Chemical and biological characteristics of low-temperature plasma treated ultra-high molecular weight polyethylene for biomedical applications

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Several low-temperature radio-frequency (RF) plasma surface treatments were performed on ultra-high molecular weight polyethylene (UHMWPE) used in biomedical applications. Process gases included Ar, C_3F_6, CH_4, hexamethyldisiloxane (HMDSO), and NH_4. These treatments were carried out at pressures in the range of 64–400 mTorr, RF powers of 240–1200 W, and temperatures well below the melting point of UHMWPE. X-ray photoelectron spectroscopy (XPS) was used to obtain information about the surface characteristics of UHMWPE treated with the HMDSO, C_3F_6, and CH_4 gases as a function of treatment conditions. XPS spectra of UHMWPE treated with C_3F_6 and CH_4 and exposed to a laboratory environment for different time periods were examined in order to assess the stability of these treatments. It was found that for the C_3F_6 process gas the amount of fluorine at the surface decreased over time, whereas the oxygen content of the CH_4 treated samples increased as a function of time. *In vitro* cytotoxicity of Ar, C_3F_6, CH_4, and NH_4 plasma treated samples was studied in light of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test results. The hemolytic nature of the various plasma treatments was evaluated using standard hemolysis tests. All of the samples tested in this study exhibited no cytotoxic and negligible hemolytic effects. The process parameters for several low-temperature plasma treatments demonstrating chemical and structural stability and good biocompatibility are discussed in conjunction with the broad applicability to other biomedical polymers.

1. Introduction

Polymers are used at an increasing rate in various biotechnology applications. The biomaterial surface at the focus of this work, ultra-high molecular weight polyethylene (UHMWPE), is the principal material currently used to replace damaged cartilage in total joint arthroplasty, or the surgical reconstruction of a diseased joint. In almost all medical applications, the surface behavior is critical to the performance of a polymer as a biomaterial. The chemical, biological, and mechanical surface properties are all important factors to consider when evaluating a potential biomaterial. The surface mechanical properties of UHMWPE have been studied extensively in recent years by several investigators [1–3].

The surface is what the body encounters first when a new device is used or implanted. The time scale for the tissue-biomaterial interaction may be on the order of several minutes for a one-time use device like a catheter, or up to several years for an implantable device like a total joint prosthesis or a cardiovascular graft. The size scales for these events range from the molecular level to the order of millimeters. Frequently, it is necessary to modify the surface chemically in order to enhance the performance of the bioimplant.

Low-temperature plasma surface modification is especially well suited for biomedical applications. Several investigators have documented reduced thrombogenicity associated with fluorocarbon monomer gas plasma treatments [4–6]. This result is particularly useful in the treatment of small diameter (less than 4 mm) vascular grafts that easily become occluded by blood clotting reactions [7]. Grafting polystyrene to polyethylene using low-temperature gas plasma has been reported to yield better adherence, viability and metabolic performance of attached rat hepatocytes (liver cells) [8]. Negligible cytotoxicity and hemolysis has been reported for poly(ethylene terephthalate) surfaces treated for improved functionality with argon and perfluorohexane plasmas [9]. Low-temperature plasma treatments have been used to promote the binding of heparin (an anticoagulant) to the surface of polyethylene [10]. Moreover, it has been demonstrated that high levels of CF_3 groups obtained after treatment with fluorocarbon plasmas yield improved binding of proteins [11], which may lead to the observed reduction in thrombogenicity.

There are several advantages to chemical surface modification by low-temperature plasma techniques. For example, the plasma surface treatment is conformal, and very complex geometries can be completely coated

because there is no line-of-sight requirement. Since plasma surface coatings are usually free of voids and pinholes [12], they can serve as barrier films limiting the diffusion of low molecular weight leachables into the body space. Films synthesized from various plasmas are relatively easy to obtain, and can be carried out as batch processes. A plasma surface modification step may also double as a sterilization step. For polymer devices in particular, low-temperature sterilization is critical as it eliminates endospore contamination without degrading the properties of the bulk polymer. In addition, these treatments use a very small amount of raw material, thus adding little cost to the manufacture of a particular device.

The main objective in the plasma surface treatments of this study was to improve the mechanical performance and biocompatibility of a polymer surface, specifically UHMWPE, by means of surface crosslinking, functionalization, and grading of the polymer surface at low temperatures. By appropriate selection of the plasma gas and treatment conditions, a particular conversion or modification can be obtained to address a particular problem or to benefit the polymeric component and the implant as a whole in various ways. While these surface changes are discussed, the focus of this paper is on the chemical stability and biocompatibility of several low-temperature radio frequency (RF) plasma treated UHMWPE surfaces that were developed in order to improve the wear resistance of the polymer. To assess the *in vitro* biocompatibility, several plasma treated UHMWPE samples were subjected to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cytotoxicity assay and hemolysis tests. X-ray photoelectron spectroscopy (XPS) studies were performed on selected plasma treated samples to determine the chemical composition and stability of the deposited films.

