Surface oxidation of polyethylene fiber reinforced polyolefin biomedical composites and its effect on cell attachment

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Three different compositions of butene–ethylene copolymer composites reinforced by polyethylene fibers and produced by filament winding are potentially suitable for biomedical applications. This study examines the effect of various processing and finishing conditions and of sterilization on the extent and composition of surface oxidation. An XPS analysis revealed only insignificant differences between the various treatments, while fibroblast cell attachment tests indicated good attachment with no signs of cytotoxity or cell degeneration for any of the materials.

1. Introduction

The advancement in orthopedic surgery is attributed, at least partially, to the continuous innovations in the field of implantable bioactive materials. The nature and degree of tissue response to implants depend on the characteristics of the material: chemical composition, surface texture, density, shape and size. Ultrahigh molecular weight polyethylene (UHMWPE) has found wide application as a load bearing material in the majority of joint endoprostheses, in combination with metal or ceramic counterparts. This undoubtedly results from its combination of good physical and chemical properties and acceptable biocompatibility [1–4]. It has very strong interatomic bonds and does not release toxic products *in vivo* [5]. Indeed, polyethylene (PE) polymers are inexpensive, easy to process and have good resistance to weathering. However, PE sterilization is not straightforward. It can be performed using steam, ethylene oxide (EtO) or high energy radiation (electron beams or gamma-radiation). A number of authors have attributed PE oxidative degradation to the sterilization process [2].

Surface chemistry and/or energy have been implicated in many aspects of cell physiology, including adhesion, proliferation and differentiation [6]. Knowledge pertaining to how surface chemistry and surface energy influence cell/surface interactions is essential for the development of agents to enhance or inhibit such interactions for tissue regeneration or biomaterials integration [7]. It is known that polyethylene oxide (PEO) containing triblock copolymers could be used to modify hydrophobic materials to generate protein and cell resistant substrates [8]. Anchorage, attachment, adhesion and spreading of cells require a surface that is not only non-toxic but also allows and favors this positive cell behavior [9].

It is difficult to examine the *in vivo* reactions of a specific cell type to the implant, because of various cell populations and biofactors present at the implantation site, therefore, *in vitro* models are used [10]. *In vitro* studies allow a more direct study of the biological response and cell/material interactions under controlled conditions. The performance of a biomaterial implant relies on the precise control of cellular interactions at the tissue/implant interface via an engineered biomaterial surface. The immediate goal of the engineered biomaterial surface is to provide a provisional matrix that promotes maximal tissue cell adhesion with minimum inflammatory cell reaction. Therefore, the critical initial design goal for a cell type-selective engineered biomaterial surface would be to minimize adsorption of biological fluid-borne proteins and formation of the non-selective, cell adhesive surface that promotes inflammatory responses [11].

Fibroblast cells are widely used cell types for the preliminary biomedical feasibility tests, for either live or dead assays, in order to test the toxicity of the material surfaces. It has also been shown that for these types of cells, their spreading is better on higher surface energy solids. Surface chemistry, or specifically the presence of certain functional groups, has been implicated in cellular attachment. In particular, surface hydroxyl, carbonyl, and carboxylate have been shown to affect cellular attachment. However, the relative effects of surface chemistry vs. surface energy are still the subjects of many debates in the literature. The study of cellular

response to specific surface functional groups requires surfaces of well-defined chemistry [7].

The objective of this work is to study the effect of processing and finishing conditions and of gas sterilization on the surface oxidation of filament wound flat strip composites of PE fiber reinforced polyolefins, destined for tendon or ligament prostheses. Then, using different ethylene–butene copolymer compositions, it is intended to investigate how the surface oxide layer affects fibroblast cell attachment and to obtain preliminary information on biological activity on the material surface [12].

