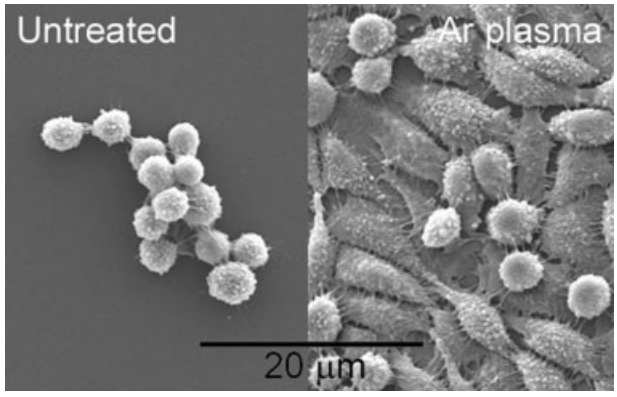
Plasma Surface Modification of Chitosan Membranes: Characterization and Preliminary Cell Response Studies

Simone S. Silva,* Sandra M. Luna, Manuela E. Gomes, Johan Benesch, Iva Pashkuleva, João F. Mano, Rui L. Reis

Surface modification of biomaterials is a way to tailor cell responses whilst retaining the bulk properties. In this work, chitosan membranes were prepared by solvent casting and treated with nitrogen or argon plasma at 20 W for 10–40 min. AFM indicated an increase in the surface roughness as a result of the ongoing etching process. XPS and contact angle measurements showed different surface elemental compositions and higher surface free energy. The MTS test and direct contact assays with an L929 fibroblast cell line indicated that the plasma treatment improved the cell adhesion and proliferation. Overall, the results demonstrated that such plasma treatments could significantly improve the biocompatibility of chitosan membranes and thus improve their potential in wound dressings and tissue engineering applications.



Introduction

Every year millions of people suffer cutaneous lesions such as burns, abrasions or wounds that need treatment. When the skin is wounded, cellular damage, loss of tissue and changes in the relationship between the tissues and the surrounding environment are correlated in a complex series of cellular and chemical events.[1] It is well established that severely damaged skin requires a protective barrier for proper healing. Effective wound dressing must not only protect the wound from the surrounding environment but also promote the healing process by providing an optimal microenvironment.[2,3] Polysaccharides have been widely used in wound management aids. Due to their relative versatility in terms of composition, structure and intrinsic properties, they can assist with the proper physiological reconstruction of the skin and reduce or prevent scar tissue formation.[4]

S. S. Silva, S. M. Luna, M. E. Gomes, J. Benesch, I. Pashkuleva, J. F. Mano, R. L. Reis
3B's Research Group - Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, Campus de Gualtar, 4710-057 Braga, Portugal
Fax: +351 253604 498; E-mail: simonesilva@dep.uminho.pt
S. S. Silva, S. M. Luna, M. E. Gomes, J. Benesch, I. Pashkuleva, J. F. Mano, R. L. Reis
IBB - Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal

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Some of the thoroughly studied natural polymers for skin regeneration are alginate, chitosan, hyaluronic acid, cellulose, collagen, gelatin and their derivatives.[3–10] These polymers can be used as gelling agents, consistency excipients in creams, matrices in patches, sponge-type wound dressings, hydrogels, membranes and skin adhesives in transdermal systems.[3–11] Among them, chitosan possesses several characteristics favorable for promoting dermal regeneration and accelerated wound healing.[5,12] Its biodegradability, adhesiveness, non-toxicity, bacteriostatic, fungistatic and haemostatic activities, and antimicrobial effects make this polysaccharide an excellent biomaterial to treat wounds.[5,12–15] Several studies[16,17] have shown that chitosan-derived membranes are not cytotoxic towards fibroblasts, but tend to inhibit cell proliferation. Thus, significant research efforts have been focused on improving the host response to these materials in terms of cellular behavior. Earlier studies used a variety of methods such as blending,[16] gamma irradiation,[18] chemical reactions[19] and plasma surface modification.[20–23] The last one of these, plasma surface modification, is a method widely used[23–25] to tailor surface functionality by working in different atmospheres. The commonly used oxygen plasma results in the formation of different oxygen containing groups, such as –OH, –C=O, –COOH. Argon and nitrogen are other examples of gases used in plasma treatment.[26–29] As a result of the introduced changes in the surface chemistry, different cell behavior on the modified material can be observed.[26–29]

The aim of this study was to improve the biocompatibility of chitosan membranes in terms of fibroblast responses in vitro. Two sets of experiments using nitrogen and argon treatments for different times were carried out. In vitro biological assays with L929 fibroblast-like cells were performed to evaluate the influence of the introduced changes on cell behavior on a preliminary basis. To the best of our knowledge, there is no other study that has compared the effect of these surface treatments of chitosan membranes on fibroblast responses.

