gas in its base form is not reactive. Emission of Ar* was used to monitor the temperature and density of the electrons which vary with plasma parameters. Hence, the ratios F*/Ar* and CF*/Ar* give a good estimate of the relative density of fluorine atoms and CF radicals (Coburn and Chen, 1980). Using this technique called actinometry, the evolution of F and CF was followed as a function of distance from the surfatron, as shown in Fig. 2b, registering a decrease in the CF concentration with the distance from the discharge, tending to zero, while F remained nearly constant. As a consequence, we can assume the presence of F atoms as active species in the post-discharge reaction chamber where the fabric samples are treated (40 cm from the surfatron).

Table 1
Static contact angle of fabrics treated with Ar–2%CF_4 (100 W, 200 sccm) post-discharge plasma for different times.

t (min)	θ_s (°)		
	Wool	Cotton	Polyamide 6
0 (UT)	118	76	[a]
2	71	46	[a]
10	[a]	[a]	[a]
20	[a]	[a]	109
40	118	[a]	124
80	122	[a]	120

[a] Fabric too hydrophilic, it absorbs drop in less than 300 ms, not allowing contact angle measurement.

3.2. Analysis of surface properties

Contact angle modifications produced by the post-discharge treatment on the surface of fabrics are shown in Table 1. Untreated wool fabrics are hydrophobic (as reported in previous works (Canal et al., 2006)), while cotton and polyamide 6 fabrics are hydrophilic (Canal et al., 2004; Topalovic et al., 2007). Contact angles vary differently with treatment time depending on the material. Short treatment times increase hydrophilic properties on the surface of wool and cotton fabrics. This could be explained by an etching action of F atoms which could totally or partially eliminate the hydrophobic components of both materials: the fatty layer on the epicuticle of wool, as well as residual waxes and pectin of the cotton cuticle, or, in the case of cotton, the activation of the surface by F and latter reaction with hydrophilic air species. The already hydrophilic surface of polyamide 6 fabrics ($\theta = 71°$) (Canal et al., 2004) does not allow static contact angle measurement on untreated samples or on samples treated for short times up to 10 min, as the fabric absorbs the water measuring liquid. At long treatment times, contact angles increase in wool and polyamide 6 fabrics, rendering the surface hydrophobic while cotton remains hydrophilic.

Chemical analysis of the surface of fabrics by XPS (Table 2) reveals the typical features expected for the untreated (UT) samples. Native WO fibre surfaces are composed of approximately 75–80% carbon, 10–12% oxygen, 6–9% nitrogen and 2–3% sulphur (Bradley and Mathieson, 1997). The N, S and O are mainly due to the protein matrix of the epicuticle (Fig. 6), which contains the diaminoacid cystine, and also to the contribution of the thioester links of the fatty acids with the protein matrix of the epicuticle. C is due to the wool protein but also the contribution of a surface lipid layer present in the outermost part of the fibre surface (fatty-layer) has to be considered (Bradley and Mathieson, 1997). Chemical composition of the UT CO fabrics, with O/C of 0.43 does not correspond to the typical structure of cellulose with O/C of 0.83, which indicates that the fabrics have been scoured in origin (Topalovic et al., 2007). The elemental composition of the surface of UT PA6 fabrics is in accordance with the chemical structure of PA6, containing C, O and N, although there is a small difference in the atomic ratio O/C (0.13), which is lower than expected for the chemical structure of

Table 2
Elemental composition in percentages and atomic ratios determined for wool, cotton and polyamide 6 fabrics UT and treated in Ar–CF_4 postdischarge.

