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Polymeric meshes for internal sutures with differentiated adhesion on the two sides

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The aim of this work is to investigate the effects of different plasma treatments on ePTFE abdominal prostheses with the final goal of obtaining a new prosthesis, made of a single strand of ePTFE, with clearly differentiated adhesion properties on the two sides, which should be able to promote tissue ingrowth on one side and prevent post surgical visceral adhesions on the other.

Samples obtained from ePTFE Bard Dulex Meshes have been treated sequentially with three different gases (N_2, O_2 and NH_3) in order to choose the optimal treatment conditions to improve ePTFE wettability. In particular, no modification was induced by N_2 treatment, while the full treatment after the final ammonia gas resulted in the best suitable candidate.

As demonstrated by scanning electron microscopy, AFM analyses and contact angle measurements, ammonia plasma treatment increases ePTFE surface roughness and renders it more hydrophilic, thus promoting adhesion without any alteration of the material's bulk properties.

The reported results also evidence the possibility to obtain the maximum wettability with a cheap treatment by optimizing plasma exposure time.

As a preliminary cell adhesion study, Swiss 3T3 fibroblasts (mouse, embryo) have been seeded on the treated and untreated materials in order to assess whether there was any difference in terms of cell attachment and spreading. Cells seeded on the ammonia plasma treated material showed a better adhesion and spreading when compared to the untreated material.

1. Introduction

In recent years, the use of polymeric meshes in hernia surgery has become essential. Their introduction has markedly improved the results with regard to the recurrence rates [1, 2] and, although the major source of concern regarding the routine use of a foreign body for hernia repair has been a perceived increase in infection risk, data from published series do not support the contention that infection is more common in open mesh repair of hernia as compared with conventional sutured repair [1, 3–5].

With the recent rise in popularity of laparoscopic surgery, in which meshes are placed intra- or preperitoneally in young individuals, several authors, reviewing this operation, have questioned the placement of mesh in contact with the peritoneum because of the risks of adhesions and fistula formation [6], underlining in this way the need for a new prosthesis with clearly differentiated adhesion properties on the two faces.

The two most frequently used prosthetic materials are polypropylene and expanded polytetrafluoroethylene (ePTFE), whose adhesion properties are almost opposite.

In fact, polypropylene meshes seem to produce the best integration in the receptor organism, while ePTFE prostheses are only partially integrated [7–9]. It was proposed [10] that the lack of anchorage associated with the use of ePTFE was related to a number of factors, and in particular to the pore size of commercially available ePTFE, which was believed inadequate to allow ingrowth of fibrocollagenous tissue.

On the other side, while polypropylene meshes have the serious drawback of a clear tendency to fistula formation and adhesion to the abdominal viscera when implanted in contact with them, ePTFE has been widely shown to generate little adhesion formation with abdominal viscera [8, 10–17].

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Most of the experimental studies performed to improve prosthetic tissue integration, avoiding adhesion formation, focused on the matching of a microporous material layer (e.g., ePTFE) with a layer of a bigger pore size material (e.g., polypropylene) [18–20]. However, techniques for the production of these prostheses are complex, which makes the products expensive, also producing a marked decrease in prosthesis mechanical strength in many cases.

The aim of this work is to obtain a new prosthesis with clearly differentiated adhesion properties on the two faces. To this aim, a "cold plasma treatment" process has been adopted in order to improve surface wettability, and consequently adhesion and tissue ingrowth properties, of just one face of a single strand of ePTFE with small pore size.

In fact, this plasma treatment produces on the treated material a surface corrosion which can be measured as superficial roughness. Moreover, all the treatment effects are limited to a maximum depth of a few microns, so the treated material's bulk properties are not altered [21].

In this work we studied how the plasma treatment can generate an additional porosity only on the treated side of the ePTFE layer, in order to increase cellular ingrowth just on one face of the prosthesis, matching with the present requirements of laparoscopic surgery.

In order to study the effects of the changes of the material with respect to cell adhesion and spreading, scanning electron microscopy studies have been performed. 3T3 fibroblasts have been seeded on the material to carry out the preliminary cell-material interactions study.

2. Materials and methods

In the present study we compare the microporous face of a Bard Dulex Mesh (a dual sided ePTFE mesh manufactured by C.R. Bard, Inc.) which minimizes adhesion formation, before and after different plasma treatments. This commercially available mesh has a smooth side to prevent adhesion and a rough side which should instead promote cellular ingrowth: our final goal is to obtain a new prosthesis, more thin and less expensive, with two smooth faces having clearly differentiated adhesion properties.