2. Experimental procedures

2.1. Plasma treatment equipment

The majority of the plasma treatments were carried out in a plasma reaction chamber (Oudenaarde, Europlasma CD400) of volume 400 mm^3 and maximum flow rate of 1 L/min. The power supply is a 13.56 MHz RF generator operating at powers up to 500 W. Some treatments were performed in an alternate chamber of work volume equal to 0.028 m^3, maximum flow rate of 0.4 L/min, and peak RF power of 1.2 kW.

2.2. Experimental materials

In each plasma process, three to six coupons of 0.9 cm^2 surface area and 2.5 mm thickness machined from medical-grade UHMWPE (GUR 4150) were sequentially polished, degreased, ultrasonically cleaned, and plasma treated. A typical plasma treatment consisted of the following steps. The samples were first subjected to a cleaning step to remove low-molecular weight impurities from the surfaces. Typically, the cleaning step was followed by a higher power density treatment, but in some cases a very high power cleaning step was used alone. In the second step, various process gases were used to crosslink the near-surface region of the UHMWPE specimens and to modify the chemical composition of the topmost layer. A schematic of the main process steps of a typical plasma treatment is shown in Fig. 1. Process parameters for selected treatments are given in tables included in each experimental section in order to clearly define which treatments were characterized, since some treatments may be quite similar and are difficult to differentiate by name. Hereafter, a treatment will be referred to by the main process gas, with the understanding that this refers to the treatment listed in that particular section. Most plasma treatment conditions yielded relatively low temperatures, well below the melting point of UHMWPE. In some of the highest power treatments, the amorphous phase of UHMWPE began to melt, but the bulk tolerances of these samples were unchanged after treatment (i.e., viscous flow did not occur).

2.3. X-ray photoelectron spectroscopy

In order to study the nature of the surface chemical environment on the plasma treated samples, XPS experiments were performed on two sets of plasma treated samples. XPS has been well documented as a highly successful tool for the analysis and chemical characterization of polymer surfaces [13, 14]. In this study, two sets of XPS experiments were carried out. First, simple survey scans were taken in order to obtain information about the atomic composition of the surfaces. The 1s peaks of the major elements present were scanned at a higher resolution (multiplex scan), and the obtained results were analyzed to determine the bonding environments of the elements, and, hence, the molecular character of the surface. In the second set of experiments, treated samples were exposed to air and scanned multiple times over several days to quantitatively document any changes in the surface character with time. The surface chemical analysis was performed to assure the presence of an altered chemical surface after plasma treatment. Survey scans were obtained for different plasma treatments, including C_3F_6, CH_4, and hexamethyldisiloxane (HMDSO)/O_2, and also untreated UHMWPE for comparison. The C_3F_6 and CH_4 treatments were examined in more detail over a two-week time period. Survey and multiplex scans were obtained within minutes of breaking the airtight seal, and then at intervals up to 12 days of air exposure.

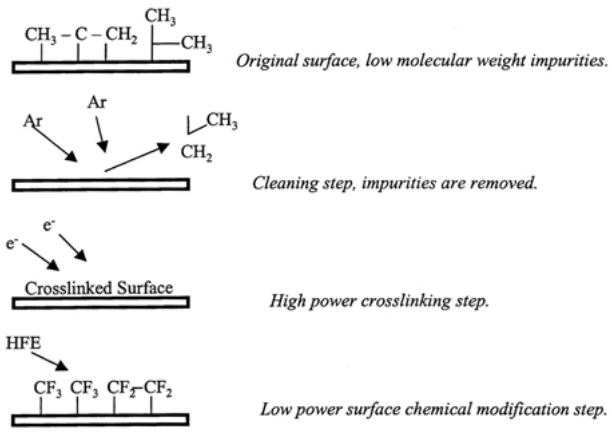

Figure 1 Schematic diagram of a typical surface modification process.

2.4. MTT assay

MTT is the abbreviated notation for tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. This salt is initially colorless and is transformed into a colored product by the activity of a living cell. It will remain colorless in the presence of dead cells and tissue culture medium [15]. The degree of color change can be detected by a spectrophotometer, and through careful calibration the number of living cells in a sample can be determined. The polyethylene samples were machined into coupons of uniform surface area. In general, the samples were mechanically polished prior to plasma surface treatment. It was assumed that the plasma treatment itself was sufficient to sterilize the samples. Since there was no problem with bacteria fouling, this assumption was justified. The sterilized samples were placed in separate wells of a 24 well plate. The samples were placed on a drop of 1% agar suspension and surrounded by agar to eliminate flotation. A 1 mL HeLa cell solution at a concentration of 3.4×10^5 cells/mL was added to each well and incubated at 37 °C. The HeLa cells were nourished with fetal bovine serum. Control wells containing only cells and no media were maintained in order to assess the general viability of the cell population. After incubating the plates for 24 h, the culture plates were retrieved and the non-agar wells were examined with a light microscope to establish cell viability. Subsequently, 0.1 mL of MTT solution was added to each well and the culture plates were returned to the incubator for 3.5 h. After the second incubation period, 1 mL of MTT solubilization solution (10% Triton X-100 plus 0.1 N HCl in anhydrous isopropanol) was added to each well and mixed thoroughly releasing the blue formazan crystals. The samples were left to sit for 15 min. The resulting solution from each well was combined with 1 mL distilled water and the absorbance was read at a wavelength of 570 nm using a spectrophotometer.