Experimental Part

Materials and Sample Preparation

Chitosan (Cht, Sigma Aldrich, CAS 9012-76-4) with a deacetylation degree of 83.8%, as determined by ^{1}H NMR,[30] was used. All other reagents were of analytical grade and used as received. Chitosan powder was dissolved at 1 wt.-% in 0.2 M acetic acid. Chitosan membranes (average thickness of 47 μm) were obtained by a solvent casting technique, followed by neutralization in a 0.1 M NaOH solution for 30 min. The plasma treatment was carried out in a radio frequency plasma reactor (PlasmaPrep5, Germany). The plasma chamber was thoroughly purged with a continuous flow of the gas used during the treatment to reduce trace amounts of air and moisture. During the treatment, the gas flow was adjusted in order to keep a constant pressure of 0.2 mbar insight the chamber. A power of 20 W was applied. The duration of the treatment was varied from 10 min to 40 min. Two different working gases, namely nitrogen (N_2) and argon (Ar) (see Table 1 that also contains designation codes for each treatment), were used in order to evaluate the effect of the working gas on the induced changes in the surface functionalities.

Table 1. Plasma conditions used for modification of chitosan membranes.

Sample	Conditions		
	Power	Time	Gas
	W	min	
ChtP1	20	10	N_2
ChtP2	20	20	N_2
ChtP3	20	30	N_2
ChtP4	20	40	N_2
ChtP5	20	10	Ar
ChtP6	20	20	Ar
ChtP7	20	30	Ar
ChtP8	20	40	Ar

Characterization Methods

Scanning Electron Microscopy (SEM)

The surface morphology of the samples was analyzed using a Leica Cambridge S-360 scanning electron microscope. All specimens were pre-coated with a conductive layer of sputtered gold. The micrographs were taken at 10 kV at different magnifications.

Atomic Force Microscopy (AFM)

The roughness of the sample surface was measured by AFM. The analyses were performed on at least three spots per sample using tapping mode (Veeco, USA) connected to a NanoScope III (Veeco, USA) with non-contacting silicon nanoprobes (ca. 300 kHz, setpoint 2–3 V) from Nanosensors, Switzerland. All images were fitted to a plane using the 3rd degrees flatten procedure included in NanoScope software version 4.43r8. The surface roughness was calculated as *Sq* (root mean square from average flat surface) and *Sa* (average absolute distance from average flat surface). The values are presented as mean ± standard deviation.

X-Ray Photoelectron Spectroscopy (XPS)

The XPS analysis was performed using an ESCALAB 200A (VG Scientific, UK) with PISCES software for data acquisition and analysis. For analysis, an achromatic Al (Kα) X-ray source operating at 15 kV (300 W) was used. The spectrometer was calibrated with reference to Ag 3d5/2 (368.27 eV) and was

operated in constant analyzer energy (CAE) mode with 20 eV pass energy. The measurements were carried out at a take-off angle of 90° (normal to the surface). Data acquisition was performed at a pressure lower than 10^{-6} Pa. The value of 285 eV of the hydrocarbon C1s core level was used as a calibration for the absolute energy scale. Overlapping peaks were resolved into their individual components by XPSPEAK 4.1 software.

Contact Angle Measurements

The surface wettability of the membranes was assessed by static contact angle (θ) measurements using the sessile drop method. Two different liquids were used: ultra-pure water (polar) and diiodomethane (non-polar). The measurements were performed using OCA20 equipment (DataPhysics, Germany) and SCA-20 software. The presented data are the average of 6 measurements. The surface energy was calculated using the Owens, Wendt, Rabel and Kaelble (OWRK) equation.[31]

in vitro Cell Culture Studies

Cell Culture

A mouse fibroblast-like cell line (L929) was selected for all the biological assays in order to evaluate the effect of surface modification on cell adhesion, viability and proliferation. The L929 fibroblast cell line was obtained from the European Collection of Cell Cultures (ECACC, UK). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, USA), supplemented with 10% of heat inactivated fetal bovine serum (FBS, Biochrom AG, Germany) and 1% antibiotic/antimycotic solution (Invitrogen, Portugal) incubated at 37 °C in a humidified atmosphere with 5% of CO_2. The culture medium was changed every 2 d.

Direct Contact Assay - Cell Adhesion and Proliferation on Chitosan Membranes

Chitosan membranes (1 cm^2 modified and non-modified) were seeded with 100 μL of a cell suspension (8×10^4 cells · mL^{-1}) and cultured for 3, 7 and 14 d at 37 °C. Tissue culture polystyrene coverslips (TCPS, Sarstedt, USA) were used as controls. After each incubation period, the samples were rinsed with a phosphate buffer saline (PBS, Sigma-Aldrich, USA) and prepared for further analysis (MTS assay and SEM observations).

