ELSEVIER

PII: S0032-3861(98)00148-7

Polymer Vol. 39 No. 25, pp. 6357–6361, 1998

0032-3861/98/$ - see front matter

An ESCA study of new collagen-based bioartificial polymeric materials

M. G. Cascone[a], G. Di Pasquale[b], A. D. La Rosa[b], C. Cristallini[a], N. Barbani[a] and A. Recca[b,*]

[a]*Department of Chemical Engineering, University of Pisa, Via Diotisalvi 2, 56126 Pisa, Italy*

[b]*Chemistry Institute, Faculty of Engineering, University of Catania, Vle A. Doria 6, 95125 Catania, Italy*

(Received 5 November 1997; revised 9 February 1998)

The surface composition of films prepared from blends containing different percentages of pure soluble collagen and poly(acrylic acid) or poly(sodium vinylsulfonate), was analysed by ESCA. The results indicate that the content of collagen on the surface of the blends is higher than expected, and that such enrichment is more evident for the blends containing a lower percentage of collagen. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: biomaterials; collagen; poly(acrylic acid))

INTRODUCTION

Blends of synthetic and natural polymers (called 'bioartificial polymeric materials') can be considered a new and interesting class of materials[1–4]. In fact the possibility of combining the desirable mechanical properties of synthetic polymers with the activity of biological polymers can be exploited to produce new materials useful for many applications in the biomedical field. For example, bioartificial polymeric materials have been already utilized as drug delivery systems[5,6], dialysis membranes[7,8] and wound dressing patches[9].

Recently, blends of soluble collagen (SC) and poly(acrylic acid) (PAA), or poly(sodium vinylsulfonate) (PSA) or poly(sodium 4-styrene sulfonate) (PSS), have been prepared with the double purpose to produce new materials and to study the interactions that occur between the collagen and the functional groups (–COO, $-XS0_3$) characteristic of the synthetic polymers[10,11].

The understanding of such interactions could be important for the application of the materials obtained in the field of artificial intraocular lenses (IOL). In fact, after some time of IOL use, about 50% of patients suffer from capsule opacification due to an uncontrolled proliferation of residual epithelial cells. This problem could be solved through an appropriate surface modification able to provide a stable interface between the IOL and the surrounding lens capsule.

Most of the commercially available IOL are made with silicones. One disadvantage of these lenses is that the adhesion between the surface and the surrounding tissue is difficult due to the extremely hydrophobic and chemically inert surface of the implant. An appropriate surface treatment (for example the plasma etching) could improve the biofunction of such materials by the generation of new functional groups (–COO, $-XS0_3$), which could allow the interactions between the lenses and the components (mainly collagen and laminin) contained in the posterior capsular membrane[12,13].

Electron spectroscopy for chemical analysis (ESCA) represents a technique of prime importance in the characterization of polymer surfaces with the possibility, in the case of polymer blends, to study the preferential segregation in the surface of one of the components[14,15]. It is very important to study this aspect in the case of biomaterials owing to the interactions of the surface with tissue, blood cells, proteins etc. In previous works[16,17] bioartificial materials based on collagen-poly(vinyl alcohol) blends were analysed by ESCA and the results indicated an enrichment of the collagen components in their surfaces.

In this paper we report an ESCA characterization of different composition blends of poly(acrylic acid) (PAA) and poly(sodium vinylsulfonate) (PVS) and collagen (*Table 1*).

EXPERIMENTAL

All materials used in this work were commercially avaliable. PAA with average molecular weight of 250 000 and PVS with low average molecular weight of 15 000–18 000 were supplied by Aldrich Chemie, Steinhem, Germany (*Figure 1a*).

Acid soluble collagen (SC), type 1 from calf skin, was supplied by Sigma Chemical Co., St Louis, MO, USA. The main ammoacids contained in the collagen are reported in *Figure 1b*.

SC–PAA and SC–PVS blends, with different composition ratios (*Table 1*), were prepared by mixing a solution of SC in 0.5 M acetic acid (1% w/v), with aqueous solutions (0.3% w/v) of PAA and PVS, respectively.

The pH of each polymeric solution was adjusted to a value of 6 before mixing. This pH value was chosen in order to avoid the immediate co-precipitation, which occurred when the blending of SC with PAA and PVS was carried out at a pH value lower than 5.

