In Vitro Monocyte Adhesion and Activation on Modified FEP Copolymer Surfaces

A. AZEEZ,[1] J. YUN,[2] K. DeFIFE,[2] E. COLTON,[2] L. CAHALLAN,[3] M. VERHOEVEN,[3] P. CAHALLAN,[3] J. M. ANDERSON,[1,2] and A. HILTNER[1,*]

[1]Department of Macromolecular Science and Center for Applied Polymer Research and [2]Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106, and [3]Medtronic Bakken Research Center, Maastricht, The Netherlands

SYNOPSIS

The functional group content and the ionic state of functional groups present on a series of surface modified poly(tetrafluoroethylene/hexafluoropropylene) (FEP) copolymers were characterized by electron spectroscopy for chemical analysis (ESCA), contact angle, and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Additionally, after a protein was preadsorbed on these surfaces, *in vitro* cell (monocyte) adhesion and activation were analyzed. The two proteins in this study were fibrinogen and immunoglobulin-G (IgG). Four modified FEP surfaces were prepared with increasing concentration of carboxyl groups relative to amide groups; ESCA was used to quantify the functional group content. To characterize the ionic state of the functional groups at physiological pH (7.1), the ATR-FTIR spectra were collected at various pH levels. Collectively, the contact angle, ESCA, and ATR-FTIR results suggested that the amide groups were unprotonated and the carboxyl groups were ionized at the physiological pH. The results from the *in vitro* studies showed that on the fibrinogen preadsorbed surfaces, monocyte adhesion was higher and monocyte activation was lower on the three surfaces that contained carboxyl groups compared to the FEP surface that had only amide groups. Conversely, the results indicated that the surface chemistry had no significant effect on monocyte adhesion or activation on the IgG preadsorbed surfaces. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

The surface characteristics of a synthetic polymer play an important role in determining the biocompatibility of the material.[1] There are several factors that define the surface properties of a material, including the functional group content, surface charge, and hydrophobic/hydrophilic character of the surface.[2-5] Therefore, one approach to improved biocompatibility of synthetic polymer surfaces has been to modify the surface chemistry and investigate the *in vivo* and *in vitro* response.

One of the most common and effective ways of controlling the surface chemistry of synthetic biomedical polymers is to alter the functional group content of the surface. However, the ionic state of functional groups attached to polymer surfaces can vary with the pH of the surrounding environment.[6-8] Holmes-Farley et al. have demonstrated pH dependent changes in the ionization state of carboxyl groups on surface modified polyethylene (PE) by contact angle and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR).[7,8] If a surface has more than one type of functional group, the ionic state of these groups may differ at a particular pH as determined by their pK_a's. In other words, the ionic state of functional groups present on the surface of a polymer at a particular pH, is dependent on the pK_a of the respective functional groups. The ionic state of functional groups on the surface of biomedical polymers is especially important at the physiological pH.

The implantation of a synthetic polymer in the body provokes an inflammatory response that is de-

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 58, 1741-1749 (1995)
 CCC 0021-8995/95/101741-09

pendent on the properties of the material.[9] Initially, protein adsorption occurs. Studies have shown that protein adsorption and conformation are surface chemistry dependent.[10-13] Protein adsorption is followed by the adhesion of white blood cells (neutrophils, monocytes, etc.).[9] These white blood cells can attract additional cells at the implant site by releasing chemicals known as cytokines.[14] It has also been reported that adhesion and activation of cells depends on protein adsorption and conformation.[10,14] Therefore, cell adhesion and cell activation ultimately depend on the polymer surface chemistry.

This article describes the characterization of the amide and carboxyl group content and the ionic state of these groups on a series of surface modified poly(tetrafluoroethylene/hexafluoropropylene) (FEP) copolymers. In addition, *in vitro* monocyte adhesion and activation were analyzed when these surfaces were preadsorbed with protein. Monocyte activation was measured in terms of the cytokines (IL-1β, IL-6, and TNF-α) released per cell.