Prior to culturing, all the membranes were sterilized with ethylene oxide under previously described conditions.[32]

MTS Assay

Cellular viability was quantitatively assessed by the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay (Promega, Madison, USA).[33] Culture medium without FBS and without phenol red was mixed with MTS in the ratio 5:1, added to the membrane/cells constructs until it totally covered them and incubated for 3 h at 37 °C in a 5% CO_2 atmosphere. After the incubation period, the optical density (OD) was read in a microplate reader (Bio-Tek, USA) at 490 nm. The measurements were made in triplicate for each treatment and time point (3, 7 and 14 d).

SEM Analysis of the Membranes

Prior to SEM measurements, the seeded membranes were fixed with 2.5% glutaraldehyde (Sigma-Aldrich, Germany) in a PBS solution and then dehydrated using a series of ethanol solutions (25, 30, 50, 70, 80, 90, 100% v/v). The samples were dried overnight at room temperature, coated with gold by sputtering and observed by SEM.

Results and Discussion

When a polymer is exposed to plasma, two competitive processes, namely functionalization and etching, take place. In a typical plasma process, the radicals created on the polymer surface by hydrogen removal combine with the radicals from the working gas to modify the surface. Alternatively, crosslinking could occur when the created radicals on the surface recombine with themselves. On the other hand, etching will also take place. The working conditions determine which processes will be dominant.[24,25] SEM images of the modified membranes did not show any significant changes in the surface morphology induced by the treatments (data not shown). Nevertheless, ongoing etching processes were confirmed by AFM. Changes in the surface topography after the performed modification were observed at the nanoscale (Figure 1). This effect was more significant after longer exposure times (30 and 40 min), when nitrogen was used as a working gas. In contrast to this, a shorter exposure time (10 and 20 min) was more effective when the modification was performed in an argon plasma atmosphere. The roughness of the argon plasma treated membranes (Figure 2B) decreased with longer exposure times (40 min) but the modified membranes were thinner compared to the untreated membranes. Additionally, the treated membranes were more brittle, which indicates crosslinking processes.

The surface chemistry of modified and non-modified samples was investigated by XPS. Since the argon atmosphere is a non-reactive gas, mainly etching was expected to be observed after this treatment. As can be seen from Table 2 a significant increase in the nitrogen content was observed for shorter treatment times (10 and 20 min). Cleavage of the remaining $-COCH_3$ groups of chitosan is most probably the reason for the obtained results. This hypothesis was confirmed by an observed decrease in the intensity of the carbon peak in the ChtP5 spectrum. However, when the treatment was extended, this relation was not that straightforward. For longer treatment times, the reactive species created by the plasma etch the already modified surface and therefore reveal a non-modified

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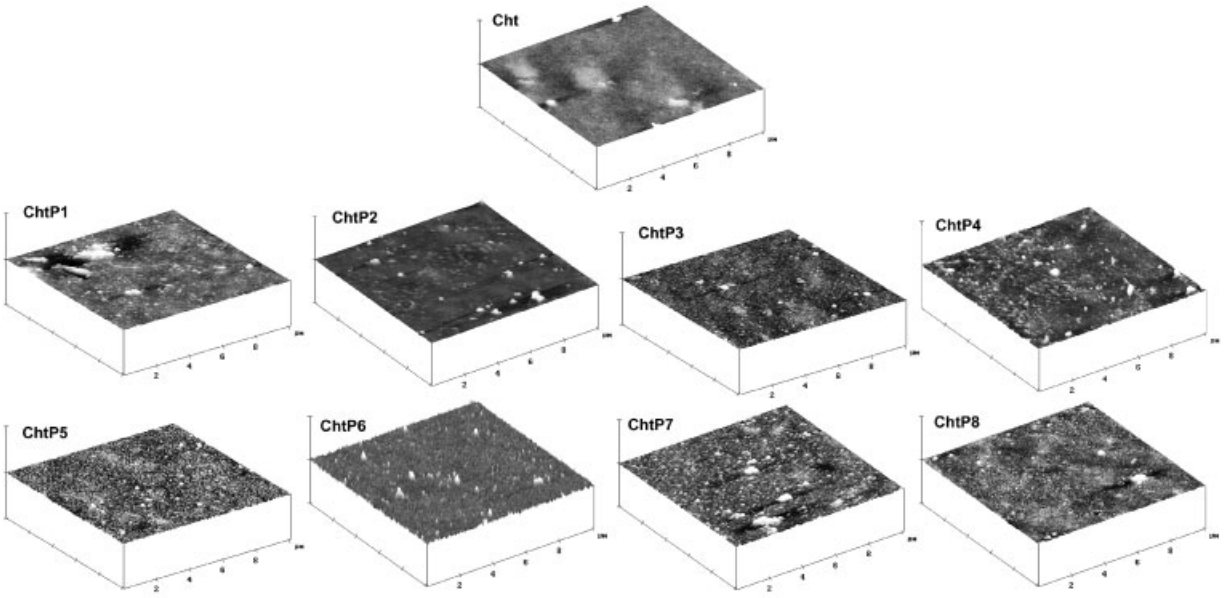