The mixtures were poured into Petri dishes at room temperature and then the films were cast.

* To whom correspondence should be addressed

Table 1 Blend composition

Sample	% Polymer	% Collagen
la	100	0
2a	70	30
3a	60	40
4a	50	50
5a	40	60
6a	30	70
lb	50	50
2b	20	80
lc	0	100

a, PAA; b, PVS; and c, SC

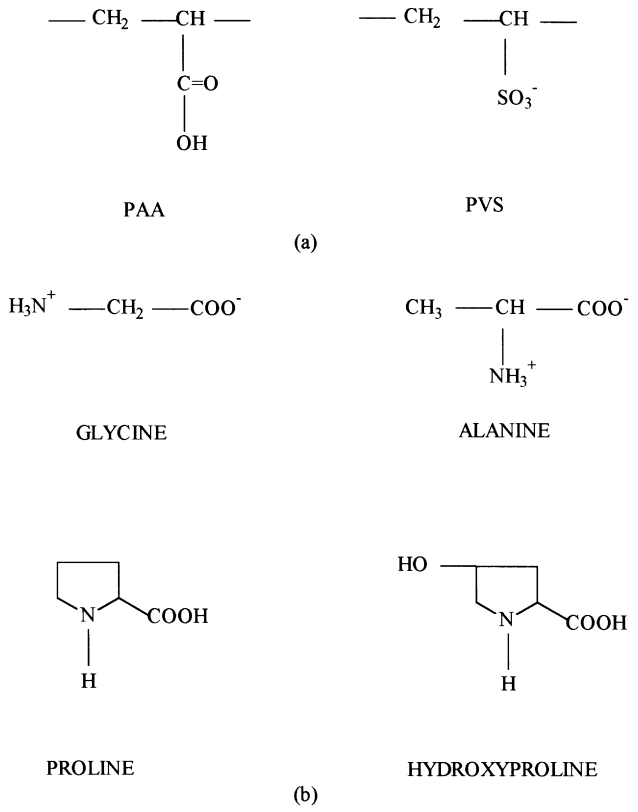

Figure 1 Structural formulas of: (a) synthetic polymers; and (b) the four main aminoacids present in collagen

These films were put in a bath of acetic acid, with pH = 2.5 for 24 h at room temperature to allow the complexation of the two polymer components, in order to avoid the dissolution in water of the materials.

ESCA spectra were recorded on a VG Instrument electron spectrometer using a Mg $K_{\alpha 1,2}$ X-ray source (1253.6 eV). The polymer films were mounted on standard sample stubs by means of double-sided adhesive type. The X-ray source under normal standard conditions was used at 300 W, 15 kV and 20 mA, but to minimize radiation damage to the polymer films, for the curve fitting, the X-ray source was operated at a reduced power of 100 W, 10 kV and 10 mA. The base pressure of the instrument was 5×10^{-10} Torr and during the measurements, it was maintained at 10^{-8} Torr. A pass energy of 100, 50 and 20 eV was used to obtain the widescans, the narrow scans for area ratio calculation and the curve fitting, respectively. The take-off angle of the electrons was 90° with respect to the sample surface. This value allows information to be obtained over a sampling depth ranging from 45 to 85 Å[18,19]. All data analysis (linear background subtraction, peak integration and curve fitting) were accomplished using VGX900x (version 6) software. To compensate for surface charging effects, all binding energies were referenced to the C_{1s} neutral carbon peak at 285.0 eV. The samples were studied as films in all cases.

RESULTS AND DISCUSSION

The survey ESCA spectrum of collagen reveals, as expected, the photoelectronic peaks of carbon, nitrogen and oxygen (*Figure 2a*). The C_{1s} peak has been deconvoluted using the three main components corresponding to aliphatic carbon atoms (285 eV), α-carbon atoms (286.4 eV) and peptidic carbon atoms (288.2 eV) (*Figure 2b*) as already reported by several authors in ESCA studies of different proteins.[20,21]

The C_{1s} envelopes of PAA and SC–PAA blends are reported in *Figure 3*. The peak fitting of the C_{1s} envelope of PAA reveals three main components that correspond to $\underline{C}H_2$–CH (285 eV), $\underline{C}$H–COOH (285.4 eV) and HO–$\underline{C}$=O (289.1 eV) atoms, respectively.