	Atomic ratio (%)							
	C	O	N	S	F	C/N	O/C	F/C
Wool								
UT	79.2	10.3	7.4	3.0	–	10.7	0.13	–
2 (min)	70.0	20.7	3.9	Undetectable	5.5	17.9	0.29	0.08
20 (min)	61.0	18.6	13.0	Undetectable	7.5	4.6	0.30	0.12
80 (min)	38.9	9.9	9.2	Undetectable	41.0	4.2	0.25	1.05
Cotton								
UT	72.8	27.2	–	–	–	–	0.37	–
2 (min)	68.4	27.4	–	–	4.2	–	0.40	0.06
20 (min)	59.9	30.8	–	–	9.3	–	0.51	0.15
80 (min)	47.6	23.8	–	–	28.7	–	0.50	0.60
Polyamide 6								
UT	79.0	11.0	10.0	–	–	7.18	0.13	–
2 (min)	70.2	18.3	7.9	–	3.7	8.88	0.26	0.05
20 (min)	63.2	16.8	10.3	–	9.8	6.45	0.26	0.15
80 (min)	39.5	9.4	5.7	–	45.4	6.93	0.24	1.15

PA6 (0.17). This was previously attributed to a possible contamination of the surface by hydrocarbons (Canal et al., 2007; Upadhyay et al., 2004).

On the three materials studied, increasing Ar–CF_4 treatment times produce progressive fluorine incorporation to the sample surface (up to about 40% in WO and PA6 and 30% in CO) with the subsequent decrease of the atomic ratio of all other elements of the sample.

According to the XPS results, WO and PA6 show different initial behaviour under the Ar–CF_4 post-discharge; On wool: the decreasing C/N ratio up to values close to 4.3 (corresponding to the aminoacids of the epicuticle) indicates the existence of an etching process of the fatty acid monolayer of the epicuticle of wool during the first stages of treatment, as already reported for other post-discharge processes (Canal et al., 2006, 2007). In the first stages the O/C ratio increases due to the elimination of the fatty acid layer, with the same values that had previously been observed in N_2 post-discharge treated wool (Canal et al., 2006), and decreases at long treatment times due to the fluorination of the surface. Globally it can be seen that the C, O and N concentrations remain mainly unaltered with the treatments carried out. On PA6, after an initial O/C increase which can be attributed to the cleaning of the surface by the post-discharge plasma, fluorine is incorporated to the surface with a ratio F/C above 1 after 80 min treatment.

Previous papers (Selli et al., 2001) suggested a substitution mechanism of H for F atoms on the polymer surface when treated in SF_6 plasmas. Taking into account that in the CF_4 post-discharge used the reactive species are F radicals, we could think that our fluorination process takes place following the same mechanism.

It has to be remarked that at long treatment times, WO and PA6 show similar behaviour, incorporating practically double F on the surface than CO, as can be seen from the C/F atomic ratio. The mechanism of fluorination is common in WO and PA6 and their different reactivity from CO can be explained through the differences in their chemical structure. Possibly the presence of the amide groups of WO and PA6 makes them more reactive to F atoms.