Figure 1. AFM images of chitosan membranes before (Cht) and after plasma treatment (ChtP1, ChtP2, ChtP3, ChtP4: nitrogen plasma; ChtP5, ChtP6, ChtP7, ChtP8: argon plasma).

surface. Hence, the values for C and N content obtained from the XPS spectra of the samples treated for 40 min (the longest time) are very similar to the initial ones for untreated chitosan (Table 2). When nitrogen is used as a working gas, not only etching but also functionalization with N-containing groups ($-NH_2$, $-NH$, $=NH$, $CONH_2$ or $C\equiv N$) must be observed.[28] Longer exposure times (30 and 40 min) resulted in higher nitrogen contents (Table 2), confirming the incorporation of these groups on the material surface.

Changes in the high resolution C_{1s} core level spectra before and after nitrogen and argon plasma treatment are shown in Figure 3. The C_{1s} core level spectrum of the chitosan membranes revealed three peaks. The C_{1s} peak at 285.0 eV was assigned to the main backbone carbon peak,

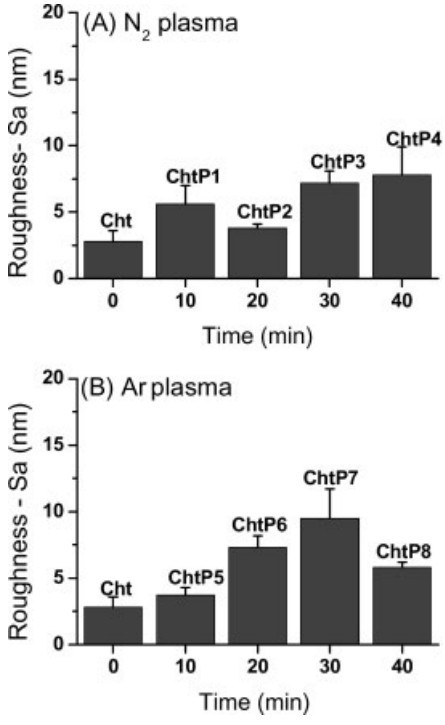

Figure 2. Mean roughness of chitosan membranes before and after nitrogen (A) and argon (B) plasma treatment.

Table 2. Surface composition and atomic ratios determined by XPS for original and modified membranes.

Treatment	Sample	Surface composition			Atomic ratio	
		% C	% O	% N	%	
					C/O ratio	C/N ratio
none	chitosan	66.41	27.97	5.62	2.37	11.82
N_2 plasma	ChtP1	64.06	30.24	5.70	2.12	11.24
N_2 plasma	ChtP2	70.33	24.24	5.33	2.90	13.20
N_2 plasma	ChtP3	70.04	21.43	8.53	3.27	8.21
N_2 plasma	ChtP4	67.88	24.61	7.50	2.76	9.05
Ar plasma	ChtP5	63.31	28.61	8.08	2.21	7.84
Ar plasma	ChtP6	66.80	24.43	8.78	2.73	7.61
Ar plasma	ChtP7	69.15	24.31	6.54	2.84	10.57
Ar plasma	ChtP8	65.61	28.61	5.79	2.29	11.33