The peak fitting of SC–PAA blends reveals four components: at 285 eV, $\underline{C}$–H and $\underline{C}$–C atoms; at 286.4 eV, α-carbon atoms; at 288.2 eV, peptidic carbon atoms; and at 289.1 eV, HO–$\underline{C}$ = O. In this case, due to the complexity of the C_{1s} peak, it was impossible to calculate with a good fit the B-shifted carboxylate feature at 285.4 eV. In any event, the total C_{1s} area ratio is not affected by introducing such an approximation.

From the amino acid composition of the calf skin collagen, it is possible to calculate the theoretical O_{1s}–C_{1s} and N_{1s}–C_{1s} area ratios for the pure collagen sample (*Table 2*) and in order to determine the collagen–polymer ratio the same calculations can be carried out for the pure artificial PAA and for the blends (*Table 2*). In all cases, the surface chemical compositions can be experimentally determined from the peak area ratios, corrected by using the appropriate sensitivity factors.

The calculated O_{1s}–C_{1s} and N_{1s}–C_{1s} area ratios are in all cases higher than the experimental values (*Table 2*) and this

Table 2 Surface composition of SC–PAA blends

Sample	Calcd. O_{1s}/C_{1s}	Exper. O_{1s}/C_{1s}	Corrected O_{1s}/C_{1s}	Calcd. N_{1s}/C_{1s}	Exper. N_{1s}/C_{1s}	Corrected N_{1s}/C_{1s}	Exper. SC%
1a	0.66	0.53	0.68				
2a	0.57	0.44	0.59	0.09	0.11	0.15	55
3a	0.54	0.33	0.47	0.12	0.12	0.16	59
4a	0.51	0.38	0.50	0.15	0.13	0.19	70
5a	0.48	0.26	0.44	0.18	0.10	0.21	77
6a	0.45	0.26	0.40	0.22	0.12	0.17	63
1c	0.36	0.31	0.37	0.31	0.23	0.27	100