The MTT assay was used to evaluate samples treated with Ar, C_3F_6, CH_4, and NH_4 plasmas. These coupons (0.9 cm^2 surface area and 2.5 mm thickness) were packaged in an airtight container immediately following plasma surface treatment and were stored in a desiccator up to the day of testing. A summary of the detailed treatment process parameters for the MTT tested samples is given in Table I.

To evaluate the effect of surface finish on the treated samples, one half of the tested samples were polished to an arithmetic mean roughness $R_a < 0.125\,\mu m$, while the other half were left with their machined surface finish ($R_a \cong 2\,\mu m$). Latex was used as a negative control (expected to kill HeLa cells), and silicone was used as a positive control (expected to be non-toxic to the cells). For completeness, two additional controls were run. A test was performed using only the HeLa cells in the sample dish to test for overall cell viability, and a second test was run using only the cells on the agar substrate to verify that the substrate material was properly constituted. Each data point in the results presented below is the mean value of three test samples. MTT tests were also performed on freshly treated samples and on samples exposed to laboratory air environment for several days. The time dependence study involved tracking the cytotoxicity of several treatments over a period of 10 days. Since it is known that the plasma surface treatments evolve after exposure to the laboratory air, it was necessary to probe the cytotoxicity of the aged surfaces.

2.5. Hemolysis assay

The destruction of red blood cells is called hemolysis. During hemolysis, a blood cell breaks apart releasing hemoglobin, which is red in color and can be separated from the whole blood. The amount of released hemoglobin in a given sample is directly related to the number of lysed cells and can be measured using a spectrophotometer. Both positive and negative control solutions were used to calibrate the spectrophotometer. Water was used as the positive control. Placing cells in water causes them to swell and eventually break apart due to the drastic concentration gradient between the inside and outside of the cell. Since almost all of the cells break apart, the spectrophotometer reading will be high. The negative control is an isotonic saline solution, commonly called isoton. As discussed previously, this solution is isotonic to the cells, and they should not shrink or swell while resident in this solution. Few cells are expected to break apart, so the spectrophotometer reading is low. The hemolysis measurements for blood in contact with the sample materials are considered only in the context of the negative and positive controls, which are defined as 0 and 100% hemolysis, respectively. These controls are tested at the same time using the same whole blood.

For the experiments described here, whole pork blood was used for the hemolysis tests. The blood was obtained within two hours from slaughtering and was transported

TABLE I Plasma process parameters of samples tested with the MTT assay

Treatment*	Steps	Process gas	Time (min)	Power (W)	Flow (sccm)	Pressure (mTorr)
PT 64	1	Ar	15	483	100	203
PT 65	1	Ar	5	483	100	—
	2	CH_4	25	400	100	—
PT 66	1	Ar	5	483	100	202
	2	C_3F_6	25	435	100	104
PT 68	1	Ar	5	384	100	189
	2	NH_4	40	384	150	190

*All samples were sterilized in a gas permeable bag with H_2O_2 in Ar carrier gas after treatment for 45 min at 200 W power and 480 mTorr working pressure.

TABLE II Plasma process parameters of samples tested with the hemolysis assay

Treatment	Steps	Process gas	Time (min)	Power (W)	Flow (sccm)	Pressure (mTorr)
PT 81	1	Ar	5	391	100	83
	2	C_3F_6	25	240	150	64
PT 67*	1	Ar	5	384	100	189
	2	C_3F_6	40	384	150	142
PT 80	1	Ar	5	392	100	80
	2	C_3F_6	40	390	150	66
PT 79	1	Ar	5	490	100	83
PT 61†	1	Ar	25	500	100	—
PT 63†	1	Ar	5	500	100	—
	2	C_2H_4	20	400	250	—
PT 76	1	Ar	5	381	100	66
	2	NH_4	40	381	150	194
PT 75	1	Ar	5	481	100	69
	2	CH_4	25	383	100	—
PT 78	1	Ar	5	492	100	85
	2	HMDSO/O_2	6	492	140	156
PT 70	1	Ar	5	384	200	106
	2	HMDSO/O_2	6	384	180	134

*Sterilized in a gas permeable bag with H_2O_2 in Ar carrier gas after treatment for 30 min at 384 W power and 412 mTorr working pressure.
†EtO sterilized.