Small rectangular Teflon slabs (3×5 cm, about 3 mm in thickness, purchased from Napolitano commercial house, Lecce, Italy) have also been used to carry out the same plasma treatments performed on Bard Dulex Mesh samples, in order to verify the real effects of such treatments on commercially available PTFE.

2.1. Plasma treatments

Plasma treatments were performed at H.T.P. UNITEX S.P.A. (Caronno Pertusella, VA, Italy) using a KPR 180 industrial system, in which a very low pressure (50–150 Pa) is obtained, while the electrodes, linked to a medium frequency electrical generator, create an electrical field that transforms the introduced gas into plasma. The fact that the temperature inside the reactor is under 65 °C implies that the ePTFE samples can be treated without modifying their mechanical properties, like mechanical resistance and elasticity.

Our samples underwent a pretreatment consisting of a brief exposure to an argon plasma, with the aim of removing organic contaminants from the surface in order to make it suitable for the subsequent structural modification treatment. For the real surface treatment three plasmas were used sequentially, obtained from three different gases (nitrogen, oxygen and ammonia) with different operative parameters choice.

All gases were applied with 180 A current intensity and 80 Pa pressure. Exposure times were set at 45 s for argon, 180 s for nitrogen, 90 s for oxygen and 120 s for ammonia. Plasma treatments were applied in the order in which these gases are listed, with samples examined after each stage.

2.2. SEM analyses

Scanning electron microscopy (SEM) was employed to visualize morphological changes in sample surface caused by different plasma treatments. We performed SEM analyses on untreated and treated samples (one analysis after each single treatment), both for Bard ePTFE and commercial Teflon, by means of a field emission scanning electron microscope (Jeol JSM-6500).

2.3. AFM analyses

We used a "tapping mode" piezoelectric scanner atomic force microscope (AFM; Bioscope Nanoscope IIIA NS3a, Digital Instruments) to perform further morphological analyses. AFM measurements were performed only on ePTFE samples and not on Teflon since, being the Teflon not the target material for the application under investigation, we decided to perform on Teflon only comparison tests consisting in SEM analyses and contact angle measurements, as discussed in the next paragraph.

The following Bard ePTFE samples were tested: untreated, exposed to argon, nitrogen and oxygen plasmas and exposed to argon, nitrogen, oxygen and ammonia plasmas.

On each sample, a first scansion (10×10 μm, 512 scansion lines per image, 512 acquisition points per line) has been carried out with the aim of confirming SEM analysis results and improving image resolution. A wider squared area scansion (50×50 μm, again with 512 scansion lines per image and 512 acquisition points per line) has also been performed on each sample, setting the maximum height value at 7 μm, the highest possible with the employed microscope, in order to appreciate surface unevenness in the vertical direction. The aim of this second series of image acquisitions was to calculate the root mean square (RMS) roughness of each scanned area using a Digital Instruments software tool. Thus, the scanned area has been enlarged and the "data scale" has been increased in order to obtain significant RMS roughness values.

2.4. Contact angle measurements

Contact angle measurements were carried out on a Costech tool, employing the software "Anglometer 2.0". A small liquid drop was injected by a microsyringe on each sample surface and drop pictures were acquired by a digital camera after a suitable time interval from drop formation. The images were analyzed by means of the Anglometer software, which allows the measurement of the contact angle between liquid and solid.

Bard ePTFE samples and commercial Teflon samples were analyzed by this technique, both the untreated ones and the ones treated with different plasmas. Several measurements have been carried out on each sample, with a constant time interval of 10 s, which was empirically evaluated to be sufficient for the drop to reach the equilibrium shape configuration.

The software analysis of stored images was performed using a technique reported in the literature [22]: contact angle was evaluated from the height and the base diameter of a sessile drop, by assuming the contour to have a circular shape and using ethylene-glycol on the samples to give the best evidence of the induced modification.

For each analyzed surface, contact angle value was finally expressed as the mean value of the single measurement results.

2.5. Material preparation for cell seeding

Three types of ePTFE sheets were used to compare cell attachment and spreading: the untreated rough side of the material, the untreated smooth side of the material and the plasma-sprayed smooth side. The samples were received as cut wafers of 2×2 cm^2. Under sterile conditions they were placed in bacteriological Petri dishes (60 mm diameter) to prevent cell adhesion to the dish, fixed to it using sterile silicone wax and then washed twice for 24 h with sterile Phosphate Buffered Saline solution (PBS, without Ca^{2+} and Mg^{2+}) containing antibiotics (penicillin 100 units/ml, streptomycin 100 μg/ml, gentamycin 1.2 μg/ml) (Invitrogen Co.) and fungizone (2.5 μg/ml) and placed in the cell incubator. Prior to cell seeding, complete culture medium (see below) was added to the dishes for 1 h.