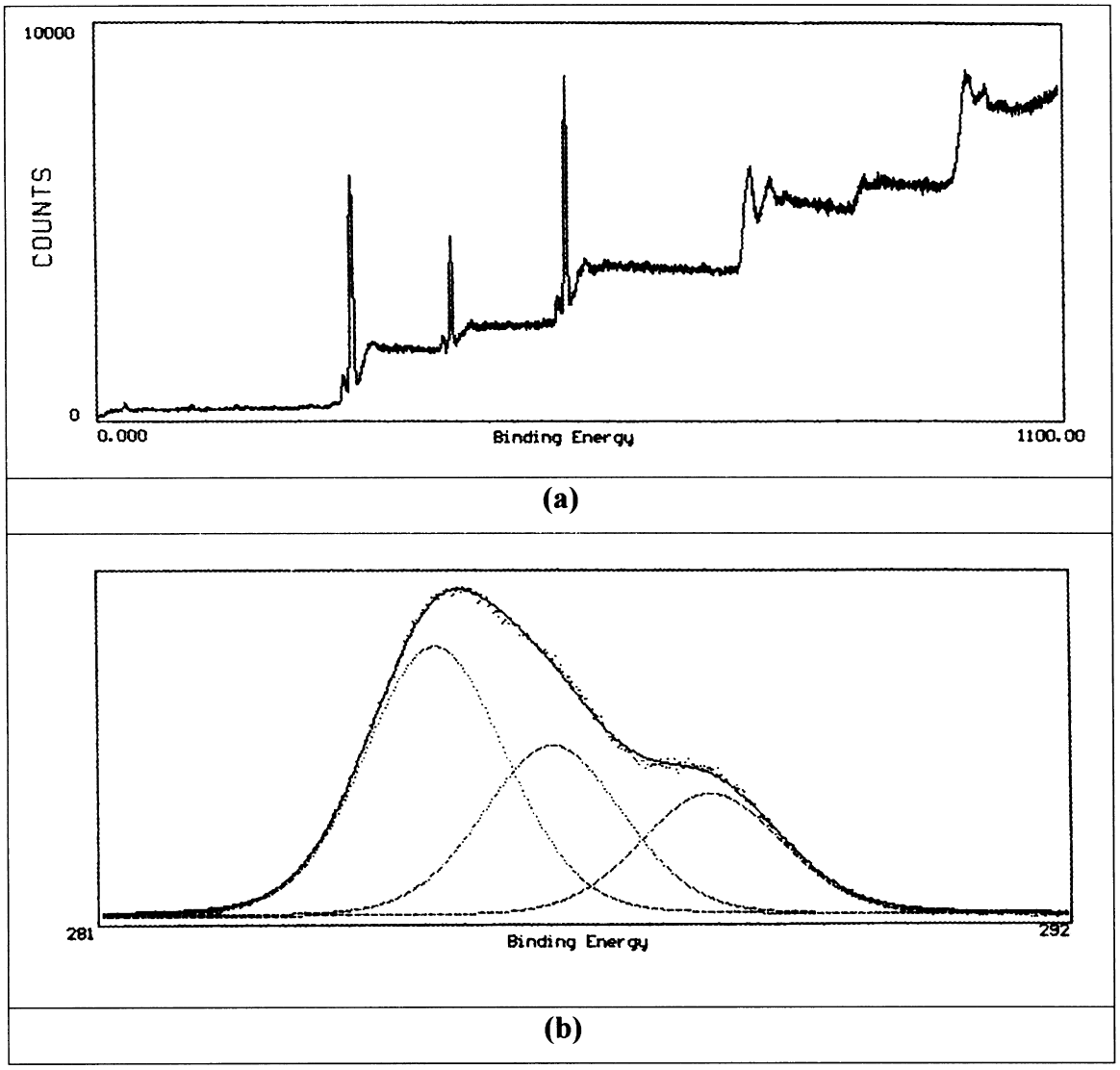

Figure 2 (a) ESCA wide scan spectrum, and (b) C_{1s} curve fitting of pure collagen

Table 3 Decomposition of the C_{1s} peak in collagen and in SC–PAA blends

Sample	C–H and C–C (%) 285 eV	α-C (%) 286.4 eV	Peptidic C (%) 288.2 eV	HO–C = O(%) 289.1 eV	Experimental SC%
2a	60.80	18.50	7.20	13.49	45
3a	69.70	10.79	7.83	11.68	51
4a	61.93	21.17	10.57	6.33	72
5a	74.96	12.96	7.34	4.74	71
6a	72.74	13.29	8.33	5.64	69
1C	47.92	30.32	21.75		100

can be ascribed to the presence of an hydrocarbon contamination on the film surface, as reported in literature[22,23]. In fact, the build-up of hydrocarbon-like material is frequently observed within the timescale of the experiments[15] (indeed this has often been used as an energy reference in the study of polymeric materials that do not present a well-resolved 'hydrocarbon' C_{1s} signal). This contamination affects the exact data quantification by increasing the area ratio of the C_{1s} profile. The contamination certainly comes from the X-ray gun window or casing in close proximity to the sample. Working with polymers routinely the problem increases because the spectrometer working pressure is significantly higher than base pressure over extended periods due to slow outgassing and loss of volatile low molecular weight constituents[15]. Anyhow, in the case of SC–PAA blends, since the peaks centered at 288.2 and 289.1 eV in the C_{1s} envelope are characteristic of the collagen and PAA, respectively (the contribution to this feature of aspartic and glutamic acids of the collagen is very small and can be neglected), it has been possible to calculate the percentage of the hydrocarbon contamination and remove it from the experimental C_{1s} area. The corrected O_{1s}–C_{1s} and N_{1s}–C_{1s} area ratios are reported in *Table 2*. The graphical representation of the corrected N_{1s}–C_{1s} area ratios