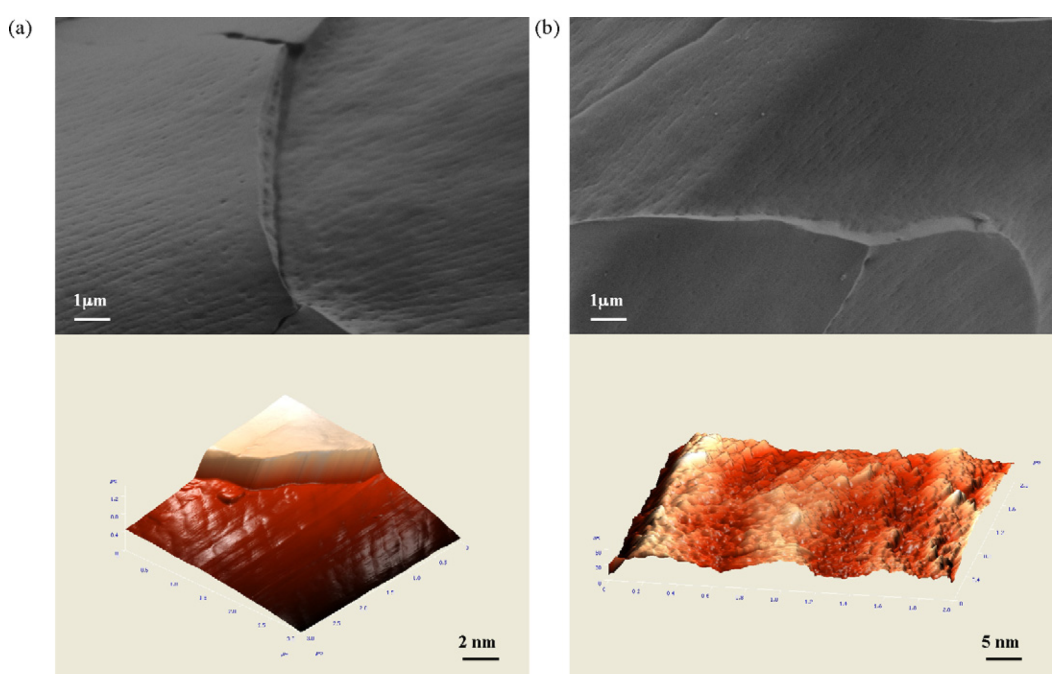

Fig. 3. SEM and AFM images of WO fibres untreated (a) and treated with an Ar–CF_4 post-discharge for 80 min (b).

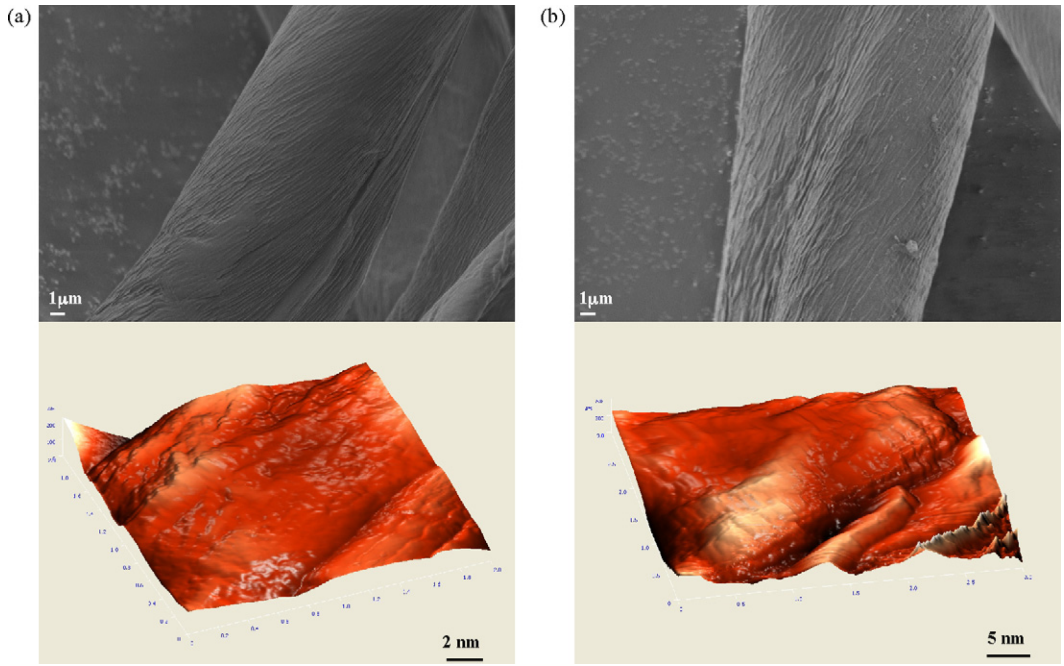

Fig. 4. SEM and AFM images of CO fibres untreated (a) and treated with an Ar–CF_4 post-discharge for 80 min (b).

Analysis of the surface topography of samples was carried out by SEM and AFM. SEM micrographs of the WO fibres Ar–CF_4 post-discharge treated for 80 min (Fig. 3a) reveal the typical structure of the cuticle of wool, with flat scales overlapping one another, and do not reveal any apparent changes with respect to the untreated WO. However, at lower scale, through AFM it can be observed that Ar–CF_4 treatment produces a certain roughness increase, which could be attributed to the elimination of the fatty layer of the epicuticle which has been confirmed by XPS.