2.6. Cell seeding on materials

Swiss 3T3 fibroblasts (mouse, embryo) were routinely cultured in Dulbecco's Modified Eagles Medium (DMEM, high glucose, with glutamax TM) supplemented with 10% (v/v) foetal calf serum, 1% nonessential aminoacids, penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (2.5 μg/mL) (Invitrogen) (*complete culture medium*). Cells were maintained at 37 °C in a 5% CO_2, 95% air, humidified atmosphere. The media were changed every 48 h.

Cells were seeded onto the materials at a density of 10^4 cells/cm^2. After 24 h, samples were processed for SEM observations.

2.7. SEM preparation of the samples

Cells grown on the three selected materials were washed with cold PBS, fixed with 2.5% glutaraldheyde/PBS solution and then postfixed with 1% Osmium tetroxide (Sigma Chemicals)/PBS solution; then they were dehydrated with ethanol/water at increasing concentrations starting from 30% and up to 100% ethanol. The samples were then dehydrated with a critical point drier (Polaron), sputter coated with gold (Polaron SC7640) and observed under SEM (Philips, 505).

3. Results and discussion

The following points will be examined: first, the surface characterization results of plasma treatment on ePTFE and on Teflon; secondly, the cell seeding results, to emphasize the influence of plasma exposure on ePTFE treated samples with respect to untreated ones.

3.1. Plasma treatment effects on Bard ePTFE

Bard ePTFE was studied by scanning electron microscope (SEM), atomic force microscope (AFM) and contact angle measurements. We analyzed the same samples before treating them and after each one of the 3 plasma treatments they had been exposed to: nitrogen plasma, oxygen plasma and ammonia plasma. Nitrogen plasma didn't produce any appreciable effect. Thus, in the following we report and discuss only the results obtained through further oxygen plasma and ammonia plasma treatment.

As shown by the SEM morphological analyses reported in Fig. 1, oxygen plasma exposure produced a destruction of ePTFE structure on the micrometric scale. SEM imaging of ePTFE after ammonia plasma exposure (Fig. 1(c)) shows, besides a further destruction of ePTFE structure, an increase in crack size, which means an increased superficial porosity.

Results obtained with SEM were fully confirmed by AFM images. Squared area scansions (10×10 μm) performed on each sample show that the characteristic ePTFE structure (Fig. 2(a)) was destroyed for a large part by oxygen plasma (Fig. 2(b)), and was completely unrecognizable after ammonia plasma exposure (Fig. 2(c)).

We used AFM also to produce images with lower magnification (50×50 μm squared areas). This second series of images emphasized the "induced superficial porosity" effect: untreated ePTFE has a quite compact structure (Fig. 3(a)), while after oxygen plasma exposure it shows a presence of holes and cracks (Fig. 3(b)), and this becomes more evident after ammonia plasma treatment (Fig. 3(c)) (all the reported AFM images correspond to unprocessed data).

Furthermore, by means of an AFM software tool, it was possible to measure the root mean square (RMS) roughness of scanned areas. Obtained values (reported in Table I) pointed out that roughness of untreated ePTFE is less than half of the value of oxygen plasma exposed ePTFE and less than one third of the corresponding value for ammonia plasma exposed ePTFE.