immediately to the laboratory for testing. The blood was incubated at 37 °C for ~ 1.5 h prior to testing. Positive and negative controls were run concurrently with all hemolysis tests. The negative control contained 10 mL of the isotonic solution and 0.2 mL of fresh, incubated pork blood in a test tube. The positive control was made by combining 10 mL of deionized water and 0.2 mL of blood. These tubes were incubated for another 30 min. The plasma treated samples were each submerged in tubes containing 10 mL of isotonic solution and 0.2 mL of blood. The plasma treatment conditions for the samples that underwent hemolysis testing are listed in Table II. These samples were also incubated for 1 h. After incubation, all samples were centrifuged at 2000 rpm for 10 min. The intact red blood cells settle to the bottom of the tube, leaving the contents of the lysed cells in the supernatant. A fraction of the supernatant (~ 10 mL) is removed and its absorbance is measured using a spectrophotometer with a wavelength of 545 nm. The spectrophotometer readings are converted to percent hemolysis values using the following equation:

$$\%\text{Hemolysis} = \frac{\text{sample} - \text{negative}}{\text{positive} - \text{negative}} \times 100$$

Sample, positive, and negative refer to the absorbance after 30 min of incubation of the plasma treated sample and the positive and negative controls, respectively [16].

3. Results

3.1. Chemical characteristics of plasma treated and untreated polyethylene

The survey scan of untreated UHMWPE (Fig. 2(a)) clearly shows the dominance of the C 1s peak and the presence of the O 1s peak at ~ 535 eV, which is an expected result of surface oxidation commonly observed in this material. The most prominent peak in the survey scan of the C_3F_6 treated sample is the F 1s peak (Fig. 2(b)). The O 1s and C 1s peaks are also distinguishable. The HMDSO/O_2 scan (Fig. 2(c)) reveals characteristic Si peaks in addition to the carbon and oxygen peaks. The Si 2p peak at ~ 102 eV is typical of the polysiloxane structure $(-OSiOR_2-)$ [13]. The intensities of all peaks in the HMDSO/O_2 scan are low, and this is most probably due to poor positioning of the sample in the vacuum chamber. In the scan of the CH_4 treated sample (Fig. 2(d)), the oxygen peak is much more prominent than in the other treatments. Oxygen is a common contaminant in CH_4 treatments, and this must be taken into consideration if surface oxidation is to be avoided. Since oxygen is not a significant contaminant in other treatments, it is postulated that the oxygen was adsorbed at the surface upon exposure to the atmosphere after plasma treatment.

The multiplex scan provides a more detailed insight into the bonding environment of each elemental component, and also determination of the relative amounts of the major surface elements, by calculating the relative signal intensities through a curve fitting routine. The multiplex scan of the C 1s peak of the C_3F_6 treated sample illustrated the presence of many sub-peaks. These peaks are attributed to the different bonding environments of different C atoms. The intensity and position of these sub-peaks can be discerned by using a curve fitting routine. Four distinct sub-peaks could be discerned for the C_3F_6 treated samples. These peaks were attributed to CF_3 (~ 294 eV), CF_2 (~ 292 eV), CF (~ 290 eV), and C–C or CH_x (~ 287 eV) bonds. The O 1s peak was examined at a higher resolution, but no sub-peaks were found, indicating that the oxygen was only involved in one type of bonding at the surface. Oxygen is probably binding with carbon, but the C–O binding energies may be obscured by the intense C–F bonds. Films produced using C_3F_6O as the starting gas have been reported to be free of oxygen [17].