2. Experimental

2.1. Materials

Composite materials were produced from Spectra 1000 UHMWPE fibers (Allied Signal) embedded in an ethylene–butene copolymer matrix of the Exact family (Exxon, Mobile). Three different copolymers were used, namely Exact 4041, 4011 and 4015 (the properties of a number of Exact copolymers were investigated in [13]). The copolymers were supplied in pellet form, from which 0.25 mm thick sheets were molded by pressing at 100 °C under a pressure of 625 MPa (Carver Laboratory Press), followed by removing them from the press and cooling in an ice-water bath. Filament winding was performed using a bench winder (Burlington Instruments Co., Vermont). A flat mandrel (2.5 mm wide, 0.5 mm thick and 135 mm long) was wrapped by a matrix film onto which the fiber was wound at a designated angle, and then wrapped by a second matrix film to produce a preform. The resulting preform was carefully removed from the mandrel and pressed at 100 °C under 15 MPa for 30 min. The specimens were then either left in the press for additional 60 min at 100 °C, followed by slow cooling to room temperature by turning off the heating (thermally treated, TT), or removed from the press and dipped in an ice-water bath (ice-water cooled, IWC). Specimens of three winding angles of 28°, 32° and 42° were produced at a fiber weight fraction of about 0.65. The final strips were 4 mm wide by 0.4 mm thick. Additional experimental details and pictures of the filament wound products are presented in Kazanci *et al*. [14].

A gas sterilization process using EtO was employed and carried out in the Hadassah Hospital Sterilization Unit (autoclave sterilization was ruled out because the required temperature was higher than the melting points of the copolymer matrices).

2.2. XPS analysis

X-ray photoelectron spectroscopy (XPS) tests were performed on samples of Exact 4015 composites in order to analyze the surface chemistry resulting from the various treatments. Four different treatments were examined with Exact 4015 composite samples, namely, (A) TT; (B) IWC; (C) TT sterilized; and (D) IWC sterilized. XPS measurements were performed on a commercial AXIS-HS Kratos setup, using a monochromatized Al (Ka) source (5 mA emission current at 15 KV) and pass energies of 20–80 eV. A flood gun was used for the neutralization of the surface. Data analysis included decomposition of the photoelectron lines into Gaussian–Lorenzian components superimposed on a Shirley background. Pronounced shifts of 3.2 eV and 3.0 eV were observed for the C lines. The detailed information about the XPS measurement technique could be found in the literature [15].

2.3. Cell culture

3T3 mouse fibroblast permanent line cells were used for the *in vitro* tests to examine cell attachment and spreading on composite samples of the three Exact compositions. The concentration of the cell culture was either 10^4 or 10^5 cells/10 µl of medium; a 10 µl portion was placed in each dish to completely cover the $5 \times 5\,cm^2$ composite sample. The cell density was optimized in preliminary experiments. Floating of samples in the medium had to be overcome because of the low density of PE fibers. Therefore, special attention was paid to seeding cells onto the samples and keeping the samples attached to the surface of the dish in the medium in order to prevent floating and drying of the cells. After seeding the cells on the samples, the dishes were left in the hood for about 20 min to attain an initial attachment, and then transferred to a 36.6 °C, 10% CO_2 incubator for cultivation for 24 h.

The specimens were prepared for scanning electron microscope (SEM) examination at predetermined periods to follow cell attachment and spreading process. First, the medium was pipetted out of the dishes. Then, the specimens were washed several times in phosphate buffered saline (PBS) and fixed with 2.5% glutaraldehyde for 1 h at room temperature and then overnight at 4 °C. The specimens were then rinsed with PBS, detached from the dishes and dried by Freon-113. After triple rinsing in 100% Freon, the specimens were mounted on stubs and dried. Thereafter, they were coated with 10 nm gold and viewed under a Philips 505 SEM operated at an accelerating voltage of 20 kV [9].

3. Results and discussions

3.1. Mechanical characterization

A previous paper [14], devoted to the elastic and viscoelastic characterization of the filament wound composites, emphasized the contribution of the matrix – in particular that of molecular branching. It showed that different selections of the reinforcement angle and of the matrix viscoelasticity, offered a wide scope of material parameter combinations, which by optimization could deliver a product of potential biomedical feasibility. In particular, the static elastic modulus and ultimate strength of the order of 3 GPa and 30 MPa, respectively, controlled by the PE fibers, and the softness and plausibility controlled by the matrix rendered it appropriate for tendon or ligament prosthesis.