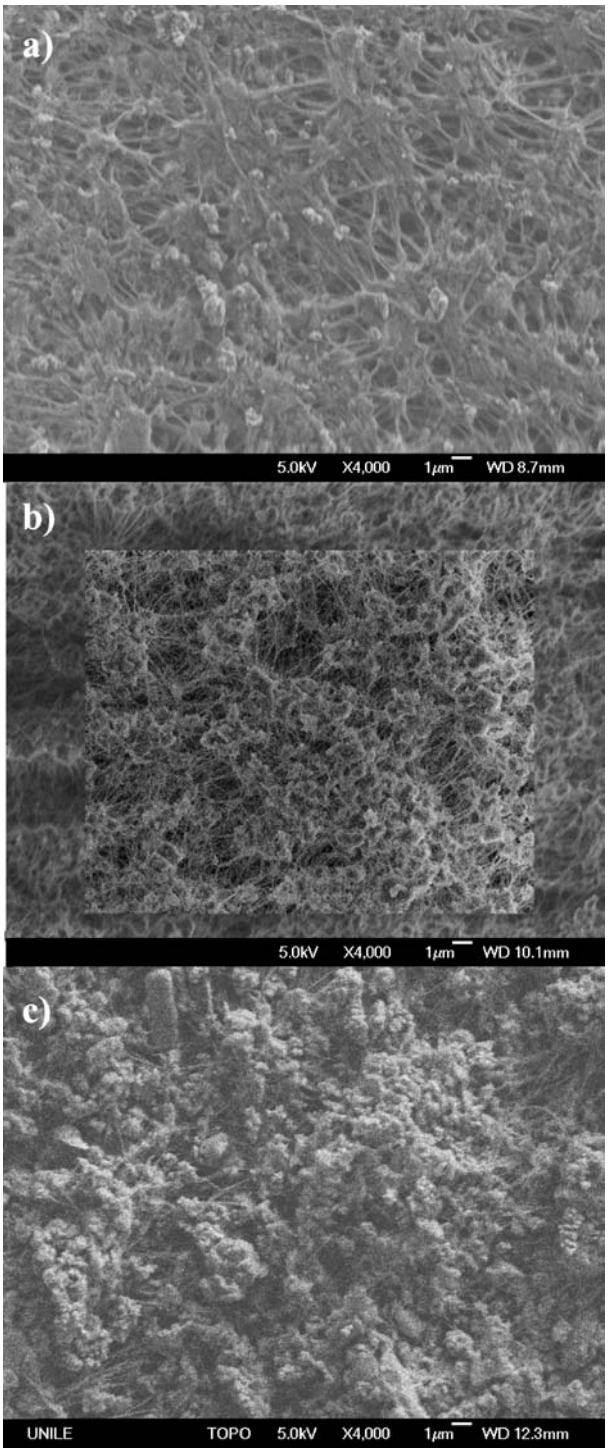

Figure 1 (a) SEM image of untreated ePTFE, showing characteristic surface structure; (b) SEM image of ePTFE after oxygen plasma treatment, showing destruction of initial structure; (c) SEM image of ePTFE after ammonia plasma treatment, showing a further destruction of initial structure and an increase in crack size.

An additional quantitative evaluation of the effects of performed treatments was achieved through contact angle measurements. Mean values of single measurement results are reported in Table II.

TABLE I RMS roughness of samples analyzed with AFM

Sample	RMS Roughness (μm)
Untreated ePTFE	0.417
ePTFE after oxygen plasma treatment	0.967
ePTFE after ammonia plasma treatment	1.403

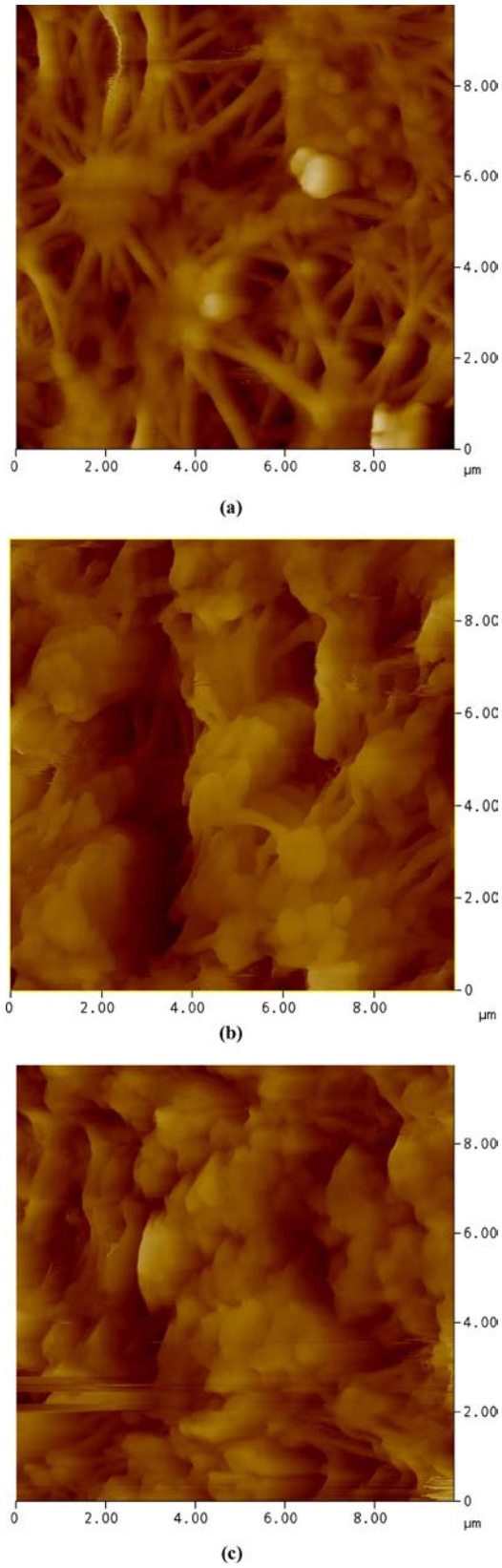

Figure 2 (a) AFM 10 × 10 μm image of untreated ePTFE, showing its characteristic surface structure; (b) AFM 10 × 10 μm image of ePTFE after oxygen plasma treatment, showing destruction of initial structure; (c) AFM 10 × 10 μm image of ePTFE after ammonia plasma treatment: initial structure is completely unrecognizable.