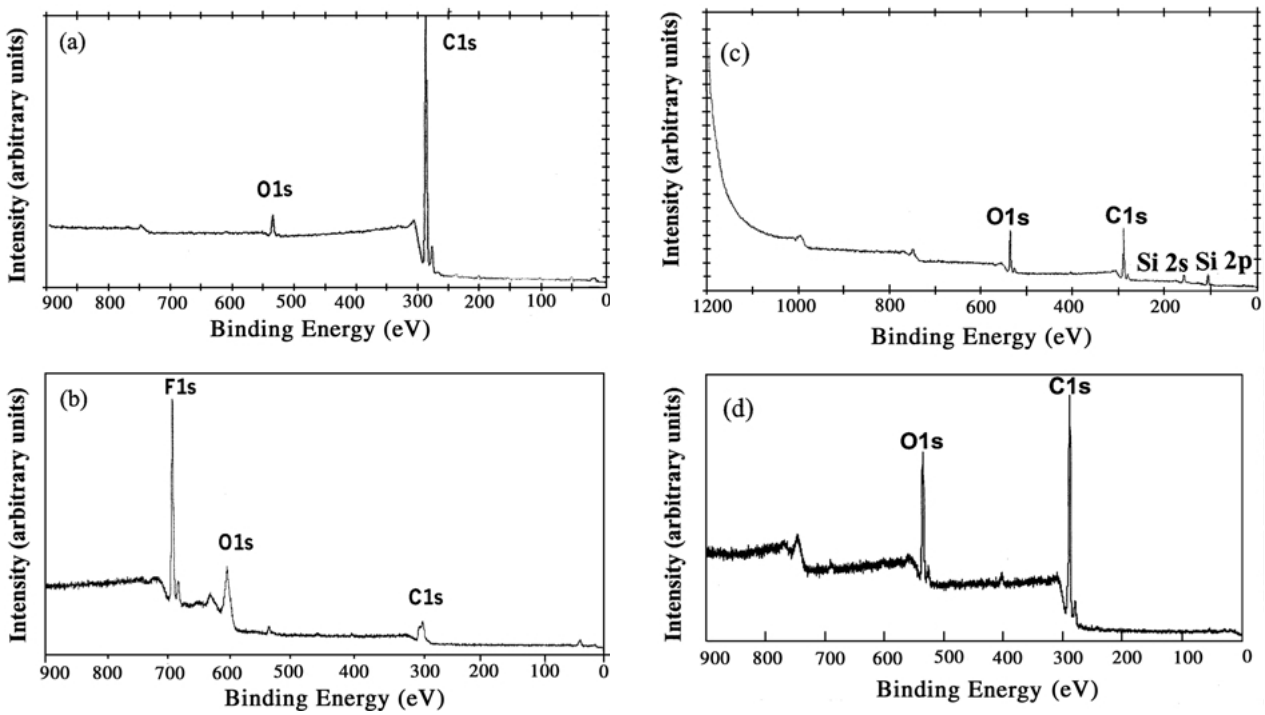

Figure 2 XPS scans of UHMWPE: (a) untreated, (b) C_3F_6 plasma treated (PT 66), (c) HMDSO/O_2 plasma treated (PT 70), and (d) CH_4 plasma treated (PT 65).

3.2. Time dependence of plasma treatments

The C_3F_6 and CH_4 treatments were examined in more detail over a two-week time period. Survey and multiplex scans were obtained within minutes of breaking the airtight seal, and then at intervals up to 12 days of air exposure. The surface chemistry of these plasma treatments did not remain constant over the two-week time period. A marked change in the surface character was observed with the C_3F_6 samples. The C/F and C/O ratios were monitored over the 12 day time period. Fluorine is expected on the modified surface, and oxygen is a known contaminant in these plasma treatments that often leads to oxidation of the plasma-polymerized films. The C/F ratio at the surface increased from 0.078 to 0.17 (Fig. 3(a)), while the C/O ratio decreased from 2.2 to 2.4 (Fig. 3(b)) over the 12 day time period. These results indicate a loss of fluorine and a gain of oxygen at the surface. The oxygen gain is quite small (it is within the error of the measurements) and could be attributed to oxidation of free radicals on the plasma-polymerized surface. A net loss of fluorine is commensurate with the qualitative contact angle observations for C_3F_6 treated samples [1]. The samples became less hydrophobic over time, indicating a loss of hydrophobic groups at the surface. A loss of fluorine would explain the evolution of such surface behavior.

The C 1s peak was examined in greater detail in order to obtain more information about the types of carbon–fluorine bonds. Under ideal experimental conditions, the C 1s peak is encountered at 285 eV. After accounting for shifts in the binding energies due to charging of the polymer and the presence of fluorine bonding, the highest C 1s bonding energy was assigned to the CF_3 group since the electrons in the CF_3 group are the most tightly bound. The next highest energy was assigned to the CF_2 groups and the lowest to the CH_x bonds. From these designations, it is possible to track the evolution of different molecular species on the surface. Over time, the number of CF_3 groups decreased from 20% to about 10% of the carbon bonds at the surface (Fig. 4(a)). The number of CF_2 groups decreased from 20% to 15% (Fig. 4(b)), while the number of CF groups appeared to increase from 22% to 27% over the 12 days in the laboratory air (Fig. 5(a)). An increase in the CH_x groups

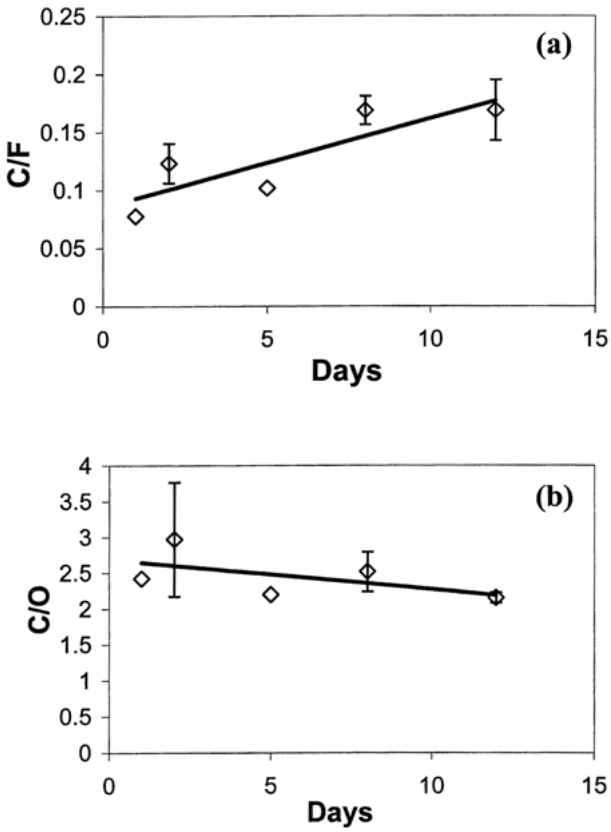

Figure 3 Variation of (a) C/F and (b) C/O ratios with time of exposure to ambient conditions for C_3F_6 plasma treated UHMWPE.