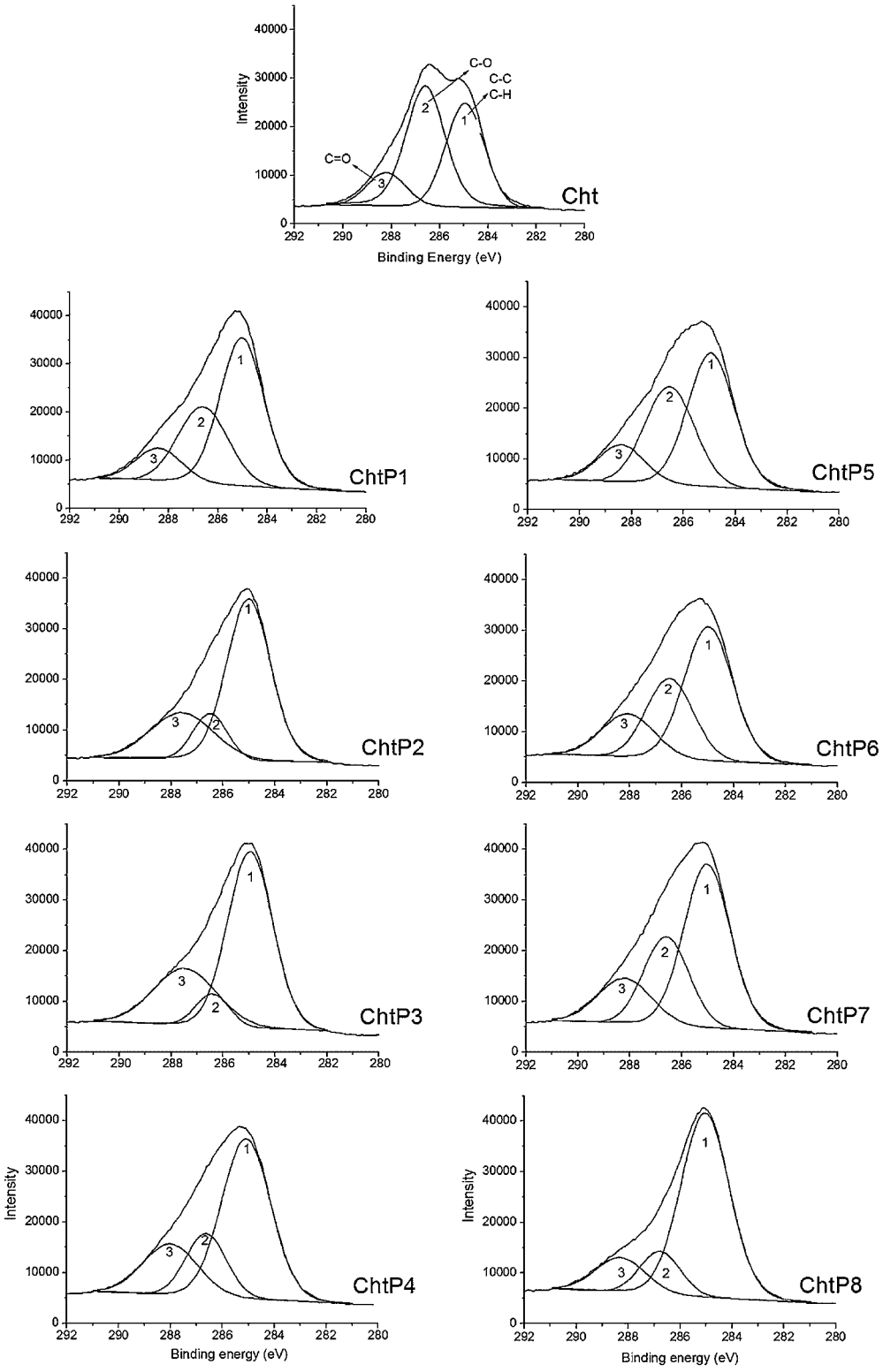

Figure 3. C_{1s} core level spectra of untreated chitosan (Cht) and modified samples (ChtP1, ChtP2, ChtP3, ChtP4 - samples after nitrogen plasma; ChtP5, ChtP6, ChtP7, ChtP8 - samples after argon plasma).

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Table 3. Relative intensities of the fitted C1s peak of untreated and modified plasma membranes.

Treatment	Sample	Binding energy (relative intensity %)	Assignments
		eV	
none	Cht	285.0 (39)	C–C, C–H
		286.6 (44)	C–O
		288.2 (16)	C–O–C, N–C=O
N_2 plasma	ChtP1	285.0 (56)	C–C, C–H
		286.6 (31)	C–O
		288.4 (12)	C–O–C, N–C=O
N_2 plasma	ChtP2	285.0 (63)	C–C, C–H
		286.5 (13)	C–O
		287.6 (25)	C–O–C, N–C=O
N_2 plasma	ChtP3	284.9 (64)	C–C, C–H
		286.4 (8)	C–O
		287.5 (28)	C–O–C, N–C=O
N_2 plasma	ChtP4	285.1 (60)	C–C, C–H
		286.6 (19)	C–O
		288.0 (21)	C–O–C, N–C=O
Ar plasma	ChtP5	284.9 (49)	C–C, C–H
		286.5 (37)	C–O
		288.4 (14)	C–O–C, N–C=O
Ar plasma	ChtP6	284.9 (52)	C–C, C–H
		286.5 (30)	C–O
		288.1 (18)	C–O–C, N–C=O
Ar plasma	ChtP7	285.0 (55)	C–C, C–H
		286.6 (28)	C–O
		288.2 (17)	C–O–C, N–C=O
Ar plasma	ChtP8	285.0 (73)	C–C, C–H
		286.8 (14)	C–O
		288.3 (14)	C–O–C, N–C=O

which also overlaps C–NH_2 chemical bindings. The peak at 286.6 eV was assigned to C–O/C–OH and the peak at 288.3 eV to O–C–O and N–C=O chemical bindings.[34,35] The same components were present in the C_{1s} core level spectra of the treated membranes, but changes in the relative intensities of all peaks were observed (Figure 3, Table 3). Generally an increase in the relative intensity of the C–H component was observed after treatment. This change was accompanied by a decrease in C–O intensity in almost all the treated membranes compared to the untreated one. These results confirm the acetate cleavage and the ongoing etching processes. Significant quantities of oxygen moieties (mainly C=O) were introduced on the surface for ChtP5 and ChtP8 (Table 2).