EXPERIMENTAL

Surface Modification Scheme

Untreated FEP copolymer films were obtained from Minnesota Plastics Inc. These materials were selected as the substrate because of their inert character.[15] Subsequent chemical grafting procedures then introduced functional groups on the surface. Results of the biological assays could then be directly correlated to these functional groups. Methods for surface modification of the FEP surfaces to introduce carboxyl and amide functional groups on the surface were previously described by Yun et al.[16]

The untreated films were cleaned with acetone and dried at 60°C. The surfaces were then tetra-etched, soap water cleaned, rinsed with distilled water, and dried at 60°C for 20 min. The dried surfaces were then treated by corona discharge at 0.52 kW. To obtain all amide groups on sample A1, a grafting solution of cerium ion (Ce^{+4}) and 40% by weight of acrylamide monomer was used to treat the FEP surface. The functional group distribution of sample A2 was obtained by using the same grafting solution that was used for sample A1, but the surface was also hydrolyzed for 5 h at 60°C in a $NaHCO_3$/NaOH solution at pH 10.5. Sample A3, which has approximately equal amounts of carboxyl and amide groups, was obtained by using a solution of Ce^{+4} ions and a 50 : 50 ratio by weight of acrylamide/acrylic acid monomer. Finally, to obtain a majority of carboxyl groups on the surface of A4, a solution of Ce^{+4} ions and only acrylic acid monomer was used to treat the FEP films.

Surface Characterization

Electron spectroscopy for chemical analysis (ESCA) was conducted for each modified FEP surface. A resolution of 1 eV was used on a SSI-M-probe spectrometer with an aluminum monochromatic x-ray source. The resulting high resolution C spectra were deconvoluted using Gaussian curves at specific characteristic peak positions.

The contact angles were measured by the sessile drop method using deionized distilled water at a pH of 5.6. The surfaces were thoroughly rinsed in deionized distilled water (pH 5.6) for 5 min and then dried overnight in a vacuum at room temperature. The advancing and receding contact angles were measured ($n = 3$) with a goniometer. The advancing contact angle was measured with five consecutive drops of deionized distilled water and then the receding contact angle was measured by removing one drop at a time from the surface.

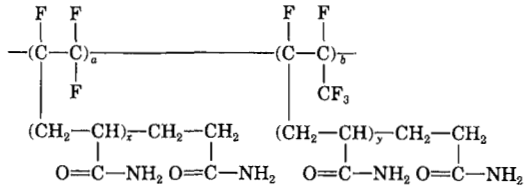
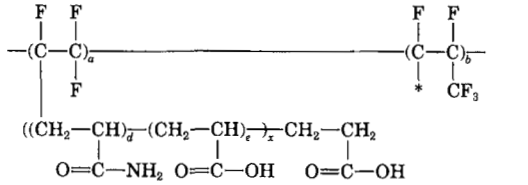
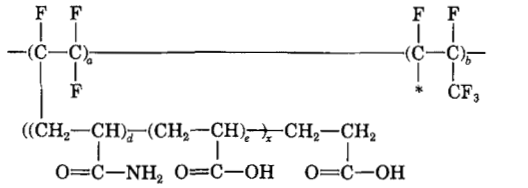
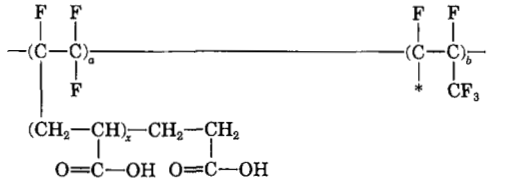
The ATR-FTIR spectra were collected at five pH levels: 0.15, 5.6, 7.1, 9.6, and 12.0 ($n = 3$ at each pH). The surfaces were treated for a period of 10 min and then cleaned by using filter papers that were equilibrated to the same pH. For a pH of 0.15, the surfaces were treated with 1*N* HCl; for a pH of 5.6, deionized distilled water was used; a pH of 7.1 was achieved by using a phosphate buffer solution (PBS buffer); a pH of 9.6 was obtained by titrating a solution of 1*N* HCl and 1*N* NaOH; and a solution at pH 12.0 was prepared by using a 0.1*N* solution of NaOH. The resulting surfaces were dried in vacuum overnight at room temperature and then the ATR-FTIR spectra was collected using a germanium crystal with a 60° incidence angle.

The region of interest in the spectra was the carbonyl region between 1800 and 1500 cm^{-1}. Deconvolution of overlapping peaks was accomplished by transferring the digitized ATR-FTIR spectra to a curve fitting program (Peakfit®, Jandel Scientific Inc.). The spectra were then curve fitted using Gaussian curves at specific peak positions with a shift range of ±5 wavenumbers. The deconvoluted curves resulted in a good fit with the observed spectra (χ^2 values $< 6.0 \times 10^{-4}$ and R^2 values > 0.9). A C—F stretch peak at 1155 cm^{-1} was selected as the normalizing peak in all the spectra.