3.2. X-ray photoelectron spectroscopy measurements

In general, XPS measurements are used to analyze the elemental composition of the outermost atom layer of the solids. XPS tests were conducted in this research in order

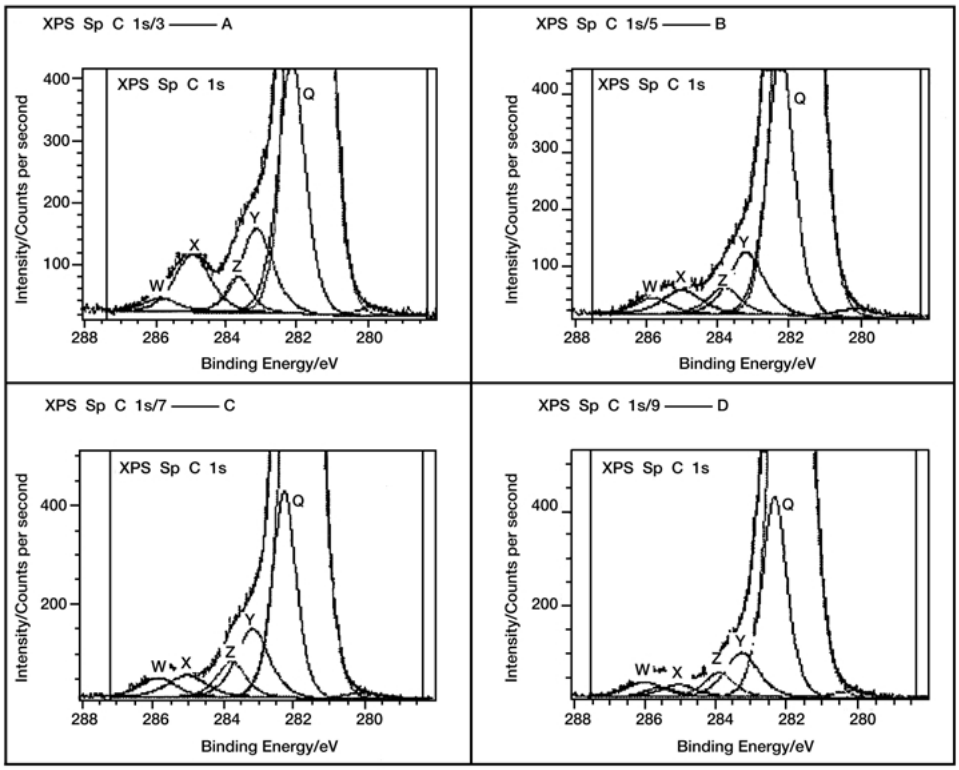


Figure 1 XPS data shown for C lines and its oxidation products for four different material treatment conditions.

to study how various processing conditions affect the surface oxidation of the composites, by determining the extent of oxidation and of its products. The results are presented graphically in Fig. 1 and numerically in Table I. The atomic concentrations of oxygen atoms were slightly higher for TT samples: A and C (14.7%) than the IWC samples B and D (13.8%). Regarding the oxidation products, their abundance is expressed by the atomic concentrations of the carbon atoms bonded to oxygen in methylene oxide, ethylene oxide, carboxyl, hydroxyl and ketone functional groups (XPS cannot distinguish between carboxylic acid and ester groups [6]). In general, the TT process is seen to produce slightly higher levels of oxidized carbon functional groups compared with the IWC process (with the exception of the methylene oxide concentration). Although, small differences in the total oxygen count and between the treatments can be detected, it is concluded that the extent of oxidation and its products are not affected by the different treatments.