Modifications in the surface chemistry and morphology can change the surface hydrophilicity,[24] which is one of the key surface parameters determining the material/

Table 4. Water contact angles (θ) and surface energy (γ) of untreated and plasma-treated chitosan membranes.

Treatment	Sample	θ_{water}	γ
		degrees	$mN \cdot m^{-1}$
none	CHT	88.5 ± 1.6	30.8 ± 0.1
N_2 plasma	ChtP1	87.7 ± 1.7	20.7 ± 0.1
N_2 plasma	ChtP2	85.1 ± 9.4	21.6 ± 0.1
N_2 plasma	ChtP3	83.5 ± 5.6	28.8 ± 0.2
N_2 plasma	ChtP4	84.7 ± 7.1	38.6 ± 0.1
Ar plasma	ChtP5	78.3 ± 9.4	21.9 ± 0.1
Ar plasma	ChtP6	88.1 ± 3.3	31.8 ± 0.1
Ar plasma	ChtP7	84.9 ± 4.4	32.4 ± 0.3
Ar plasma	ChtP8	93.7 ± 2.6	18.0 ± 0.2

bioenvironmental interactions. The measured contact angles and calculated surface energies of the prepared membranes are summarized in Table 4. Generally, all the treatments resulted in more hydrophilic surfaces. Previous works[29,36] have demonstrated that short time (1–3 min) treatments in a nitrogen atmosphere result in more hydrophilic surfaces. In contrast, longer treatments (i.e., >3 min) decrease the surface hydrophilicity. The obtained results for the surface energy are in agreement with the ones from XPS analysis. Different trends were observed for the used working atmospheres. A slight increase of the surface energy with the treatment time was observed for the samples treated by nitrogen plasma. An intermediate exposure time was found to result in highest surface energy, when argon was used as a working gas. Earlier studies[37,38] reported that materials with a high surface energy promote rapid cellular adhesion and spreading, whereas low surface energy does not favor such behavior. It has been also shown by different studies[24,39] that changes in the surface charge, chemical surface composition and roughness can affect the biocompatibility of a polymer.

The results obtained from the MTS assay (Figure 4) showed that both nitrogen and argon plasma treated membranes promoted a higher cell viability than untreated chitosan membranes. This effect was observed for all the studied time periods. After 14 d of culture, the ChtP2 sample presented the highest cell viability among the nitrogen plasma treated membranes. The values obtained for the membranes treated with argon plasma were more consistent, regardless of the culture time.

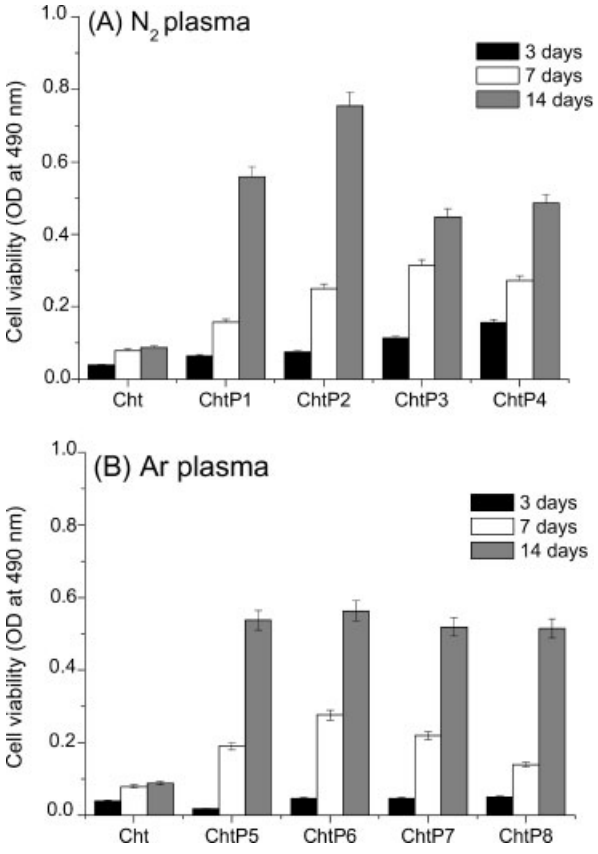

Figure 4. Viability levels of L929 fibroblasts-like cells on the untreated and treated plasma membranes assessed by MTS assay. (A) ChtP1, ChtP2, ChtP3, ChtP4 - samples after nitrogen plasma, and (B) ChtP5, ChtP6, ChtP7 and ChtP8 - samples after argon plasma.