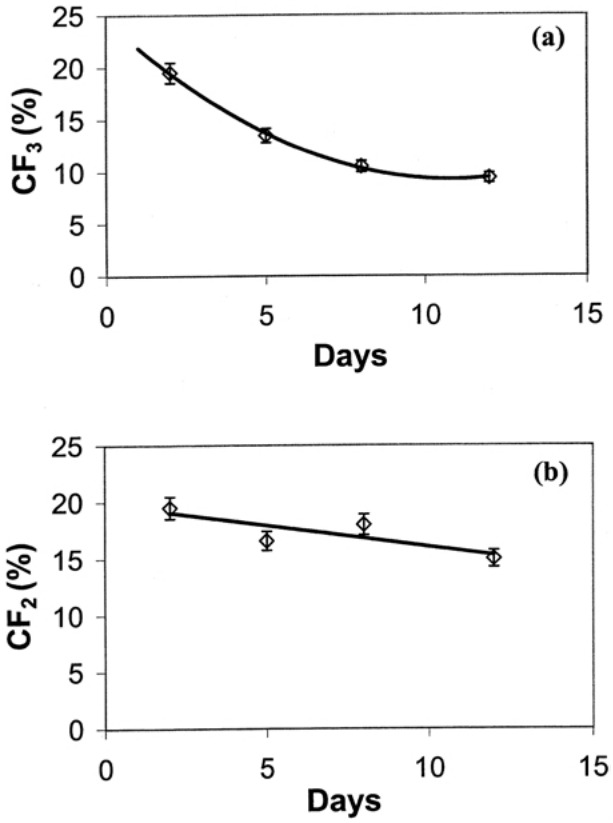

Figure 4 Percentage of (a) CF_3 and (b) CF_2 groups versus time of exposure to ambient conditions for C_3F_6 plasma treated UHMWPE.

from 39% to 49% was also observed (Fig. 5(b)). These findings are in agreement with qualitative observations. The CH_4 treatment also exhibited a change in the surface elemental character. The C/O ratio of these samples

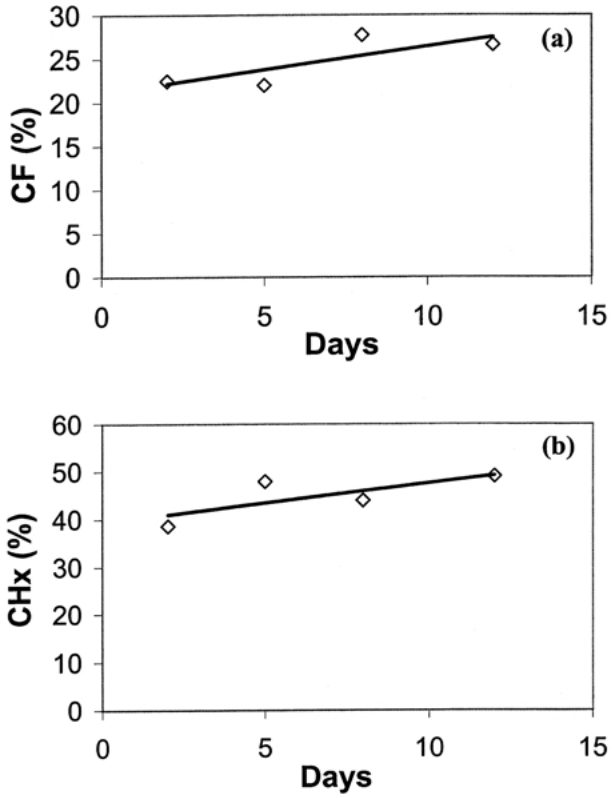

Figure 5 Percentage of (a) CF and (b) CH_x groups versus time of exposure to ambient conditions for C_3F_6 plasma treated UHMWPE.

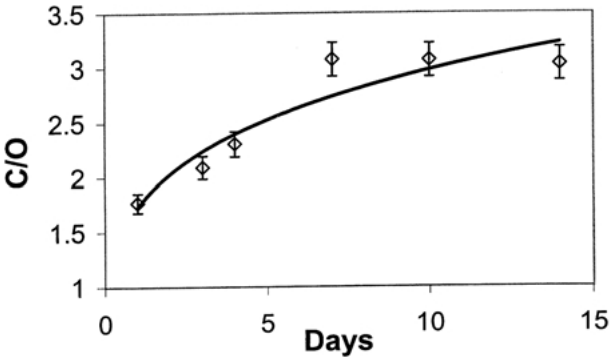

Figure 6 Variation of C/O ratio with time of exposure to ambient conditions for CH_4 plasma treated UHMWPE.

increased over the two-week period (Fig. 6). This result indicates that oxygen from the atmosphere was probably diffusing into the polymer from the surface.