3.3. Cell culture tests

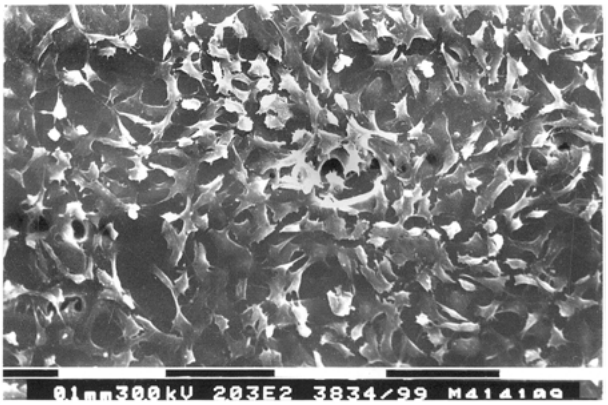
This test was conducted in order to evaluate the effect of different ethylene–butene copolymer compositions and different processing conditions on cell attachment, spread and well-being. Fig. 2(a)–(c) indicates that after a 24 h exposure, a good cell attachment was obtained. The cells were well spread, displaying a flat configuration, and colonized large areas of the substrate surface. They also maintained physical contact with one another through multiple extensions. When seeded in the higher density of 10^5 cells per substrate, they formed a multilayer sheet, as seen at a higher magnification in Fig. 3. They overlapped and superimposed one another, making it difficult to distinguish the borders of individual cells. No signs of cytotoxicity or cell necrosis and degeneration were evident throughout the experiment for all the samples. As PE based compositions are relatively inert in biological environments, the good cell attachment onto the PE-polyolefin composite observed here is considered encouraging. It is attributed mostly to the oxide layer formed on the composite surface, however, it could also result from the specific surface topography of the materials, resulting from the molding conditions.

TABLE I Atomic concentrations of carbon atoms bonded to oxygen in different functional groups: *Q*=*(C–O–C), W*=(COOH), *X*=(C=O), *Y*=(C–OH), *Z*=(C–C–O)

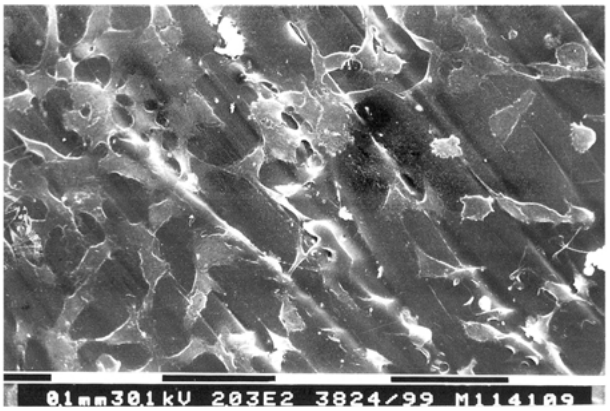
Treatment	*Q* (%)	*W* (%)	*X* (%)	*Y* (%)	*Z* (%)	Total (%)
A	14.4	1.0	4.5	5.8	1.9	27.6
B	15.3	2.0	2.0	4.8	1.8	25.1
C	14.1	2.4	2.4	6.5	2.9	27.8
D	15.0	1.3	1.3	4.5	2.1	24.5

4. Conclusion

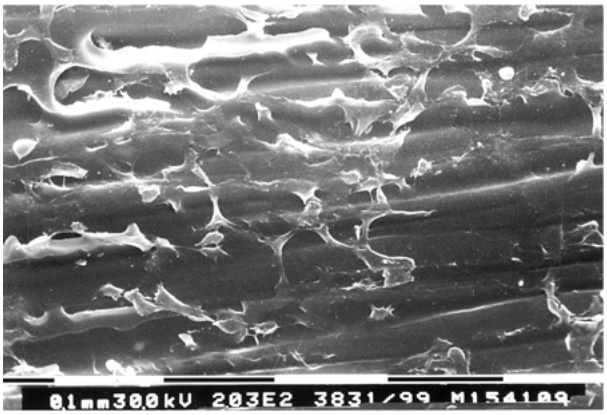
This research studied the interaction between a fibroblast cell culture and filament wound PE fiber reinforced ethylene–butene copolymer composites, destined for ligament or tendon prostheses. The total surface concentration of oxygen carrying functional groups and their nature were only moderately affected by the processing conditions. The *in vitro* cell culture tests indicated no signs of cytotoxicity or cell necrosis and degeneration, wherein the cells retained their typical broad and flattened morphology. This result corresponds



(a)



(b)



(c)

Figure 2 SE photomicrographs of composite samples after cell culture exposure: (a) Exact 4041; (b) 4011; (c) 4015.