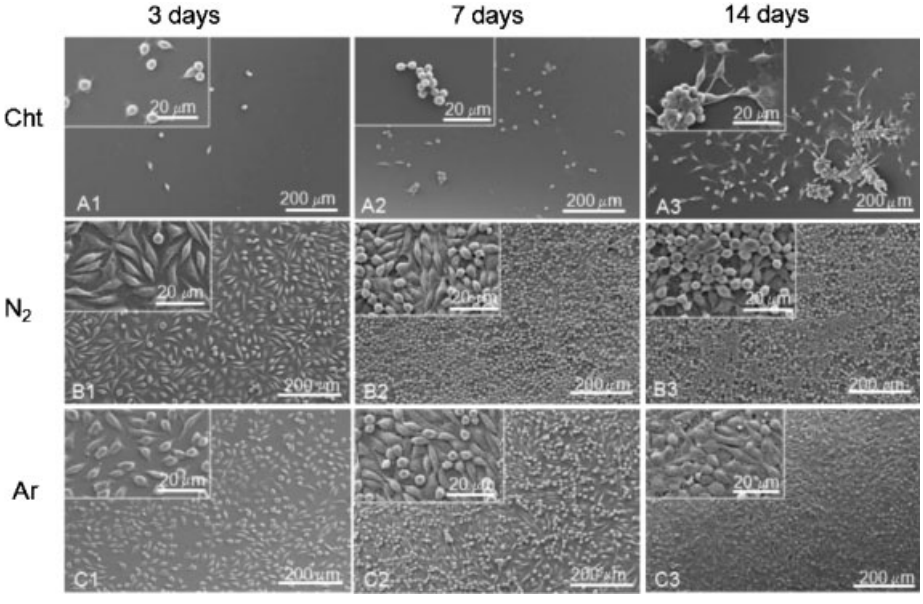

Figure 5. SEM micrographs of L929 fibroblast-like cells cultured on: (A) Cht (untreated membrane - control); (B) ChtP2 (chitosan membranes modified by nitrogen plasma); (C) ChtP6 (chitosan membranes modified by argon plasma), after 3, 7 and 14 d of culture.

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Cell morphology on both untreated and modified membranes was observed by SEM. Fibroblast-like cells were able to attach and stretch on all the types of modified membranes after different culture times. The ChtP2 sample, which presented the best results among nitrogen treated membranes, was selected to be studied for different culture periods. The ChtP6 from the argon modified membranes was also chosen in order to evaluate the effect of the working gas on cell morphology. After 3 d of culture, there was a significant number of L929 cells on the surface of both nitrogen and argon treated plasma membranes (Figure 5).

The attached cells presented a typical morphology for fibroblasts with an elongated shape. The number of attached cells increased with culture time and, after 14 d, a dense cellular monolayer covered both modified membranes. On the contrary, and as previously described,[16,17] poor cell attachment was observed for the untreated membranes. After 3 d of culture, only a few rounded cells could be seen on their surface. This number increases with the time of culture, but at the end of the studied period it was still insignificant compared to the number of cells adhered to the modified membranes.

Conclusion

Surface modification of chitosan membranes was performed using nitrogen and argon plasma. Higher surface roughness (nanoscale) measured for the modified materials indicated etching processes. This effect increased with the exposure time and did not depend on the working atmosphere used. The surface energy increased for the treated membranes compared to the untreated ones. XPS measurements confirmed the incorporation of oxygen- and nitrogen- containing groups on the surface after treatments. In vitro preliminary biological studies showed that modified chitosan membranes displayed higher cell viability on the surface when compared to untreated chitosan membranes. The results demonstrated that either nitrogen or argon plasma treatments can be used as a way to improve the fibroblast adhesion and proliferation of chitosan membranes. The proposed modifications would facilitate the use of chitosan and chitosan based materials in wound dressing and tissue engineering applications.