Table II shows that oxygen plasma induces only a slight modification of contact angle value, while ammonia plasma seems to be a more suitable treatment in terms of roughness induction.

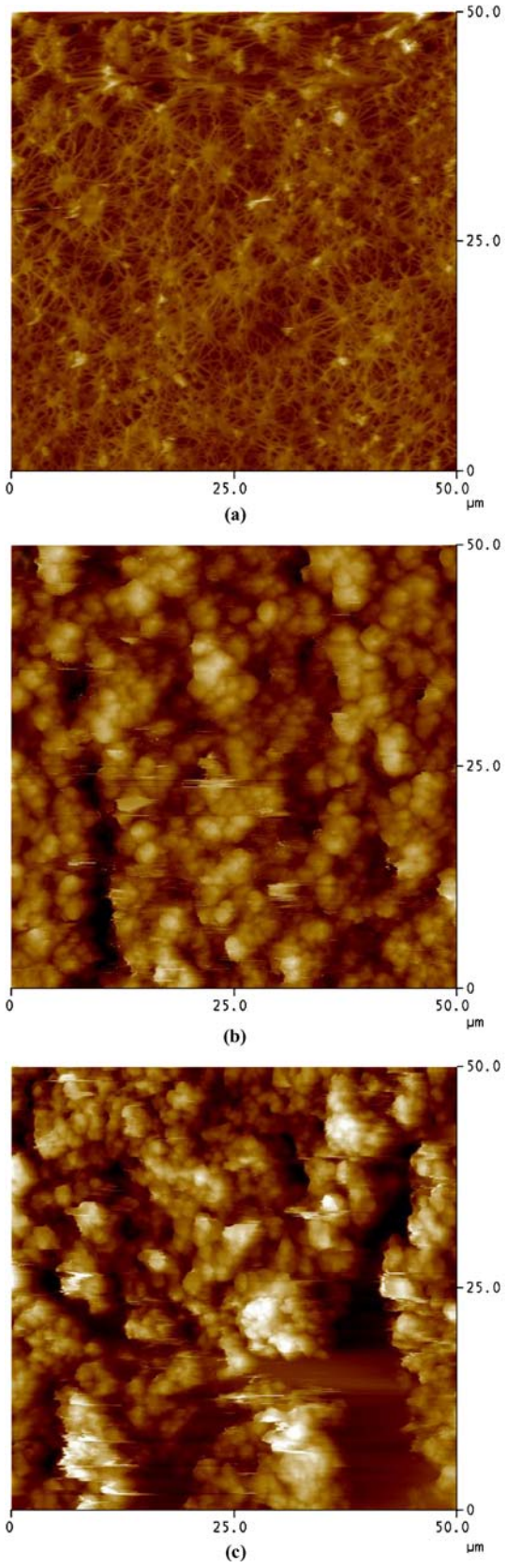

Figure 3 (a) AFM 50×50 μm image of untreated ePTFE, showing a quite compact structure; (b) AFM 50×50 μm image of ePTFE after oxygen plasma treatment, showing the presence of holes and cracks; (c) AFM 50×50 μm of ePTFE after ammonia plasma treatment: holes and cracks are more evident.

The lack of efficacy showed by nitrogen and oxygen plasmas in modifying the ePTFE surface is probably due to the high separation energy of the C─F bond (about 5.35 eV). The more significant effect of last