In parallel, the treatment carried out does not produce any significant topographical surface changes on CO (Fig. 3b) or on PA6 (Fig. 3c), either, confirming the benignity of the post-discharge plasma used.

SEM micrographs of the untreated WO fibres (Fig. 3a) reveal the typical structure of the cuticle of wool, with flat scales overlapping one another, and not apparent changes are found after 80 min of treatment. However, at lower scale, through AFM it can be observed that Ar–CF_4 treatment produces a certain roughness

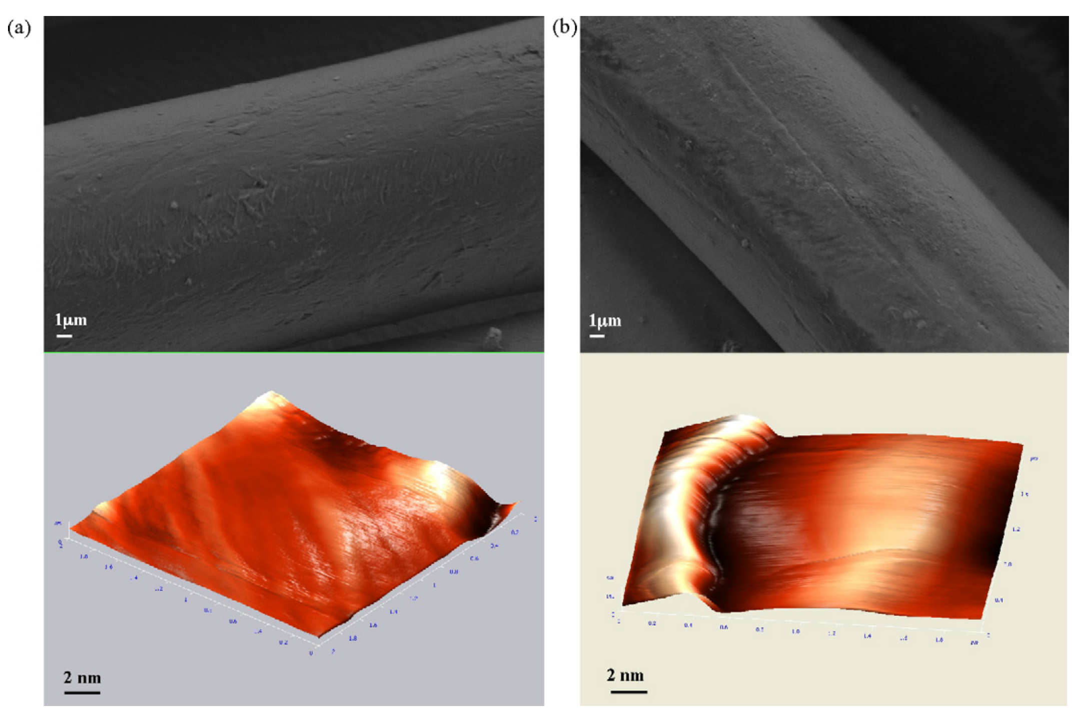

Fig. 5. SEM and AFM images of PA6 fibres untreated (a) and treated with an Ar–CF_4 post-discharge for 80 min (b).