3.3. MTT assay

The MTT assay was used to test samples that were freshly plasma treated, as well as to conduct a study of selected plasma treatments as a function of days exposed to laboratory air. Both polished and unpolished UHMWPE samples treated with Ar, C_3F_6, CH_4, and NH_4 plasmas were used in the MTT studies. Since there was no discernible difference in the behavior of polished and unpolished samples, as shown in Fig. 7, all the data are presented together regardless of surface finish. The results shown in Fig. 7 suggest that none of the plasma treated samples appeared to affect cell growth in a negative way. All samples had final cell counts greater than or equal to the positive control (silicone) material. A visual check of representative cells from each test well was made as a check of the MTT assay. None of the plasma treated samples performed statistically better than the positive control material in the MTT cytotoxicity tests.

The second round of experiments involved selecting three plasma treatments and performing the cytotoxicity assay after 1, 5, and 10 days in laboratory air. The plasma treatments chosen for this study were those performed with Ar, C_3F_6, and NH_4 processing gases. Again, only the HeLa, agar, and positive and negative controls were run. As shown in Fig. 8, none of the plasma treatments

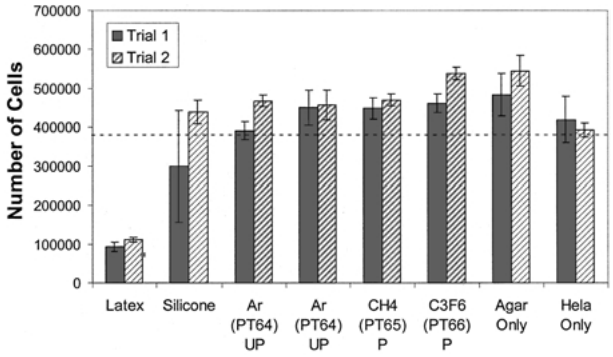

Figure 7 MTT results for selected plasma treated UHMWPE samples (P = polished; UP = unpolished). Two sets of three samples were used for each treatment. The dashed line represents the starting cell count.

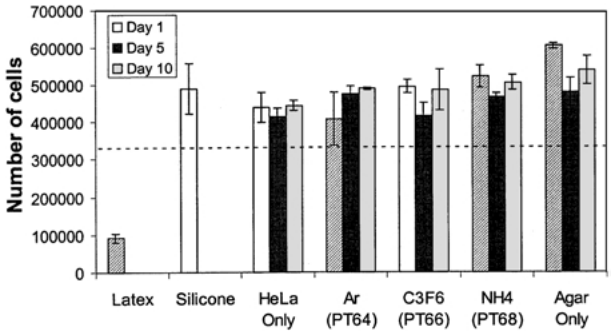

Figure 8 MTT results for plasma treated UHMWPE exposed to ambient conditions for 1–10 days. The dashed line represents the starting cell count.

yielded statistically different cytotoxicity results after exposure to the atmosphere for 1, 5, and 10 days. This data is encouraging, since it supports the hypothesis that even though the surface chemical character may change with increasing time of exposure to a humid or aqueous environment, the new surface moieties are nontoxic to the cells used in this study.

3.4. Hemolysis assay

Samples treated with Ar, C_3F_6, NH_4, CH_4, and HMDSO were analyzed using the hemolysis assay. Some of the samples were sterilized after treatment with ethylene oxide gas (EtO), which is known to be toxic to cells, for several weeks after treatment [18]. Samples sterilized in this manner were allowed to outgas for a period of time before implantation. These samples were placed in gas per-meable bags inside a desiccator for approximately 3 months prior to testing. The remaining samples were kept inside airtight bags in a desiccator prior to testing. Details of the plasma treatments and sterilization steps are given in Table II.

A minimum of three samples were tested for each treatment. The absorbance readings were averaged, and the standard deviation was obtained. Since a direct calibration was not possible for whole pork blood, there was a measurement error associated with each set of positive and negative controls. To obtain the experimental error, the error of each of the controls was combined with the error of each set of experimental samples.

Fig. 9 shows that all of the C_3F_6 treatment variations

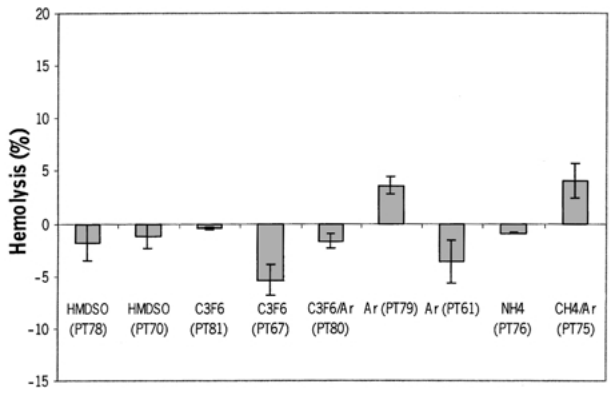

Figure 9 Percent hemolysis for plasma treated UHMWPE after contact with pork blood for 1 h.