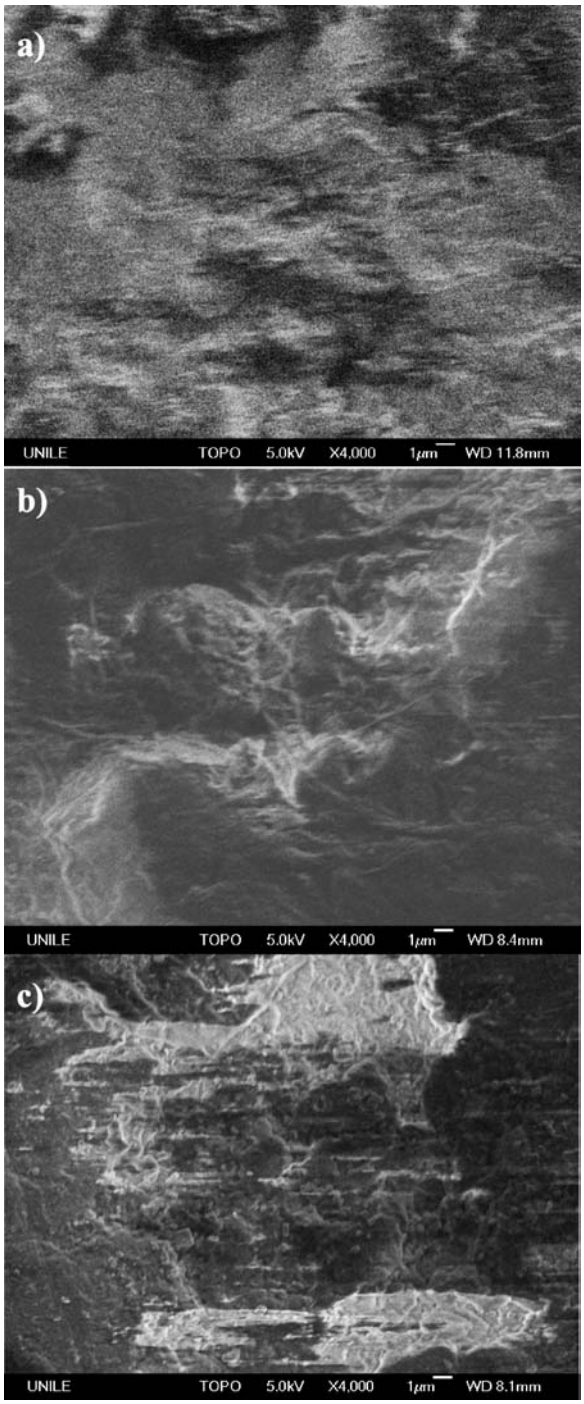

Figure 4 (a) SEM image of untreated Teflon, showing initial surface structure; (b) SEM image of Teflon after oxygen plasma treatment, showing an increased surface roughness; (c) SEM image of Teflon after ammonia plasma treatment, showing the surface crumbling produced by the treatment.

treatment instead, is almost certainly due to a specific ability of ammonia plasma: it is capable of introducing polar groups on ePTFE surface, whose wettability can thus be increased by the groups' reactivity [22].

TABLE II Contact angle with ethylene glycol for different ePTFE samples

Sample	Contact angle (mean ± SD)
Untreated ePTFE	104 ± 5°
ePTFE after oxygen plasma treatment	100 ± 6°
ePTFE after ammonia plasma treatment	90 ± 6°

TABLE III Contact angle with ethylene glycol for different Teflon samples

Sample	Contact angle (mean ± SD)
Untreated Teflon	102 ± 3°
Teflon after oxygen plasma treatment	94 ± 3°
Teflon after ammonia plasma treatment	87 ± 4°

However, ammonia plasma was employed only on samples already treated with oxygen plasma, since, as reported in literature [23], the use of oxidative plasmas is recommended for all treatments aimed to adhesion improving.

3.2. Plasma treatment effects on Teflon

The same treatments performed on Bard ePTFE samples were performed on Teflon slabs, and these slabs were in turn studied by means of SEM and contact angle measurements.

Reported SEM images of untreated Teflon and Teflon after oxygen plasma exposure (Fig. 4(a) and (b)) underline the increase in surface roughness caused by this treatment. Subsequent ammonia plasma treatment then produces a real surface crumbling (Fig. 4(c)).

Contact angle measurements were performed on Teflon samples and the mean values of the single measurements are reported in Table III.

Comparing Table III values with the corresponding values in Table II, the contact angle trend for Teflon seems to be close to the one displayed by ePTFE and, in particular, the total contact angle variation produced after ammonia plasma exposure with respect to untreated samples values is similar.

The only difference between the two groups of samples consists in the better effect, in terms of surface modification, that the oxygen plasma seems to have on Teflon rather than on ePTFE. This discrepancy has been attributed to the statistical distribution of the measured values. In fact, for oxygen treated Teflon all the measured values are quite close together, while in the case of oxygen treated ePTFE corresponding values are dispersed on a wider interval, which nevertheless has the same minimum value as the Teflon interval. Actually, performing quantitative error analysis for contact angle data from the standard deviation of measurements reported in Tables II and III, we can say that 95% confidence interval for contact angle measurements on Teflon after the oxygen plasma treatment is 94 ± 6°, while in the case of ePTFE the same treatment produces a 95% confidence interval of 100 ± 12°: both of the two materials have the same likelihood ($p < 0.03$) to have the contact angle measured value above 88°; otherwise we can state that contact angle measured value is less than 100° with good significance level ($p < 0.03$) only in the case of Teflon, while in the case of ePTFE the same statement has a very bad significance level ($p < 0.5$). This suggests that the oxygen plasma exposure time (90s) is not enough to produce an homogeneous surface treatment, especially in the case of ePTFE: in all probability, by increasing the exposure time it would be possible to optimize the treatment effects on both surfaces and to enhance the coherence of results.