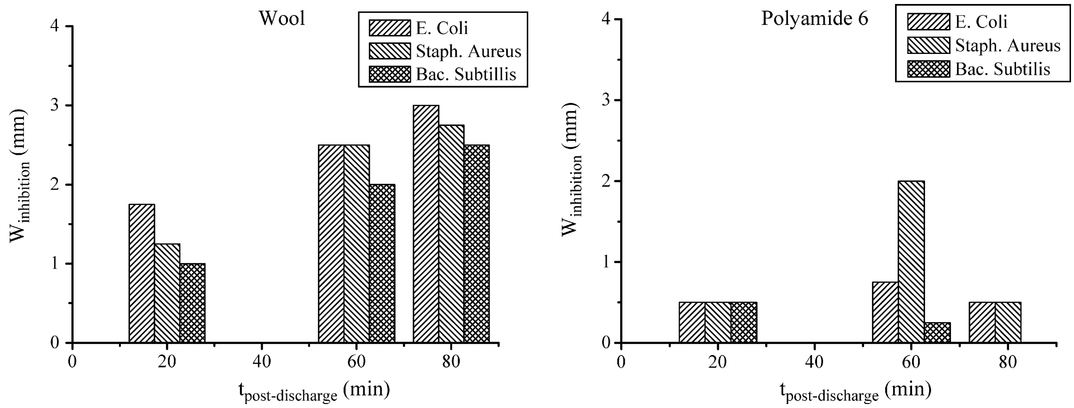

Fig. 6. Width of the zone of bacterial inhibition in wool and polyamide fabrics with respect to treatment time.

increase, which could be attributed to the elimination of the fatty layer of the epicuticle which has been confirmed by XPS.

On the other hand, Figs. 4 and 5 confirm the mild conditions of treatment in the post-discharge, which produce no significant topographical surface changes on CO or on PA6.

3.3. Antibacterial activity

The effects of the Ar–2%CF_4 post-discharge were evaluated on the antibacterial activity of wool (WO), cotton (CO) and polyamide 6 (PA6) fabrics. They were therefore treated in the Ar–2%CF_4 post-discharge for different times, and were then tested against *E. coli*, *S. aureus*, *B. subtilis* and *C. albicans*.

Qualitative antibacterial evaluation revealed different behaviour depending on the fabric tested; while CO fabrics do not show any antibacterial activity with any of the microorganisms studied at any treatment time, WO and PA6 display bacterial inhibition with all microorganisms tested except for *C. albicans*, as shown in Fig. 6. It can therefore be inferred that the surface obtained for WO and PA6 is active for bacteria but not for fungus.

WO fabrics treated with an Ar–CF_4 post-discharge show antibacterial activity from 20 min and on, and the width of bacterial inactivation increases with treatment time on *E. coli*, *S. aureus* and *B. subtilis*. PA6 fabrics show a slight antibacterial activity in the same species as wool, also from 20 min treatment. However, its effect is feebler and does not increase with time.

It is known that plasma and post-discharge treated fabrics tend to undergo an ageing process, highly dependent on the plasma gas used, in the hours which follow treatment (Canal et al., 2004, 2008). Therefore, the antibacterial activity of fabrics treated with an Ar–CF_4 post-discharge and stored for 24 h was tested, displaying the same antibacterial activity as fresh samples, so it can be concluded that the functional groups created on the surface of wool and polyamide 6 fabrics with this treatment do not undergo fast ageing.

The quantitative analysis of the antibacterial activity of WO and PA6 fabrics was carried out with *E. Coli* (see Table 3). The Ar–CF_4 post-discharge treatment has an effective bactericidal effect, showing a reduction of bacteria around 90% in WO and 85% in PA6. The differences obtained for the increasing treatment times are not significant taking into account that the associated error to this method is of 8%.

Table 3
Percent reduction of *E. coli* inoculated to fabrics treated in Ar–CF_4 post-discharge for different times.

Ar–CF_4 treatment time (min)	% Reduction of bacteria	
	Wool	Polyamide 6
20	89	87
40	90	84
80	94	87

The antibacterial results obtained point out to a binding between WO or PA6 and F which dissociates in contact with aqueous media, releasing fluorine ions which interact with the bacterial metabolism (Pellat et al., 2002).

4. Conclusions

Optical emission spectroscopy indicated that it can be assumed that mainly fluorine atoms are present in the post-discharge, while CF disappears in the near afterglow. Ar–CF_4 post-discharges modify the adhesion of wool, cotton and polyamide 6 fabrics without altering their topography. Surface chemistry, with the incorporation of F is the key parameter for the antibacterial activity of the fabrics. The quantity of F incorporated to the surface is not the only requirement for antibacterial activity, according to the cotton behaviour, but also the kind of bonding established between F and the surface. This will have to be further investigated.

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