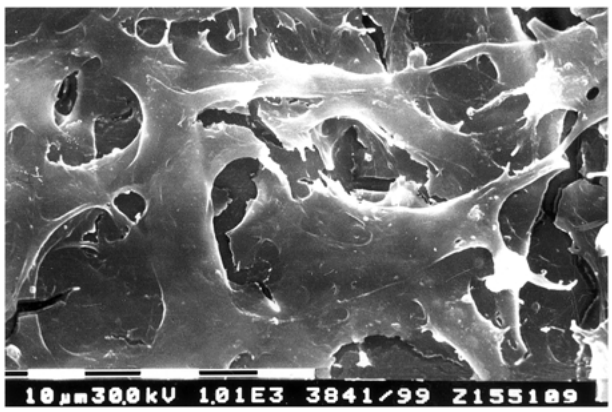


Figure 3 A higher magnification SE photomicrograph of an Exact 4015 sample after cell culture exposure.

to the relatively high oxygen surface concentration generated by the processing conditions.

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References

1. B. M. DALY and J. YIN, *J. Biomed. Mater. Res.* **42** (1998) 523.
2. L. COSTA, M. P. LUDA, L. TROSSARELLI, E. M. BRACH DEL PREVER, M. CROVA and P. GALLINARO, *Biomaterials* **19** (1998) 659.
3. C. JAMA, J.-D. QUENSIERRE, L. GENGEMBRE, V. MOINEAU, J. GRIMBLOT, O. DESSAUX and P. GOUDMAND, *Surf. Inter. Analysis* **27** (1999) 653.
4. M. DENG and S. W. SHALABY, *Biomaterials* **18** (1997) 645.
5. S. L. EVANS and P. J. GREGSON, *ibid.* **19** (1998) 1329.
6. R. M. FRANCE, R. D. SHORT, R. A. DAWSON and S. MACNEIL, *J. Mater. Chem.* **8** (1998) 37.
7. R. M. FRANCE, R. D. SHORT, E. DUVAL, F. R. JONES, R. A. DAWSON and S. MACNEIL, *Chem. Mater.* **10** (1998) 1176.
8. J. A. NEFF, P. A. TRESCO and K. D. CALDWELL, *Biomaterials* **20** (1999) 2377.
9. H. BEN-BASSAT, B. Y. KLEIN, E. LERNER, R. AZOURY, E. RAHAMIM, Z. SHLOMAI and S. SARIG, *Cells and Mater.* **4** (1994) 37.
10. H. BEN-BASSAT, B. Y. KLEIN, I. LEICHTER, M. LIEBERGALL, D. SEGAL, F. KAHANA and S. SARIG ''Biomaterials Engineering and Devices: Human Applications'' vol. 2, edited by D. L. Wise *et al.* (Human Press, Inc., Totowa, New Jersey) p. 155.
11. S. P. MASSIA, J. STARK and D. S. LETBETTER, *Biomaterials* **21** (2000) 2253.
12. O. J. OJI, J. V. WOOD and S. DOWNS, *J. Mater. Sci.: Mater. Med.* **10** (1999) 869.
13. D. FEIGELSHTEIN, H. HAREL, A. LUSTIGER and G. MAROM, *Advanced Composites Letters* **9** (2000) 349.
14. M. KAZANCI, D. COHN and G. MAROM, *J Mater. Sci.* (submitted for publication.)
15. I. WEISSBUCH, P. N. BAXTER, I. KUZMENKO, H. COHEN, S. COHEN, K. KJAER, P. B. HOWES, J. ALS-NIELSEN, J-M. LEHN, L. LEISEROWITZ and M. LAHAV, *Chem. Eur. J.* **6** (2000) 725.

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