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[1] R. Lanza, R. Langer, J. Vacanti, "*Principles of Tissue Engineering*", Academic Press, London 2000, p. 857.
[2] M. Bradley, N. Cullum, E. A. Nelson, M. Petticrew, T. Sheldon, D. Torgerson, *Health Technol. Asses.* **1999**, *3*, 1.
[3] Y. Qin, *J. Appl. Polym. Sci.* **2004**, *91*, 953.
[4] L. L. Lloyd, J. F. Kennedy, P. Methacanon, M. Paterson, J. Knill, *Carbohydr. Polym.* **1998**, *37*, 315.
[5] W. Paul, C. P. Sharma, *Trends Biomat. Artif. Organs* **2004**, *18*, 18.
[6] R. M. Silva, J. F. Mano, R. L. Reis, *J. Mater. Sci: Mater. Med.* **2004**, *15*, 105.
[7] A. K. Azad, N. Sermsintham, S. Chandrkrachang, W. F. Stevens, *J. Biomed. Mater. Res. B* **2004**, *69B*, 216.
[8] K. R. Kirker, Y. Luo, J. H. Nielson, J. Shelby, G. D. Prestwich, *Biomaterials* **2002**, *23*, 3661.
[9] M. G. Jeschke, G. Sandmann, T. Schubert, D. Klein, *Wound Repair Reg.* **2005**, *13*, 324.
[10] W. H. Chang, Y. Chang, P. H. Lai, H. W. Sung, *J. Biomat. Sci.: Polym. E* **2003**, *14*, 481.
[11] C. Valenta, B. G. Auner, *Eur. J. Pharm. Biopharm.* **2004**, *58*, 279.
[12] M. N. V. R. Kumar, *Chem. Rev.* **2004**, *104*, 6017.
[13] M. G. N. Campos, C. R. F. Grosso, G. Cardenas, L. H. I. Mei, *Macromol. Symp.* **2005**, *229*, 253.
[14] H. Zheng, Y. Du, J. Yu, R. Huang, L. Zhang, *J. Appl. Polym. Sci.* **2001**, *80*, 2558.
[15] C. Peniche, W. A. Monal, H. Peniche, N. Acosta, *Macromol. Biosci.* **2003**, *3*, 512.
[16] S. S. Silva, M. I. Santos, O. P. Coutinho, J. F. Mano, R. L. Reis, *J. Mater. Sci.: Mater. Med.* **2005**, *16*, 575.
[17] C. Chatelet, O. Damour, A. Domard, *Biomaterials* **2001**, *22*, 261.
[18] F. Yang, X. Li, M. Cheng, Y. Gong, N. Zhao, X. Zhang, *J. Biomater. Appl.* **2002**, *16*, 215.
[19] V. Tangpasuthadol, N. Pongchaisirikul, V. P. Hoven, *Carbohydr. Res.* **2003**, *338*, 937.
[20] H. T. Wang, Y. E. Fang, Y. S. Yan, *J. Mater. Chem.* **2001**, *11*, 1374.
[21] Y. P. Li, L. Liu, Y. E. Fang, *Polym. Int.* **2003**, *52*, 285.
[22] X. Zhu, K. S. Chian, M. B. E. Chan-park, S. T. Lee, *J. Biomed. Mater. Res. A* **2005**, *73A*, 264.
[23] P. M. L. Pérez, A. P. Marques, R. M. P. Silva, I. Pashkuleva, R. L. Reis, *J. Mater. Chem.* **2007**, *17*, 4064.
[24] P. K. Chu, J. Y. Chen, L. P. Wang, N. Huang, *Mat. Sci. Eng. R* **2002**, *36*, 143.
[25] F. A. Khonsari, M. Tatoulian, N. Shahidzadeh, J. Amouroux, "*Study of the plasma treated polymers and the stability of the surface properties*", Springer-Verlag, New York 1998, p. 165.
[26] C. M. Alves, Y. Yang, D. L. Carnes, J. L. Ong, V. L. Sylvia, D. D. Dean, C. M. Agrawal, R. L. Reis, *Biomaterials* **2007**, *28*, 307.
[27] A. A. M. Plath, K. Schroder, B. Finke, A. Ohl, *Vacuum* **2003**, *71*, 391.
[28] L. J. Gerenser, J. M. Grace, G. Apai, P. M. Thompson, *Surf. Interface Anal.* **2002**, *29*, 12.

[29] N. V. Bhat, D. J. Upadhyay, *J. Appl. Polym. Sci.* **2002**, *86*, 925.
[30] A. Hirai, H. Odani, A. Nakajima, *Polym. Bull.* **1991**, *26*, 87.
[31] D. K. Owens, R. C. Wendt, *J. Appl. Polym. Sci.* **1969**, *13*, 1741.
[32] R. L. Reis, S. C. Mendes, A. M. Cunha, M. J. Bevis, *Polym. Int.* **1997**, *43*, 347.
[33] A. J. Salgado, M. E. Gomes, A. Chou, O. P. Coutinho, R. L. Reis, D. W. Hutmacher, *Mater. Sci. Eng. C* **2002**, *20*, 27.
[34] G. Beamson, D. Briggs, "*High Resolution XPS of Organic Polymers*", John Wiley & Sons, Chichester 1992.
[35] L. J. Matienzo, S. K. Winnacker, *Macromol. Mater. Eng.* **2002**, *287*, 871.
[36] C. M. Chan, T. M. Ko, H. Hiraoka, *Surf. Sci. Rep.* **1996**, *24*, 1.
[37] S. A. Mitchell, M. R. Davidson, R. H. Bradley, *J. Colloid Interface Sci.* **2005**, *281*, 122.
[38] K. Anselme, *Biomaterials* **2001**, *21*, 667.
[39] B. D. Ratner, A. S. Hoffman, F. J. Schoen, J. E. Lemos, "*Biomaterials Science: An Introduction to Materials in Medicine*", Academic Press, New York 1996, p. 141.

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