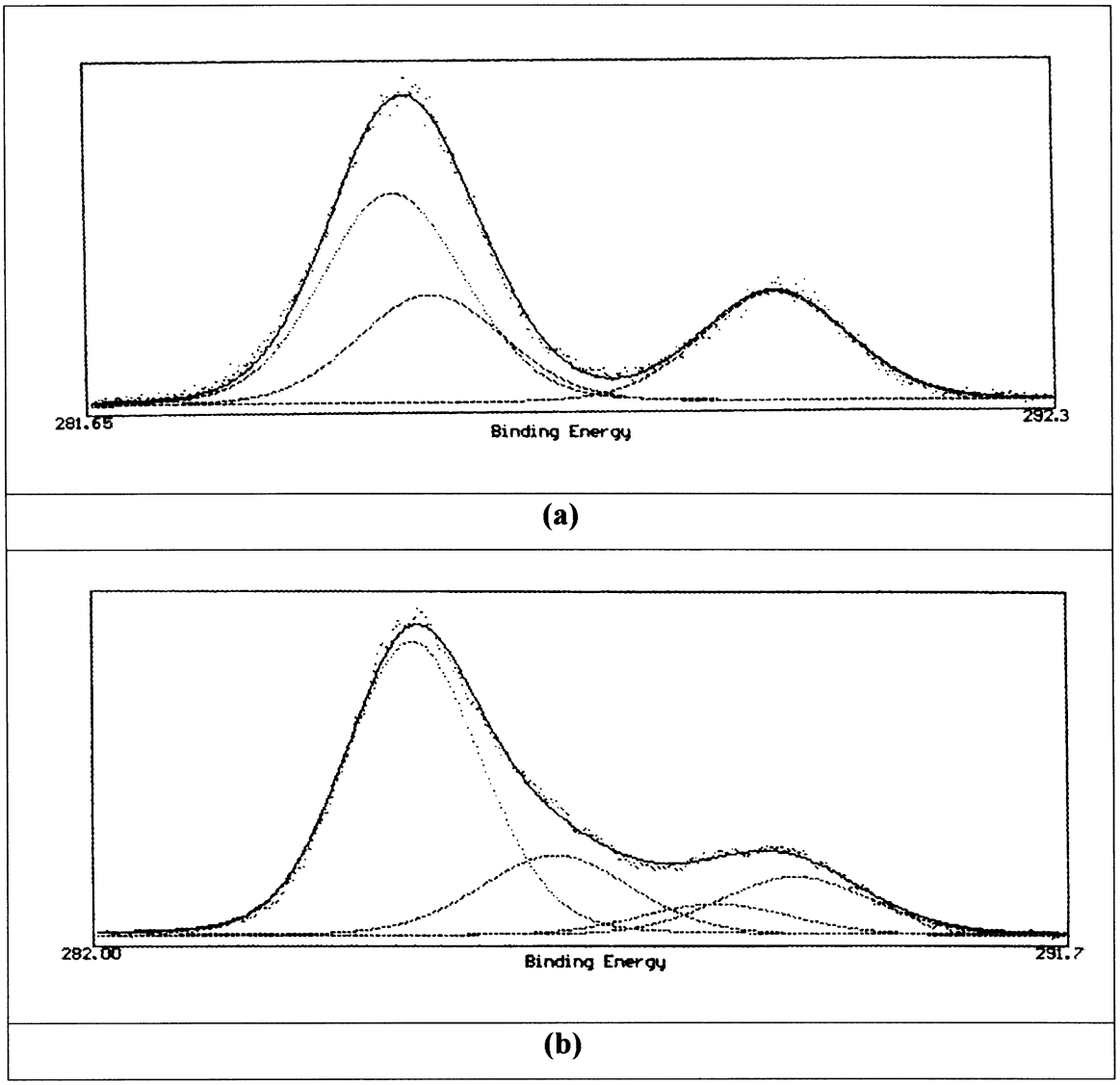

Figure 3 C_{1s} curve fitting of: (a) PAA; and (b) sample 2a

as a function of the collagen weight percentage in the SC–PAA blends (*Figure 4*) shows a surface enrichment of collagen on the samples with decreasing collagen content in the blend (in the graph the diagonal line indicates a surface composition that is equivalent to the bulk composition of the blend).

In order to confirm such result, the surface composition was also determined by curve fitting the C_{1s} region (*Table 3*). The experimental collagen weight percentage in each blend has been determined by taking into account that in the pure collagen the percentage of carbon functionality deriving from peptidic groups is 21.75%. The graph in *Figure 5*, which reports the experimental collagen weight percentages as a function of the theoretical values, confirms an enrichment of collagen in the surface of the blends. Such enrichment is higher when lower concentrations of collagen are used in the initial blend (the diagonal line corresponds to the calculated surface composition).