A less varied behavior has been observed for ammonia plasma exposure. In this case, the two mean values are quite close together (87° for Teflon and 90° for ePTFE), but both measurement groups are dispersed on a relatively wide interval, having almost the same minimum value: ammonia plasma treatment produces a 95% confidence interval of 87 ± 8° for contact angle measurements on Teflon and of 90 ± 12° for contact angle measurements on ePTFE. Even in this case, exposure time should be increased to optimize treatment effects.

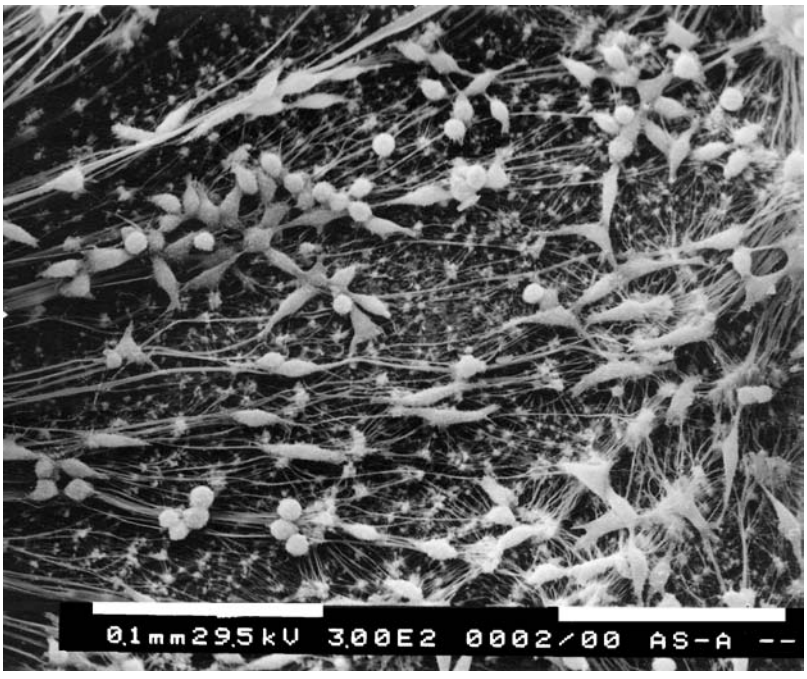

Figure 5 SEM micrograph of 3T3 fibroblasts on untreated ePTFE -rough side- 24 h after cell seeding: cell adhesion is low.

3.3. Comparison of obtained results

Comparing all the experimental results obtained for Bard ePTFE and Teflon surfaces exposed to plasma treatments, it is evident that the treatment induced effects are similar on both materials.

In particular, in the case of ePTFE, SEM and AFM images show that surface microporosity, which according to Simmermacher *et al.* [10] is responsible for the lack of anchorage associated to the use of ePTFE, remains of the same order of magnitude after plasma treatments. This suggests that plasma treatment mainly generates a better wettability on treated surfaces, as demonstrated by the decrease in contact angle value, probably due to an increase in surface energy.

3.4. Cell seeding results

After 24 h cells seeded on the materials showed different adhesions and spreading: cells seeded on the ePTFE rough side showed -as expected- low adhesion (Fig. 5). Regarding the untreated and treated smooth ePTFE, the electron microscopy images obtained revealed that cells seeded on the untreated ePTFE adhered and spread over the material (Fig. 6), although wide areas without cells are present. Cells seeded on plasma-sprayed ePTFE showed more cell adhesion and spreading than that observed for either untreated ePTFE (Fig. 7). Cells displayed a good morphology on both of the smooth side materials, with higher cellular density for cells seeded on the modified ePTFE. This preliminary cell adhesion