(with and without Ar cleaning, and with and without EtO sterilization) yielded negative hemolysis results. The HMDSO and NH_4 treated samples also resulted in negative percentage hemolysis values. These results are within experimental error, and indicate that none of these treatments affects the viability of pork red blood cells. Samples treated only with Ar and Ar/CH_4 plasmas yielded positive percentage hemolysis values that were statistically higher than the negative control sample. All of these measurements were less than or equal to 5% hemolysis, except for one set of Ar treated samples, which had a measurement of 12% hemolysis. These values are all very low, and indicate that very little red blood cell destruction occurred on any of the plasma treated sample surfaces.

4. Discussion

The surface spectra of the plasma treated polymers revealed the presence of various surface groups. An interesting result is the incorporation of large amounts of oxygen into the CH_4-treated polymers, while there is little oxygen contamination in the C_3F_6-treated polymers. Other investigators that used C_3F_6O as a starting gas also noted that oxygen was not incorporated into the films [17]. The electronegativity of fluorine is higher than the electronegativity of oxygen, and it may preferentially bond with carbon both in the plasma and on the substrate, thus allowing oxygen to bond with displaced hydrogen and leave the chamber as water vapor. In the methane plasma, this competition does not exist, and the oxygen is readily incorporated into the surface.

There are several hypotheses to explain the transient behavior of plasma surface treatments of polymer substrates. If a sample is highly hydrophobic and must reside in a humid atmosphere or an aqueous solution, there will be a high thermodynamic tendency for the surface to reduce its energy by eliminating the water contact [19]. Bond rotation about the polymer backbone to place the hydrophobic group in the bulk is one possible mechanism. Grafted polymer branch structures may fold up in an attempt to eliminate the contact with polar species. The other mechanism is chain reptation. Through vibrational, rotational, and translational motion, a lower molecular weight polymer chain might bury itself into the bulk material. Both of these mechanisms have been observed in previous studies. For example, the initially high water contact angles of polystyrenes with perfluorosilane end groups decrease as a function of time when exposed to water vapor. The opposite trend was found when the same films were exposed to a non-polar argon gas environment [19]. This result is clearly due to rotation of the polystyrene backbone. It is almost certain that this type of segregation occurred in the fluorocarbon treated surfaces.

The changes in the CH_4-treated surfaces are more likely due to adsorption of oxygen onto the polymer surface from the atmosphere. Over time, oxygen from the atmosphere is known to oxidize the UHMWPE surface. This process is accelerated by high-energy sterilization techniques, such as gamma radiation, that create free radicals near the UHMWPE surface [20, 21]. The result

after years from post radiation is a severely damaged surface layer of thickness up to several millimeters. It is not unreasonable to expect that some free radicals may be present on the surface of the plasma treated polymers, and that progressive surface oxidation may proceed in this way.

In summary, none of the plasma surface treatments significantly affected the two cell types (HeLa and pork red blood cells) used in this study. This result is positive, and leads the way for more specific biocompatibility testing. *In vitro* biocompatibility tests provide a means of screening potentially useful surface treatments and materials for use in the body. Only after subsequent successful and repeatable testing using animal models can a material be considered as a candidate for human trials. The cytotoxicity and hemolysis tests described here represent a small fraction of the range of tests necessary to qualify a material for use in clinical trials.

Blood compatibility is a very complex concept, consisting of much more than non-hemolytic behavior. The thrombogenic pathway can be triggered in a variety of ways, and it is important that a new blood contacting material is fully tested *in vitro* before tests on mammals are performed. Although the surface roughness differences examined in this study showed no difference in cytotoxicity, adherent cells are known to behave differently on surfaces with geometric patterns of their size scales. HeLa and pork red blood cells are not adherent cells, and therefore are not expected to behave differently in the presence of micrometer sized surface features.

5. Conclusions

The surface chemical behavior and biocompatibility characteristics of UHMWPE subjected to low-temperature plasma treatment with Ar, C_3F_6, CH_4, HMDSO, and NH_4 gases were investigated in light of XPS, *in vitro* cytotoxicity, and hemolysis results. In view of the presented results and discussion, the following main conclusions can be drawn:

1. Plasma treatment with fluorocarbon gases, such as C_3F_6, produces a surface chemical state characterized by the dominance of CF_3, CF_2, CF, and C–C (or CH_x) bonds. Oxygen contamination was prominent only in the CH_4 treatments.

2. Significant changes in the surface chemistry of the C_3F_6 and CH_4 plasma treated polyethylene surfaces occurred with the time of exposure to the ambient conditions. A loss of fluorine and a gain of oxygen were observed with time, thus decreasing the surface hydrophobicity. The percentages of CF_3 and CF_2 groups decreased and those of CF and CH_x groups increased with time.

3. Despite changes in the chemical behavior of the plasma treated surfaces with time of exposure to ambient conditions, cytotoxicity results demonstrated that the produced surface moieties are nontoxic to the cells.

4. Hemolysis tests revealed that plasma treatment with C_3F_6, HMDSO, and NH_4 yielded negative results as opposed to treatments with Ar and Ar/CH_4 that yielded positive results. However, the overall effect on the two cell types examined was marginal.

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