In *Table 4* are reported the data concerning the SC–PVS blends. In this case, due to their low intensity, it is not possible to determine the area corresponding to photoelectronic peaks of sulfur atoms. In addition, it is impossible to calculate the hydrocarbon contamination because in the C_{1s} region the peaks of collagen overlap with those of the synthetic polymer. However, from consideration of the experimental N_{1s}–C_{1s} ratio it is reasonable to believe also that in the SC–PVS blends the content of collagen in the surface is higher than in the bulk.

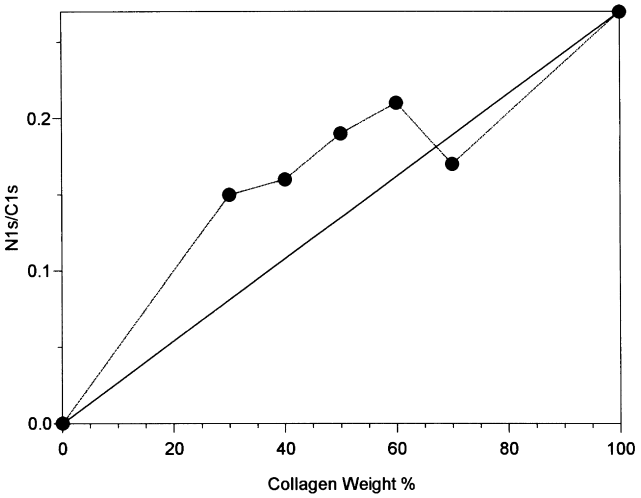

Figure 4 Surface composition from N_{1s}–C_{1s} ratios for PAA–SC blends

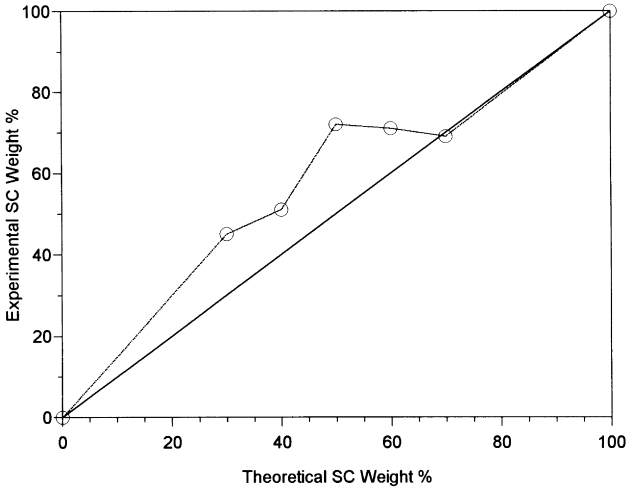

Figure 5 Surface composition of collagen in PAA–SC blends calculated from C_{1s} curve fitting

Table 4 Surface composition of SC–PVS blends

Sample	Calcd. O_{1s}/C_{1s}	Exper. O_{1s}/C_{1s}	Calcd. N_{1s}/C_{1s}	Exper. N_{1s}/C_{1s}
lb	0.93	0.33	0.15	0.20
2b	0.59	0.31	0.25	0.21
1c	0.36	0.31	0.31	0.23

Thermogravitnetric analysis (TGA) of the samples shows that the thermal behaviour of the blends is intermediate between those of the pure components and the scanning electron microscopy analysis (SEM) do not show any significant phase separation[24].

CONCLUSIONS

The results indicate, for all the investigated materials, an enrichment of collagen in the surface with respect to the initial percentage utilized to prepare the blends. Therefore a difference seems to exist between the bulk and the surface composition.

Since TGA and SEM indicate a good compatibility between the two components, the surface collagen enrichment could be explained with a loss of the other component during the sample preparation procedure. For example the treatment in acetic acid, performed to favour the complexation of the two components, could induce a variation in the blend surface composition.

Future work will be carried out to investigate if collagen migration toward the surface is a real phenomenon or not. However, if a real difference exists between the bulk and the surface composition, this should be taken into account in consideration of the potential biomedical applications of these materials.

In fact the success in implanting a foreign material in the human body depends on the interactions which occur between the material and the tissue components. These interactions depend mainly on the presence of the biocompound on the surface of the material. Therefore the investigation of the surface composition represents a very useful tool to evaluate any differences with the bulk and to establish the potential behaviour of the new material when interacting with a living system.