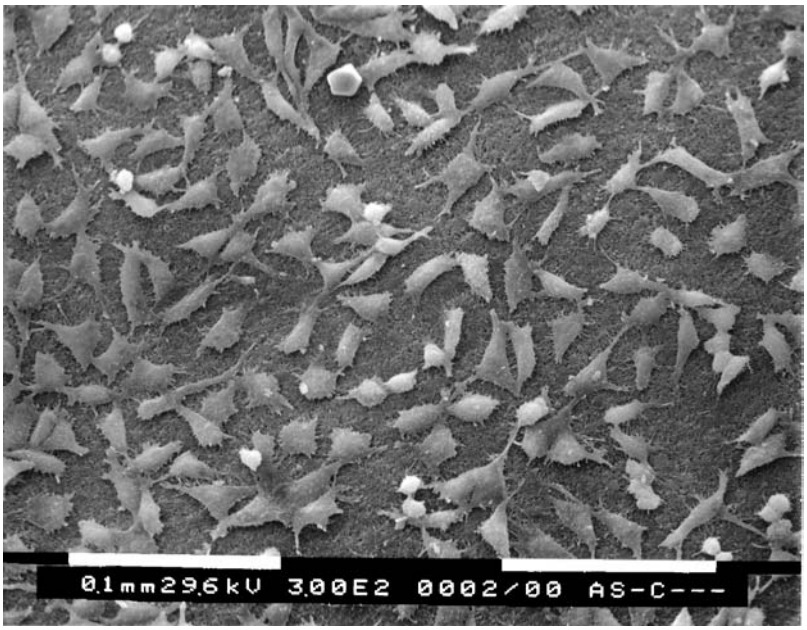

Figure 6 SEM micrograph of 3T3 fibroblasts on untreated ePTFE -smooth side- 24 h after cell seeding: cells adhered and spread over the material, although wide areas without cells are present.

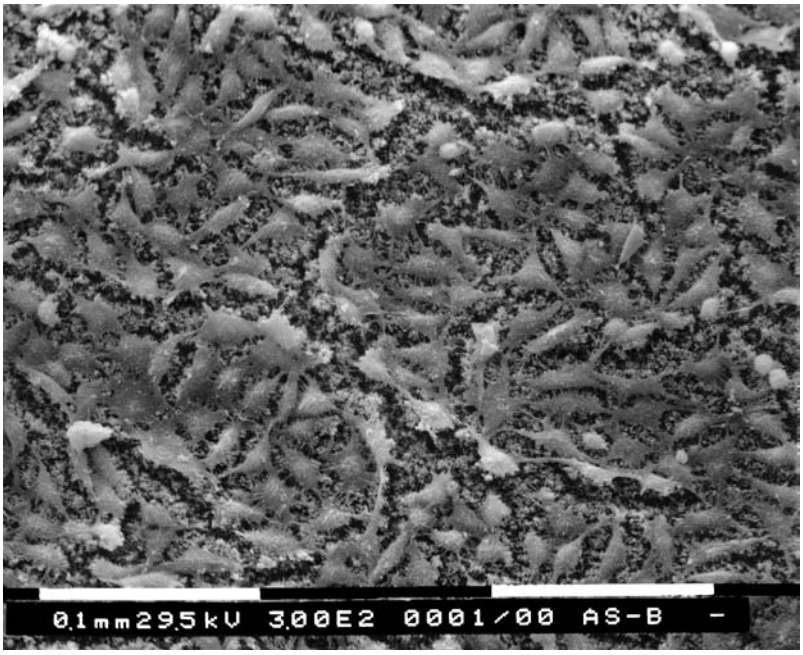

Figure 7 SEM micrograph of 3T3 fibroblasts on ammonia plasma treated ePTFE -smooth side- 24 h after cell seeding: cell adhesion is higher than that observed for other untreated samples.

assay will be followed by further studies aimed to obtain more information concerning long-term compatibility and cellular response to the modified material.

4. Conclusions

Bard ePTFE prosthesis surface was modified by different plasma treatments and the outcome was an increase in roughness and, consequently, in wettability and cell adhesion.

It has been shown that the nature of the gas is an important parameter. In particular, among the performed treatments, ammonia plasma treatment seems the most promising, as after this treatment contact angle value between ethylene glycol and ePTFE decreased from 104° to 90°.

The improved wettability is due to the increased surface roughness, which was in turn measured through the AFM software tool. In fact, by software analysis of AFM stored images, it was demonstrated that oxygen plasma treatment is capable of doubling ePTFE RMS surface roughness, which is increased to a factor of 3, with respect to the untreated surface value, after further ammonia plasma exposure.

In order to give better evidence of treatment effects on surface morphology, we produced AFM images with augmented magnification and SEM images: both techniques showed that oxygen and ammonia plasma exposures progressively destroy an ePTFE surface, increasing its roughness.

The same treatment procedures used with Bard ePTFE were also performed on Teflon slabs. By SEM analyses and contact angle measurements it was possible to verify that treatment effects are similar to those produced on ePTFE prostheses.

In vitro preliminary studies on 3T3 fibroblasts confirmed increased cell adhesion to the treated surface of the mesh samples.

In conclusion, we can state that the described surface modification technique has several potential clinical applications, not only in hernia surgery, but in general in all kinds of surgery involving internal sutures which require the application of prostheses with differentiated adhesion properties on the two sides.

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