REFERENCES

1. Barbani, N., Lazzeri, L., Lelli, L., Bonaretti, A., Seggiani, M., Narducci, P., Pizzirani, G. and Giusti, P., *J. Mater. Sci: Mater. Med.*, 1994, **5**, 882.
2. Giusti, P., Lazzeri, L. and Lelk, L., *Trends Polym. Sci.*, 1993, **1**, 261.
3. Giusti, P., Lazzeri, L., De Petris, S., Palla, M. and Cascone, M. G., *Biomaterials*, 1994, **15**, 1229.
4. Giusti, P., Lazzeri, L., Barbani, N., Lelli, L., Petris, S. and Cascone, M. G., *Makromol. Chem. Macromol. Symp.*, 1994, **78**, 285.
5. Cascone, M. G., Disilvio, L., Sim, B. and Downes, S., *J. Mater. Sci.: Mater. Med.*, 1994, **5**, 770.
6. Cascone, M. G., Sim, B. and Downes, S., *Biomaterials*, 1995, **16**, 569.
7. Seggiani, M., Lazzeri, L., Cascone, M. G., Vitolo, S. and Palla, M., *J. Mater. Sci.: Mater. Med.*, 1994, **5**, 868.
8. Seggiani, M., Lazzeri, L., Giusti, P., Barbani, N., Lelli, L., Palla, M. and Taveri, M., *J. Mater. Sci.: Mater. Med.*, 1994, **5**, 872.
9. Lazzeri, L., Barbani, N., Lelk. L., Bonaretti, A., Centonze, P. and Giusti, P., *10th European Conference on Biomaterials*, Davos, Switzerland, September 8-11, 1993, Abs. P. 78.
10. Lazzeri, L., *Trends Polym. Sci.*, 1996, **4**, 249.
11. Giusti, P., Lazzeri, P. and Cascone, M. G., *Polymeric Materials Encyclopedia*, Vol. 1. CRC Press, Boca Raton, FL, 1996, p. 538.
12. Hagelstein, M., Valdor, M., Kaden, P., Richter, H. A. and Ifittermeyer, Ch. *11th European Conference on Biomaterials*, Pisa, Italy, September 10–14, 1994, Abs. P. 505.
13. Bienert, H., Lorenz, G., Richter, H. A., Hocker, H. and Mittenneyer, Ch., *12th European Conference on Biomaterials*, Porto, Portugal, September 10–13, 1995, Abs. P. 19.
14. Clark, D. T., *Handbook of X-ray and Ultraviolet Photoelectron Spectroscopy*, ed. D. Briggs. Hayden, London, 1977, p. 233.
15. Briggs, D., *Practical Surface Analysis*, Vol. 1, eds D. Briggs and M. P. Seah. Wiley, New York, 1990, pp. 437–438.
16. Cascone, M. G., Lazzeri, L., Barbani, N., Polacco, G., Pollicino, A. and Giusti, P., *J. Mater. Sci: Mater. Med.*, 1996, **7**, 297.
17. Cascone, M. G., Giusti, P., Lazzeri, L., Pollicino, A. and Recca, A., *J. Biomat. Sci. Polym. Ed.*, 1996, **7**(10), 917.
18. Clark, D. T. and Thomas, H. R., *J. Polym. Sci., Polym. Chem. Ed.*, 1977, **15**, 2483.
19. Seah, M. P. and Dench, W. A., *Surf. Interface Anal.*, 1979, **1**, 2.
20. Ratner, B. D., Florbett, T. A., Shuttleworth, D. and Thomas, H. R., *J. Colloid Interface Sci.*, 1981, **83**, 630.
21. Clark, D. T., Peeling, J. and Colling, L., *Biochim. Biophys. Acta*, 1976, **453**, 533.
22. Beamson, G. and Briggs, D., *High Resolution APS of Organic Polymers*. Wiley, New York, 1992, pp. 23 and 43.
23. Briggs, D. and Seah, M. P., *Practical Surface Analysis*. Wiley, New York, 1990, p. 543.
24. Barbani, N., Lazzeri, L., Cristallini, C., Cascone, M. G., Polacco, E. and Pizzirani, G., *J. Mater. Sci.: Mater. Med.* (in press).