Monocyte Adhesion and Cytokine Production Assays

Details of the protein preadsorption procedure, monocyte adhesion, and cytokine production assays

Table I Structural Information, Contact Angle Data, and ESCA Data

Name Modified FEP Surface	Relevant Information	Structure	Contact Angle		ESCA Data				
			Advancing[a]	Receding[b]	%—C(=O)—NH_2	%—C(=O)—OH	% COC	% CN	% C—C, C—H
A1	Neutral; no COOH Groups		37 ± 3 – 43 ± 4	11 ± 3 – < 10	15	—	4	9	72
A2	Anionic; low concentration of COOH groups compared to $CONH_2$ groups ($COOH \ll CONH_2$)	 $d \gg e$	27 ± 0 – 27 ± 1	< 10	20	3	—	9	68
A3	Anionic; equal COOH and $CONH_2$ groups ($COOH = CONH_2$)	 $d = e$	32 ± 1 – 37 ± 3	< 10	11	9	—	11	68
A4	Anionic; all COOH groups		57 ± 2 – 66 ± 3	32 ± 4 – < 10	4	15	4	6	71

* = same substituent as on FE portion of polymer.

[a] Drop 1–drop 5, mean ± SD, $n = 3$.

[b] Drop 4–last drop, mean ± SD, $n = 3$.

Table II Functional Groups on Modified FEP Surfaces at pH 7.1

FEP Surface	Functional Groups from ESCA (Based on 100)		Calculated Ionization State of Functional Groups at pH 7.1 (Based on 100)		
	Carboxyl	Amide	CO_2^-	COOH	$CONH_2$
A1	0	100	0	0	100
A2	13	87	11.2	1.8	87
A3	45	55	39.0	6.0	55
A4	79	21	68.2	10.8	21

have been described elsewhere.[16] Briefly, the surfaces were equilibrated in a PBS solution for 1 h at room temperature and then preadsorbed with fibrinogen or immunoglobulin-G (IgG) for 2 h at 37°C. Monocytes were isolated from human blood and seeded in culture wells containing the protein preadsorbed samples. After a 24-h incubation in 5% CO_2 at 37°C, the culture plates were centrifuged for 5 min to remove nonadherent cells and the supernatant was harvested ($n = 3$). The monocytes adhered on the polymer surfaces were stained and counted using an Olympus BH-2 microscope and a JAVA image analyzer. Cytokines (IL-1β, IL-6, and TNF-α) released in the supernatant were quantified by direct enzyme-linked immunosorbant assay (ELISA) (R&D Systems, Minneapolis, MN).

RESULTS AND DISCUSSION

Surface Characterization

Functional groups on the modified FEP surfaces were identified by ESCA (Table I). The ionizable groups were carboxyl and amide groups; the relative amount of these groups on each surface is expressed as the ratio of carboxyl groups to the total carboxyl and amide groups (Table II). As expected, the results showed that sample A1 has only amide groups and that the carboxyl groups relative to amide groups increases from sample A2 to sample A4 that has a majority of carboxyl groups and only trace amounts of amide groups.

The results of the sessile drop water contact angle study are summarized in Table I. The receding contact angles indicated that all the surfaces were wettable with a contact angle $\theta < 10$. The advancing contact angle varied with surface modification but did not show a consistent trend with carboxyl group content. However, there did seem to be a correlation between contact angle and the number of amide groups. Surfaces with higher amide group content were more wettable than the surfaces with less amide groups. Sample A2 had the most amide groups and was also the most hydrophilic, and sample A4 had the fewest amide groups and was the least hydrophilic.

To determine the ionization state of the carboxyl and amide groups, ATR-FTIR spectra were collected from surfaces treated at pH levels of 0.15, 5.6, 7.1, 9.6, and 12.0. The region of interest in these spectra was the carbonyl region from 1800 to 1500 cm^{-1}. The peaks in this region were assigned to protonated and hydrogen bonded carboxyl groups, protonated and nonhydrogen bonded carboxyl groups, ionized carboxyl groups, and amide groups (Table III).

The carbonyl region in the IR spectra of each surface at pH 7.1 is shown in Figure 1. The deconvoluted spectra show that sample A1 has only amide peaks, samples A2 and A3 have both amide and carboxyl peaks, and sample A4 has only carboxyl peaks. Furthermore, Figure 1 shows that the ionized carboxyl peak (1550 cm^{-1}) is significantly larger than the protonated carboxyl peak (1709 cm^{-1}) on samples A2, A3, and A4. This suggests that at pH 7.1 most of the carboxyl groups are ionized.

To observe changes in the ionization state of the functional groups, the ATR-FTIR spectra of sur-

Table III IR Peak Assignments Used in Deconvolution of Spectra

Group	Frequency (cm^{-1})
CO_2^{-1} asymmetric stretch	1550
N—H deformation, primary amide	1602
C=O stretch, primary amide	1660 and 1680
C=O stretch, protonated and hydrogen bonded carboxyl group	1710
C=O stretch, protonated carboxyl group	1730

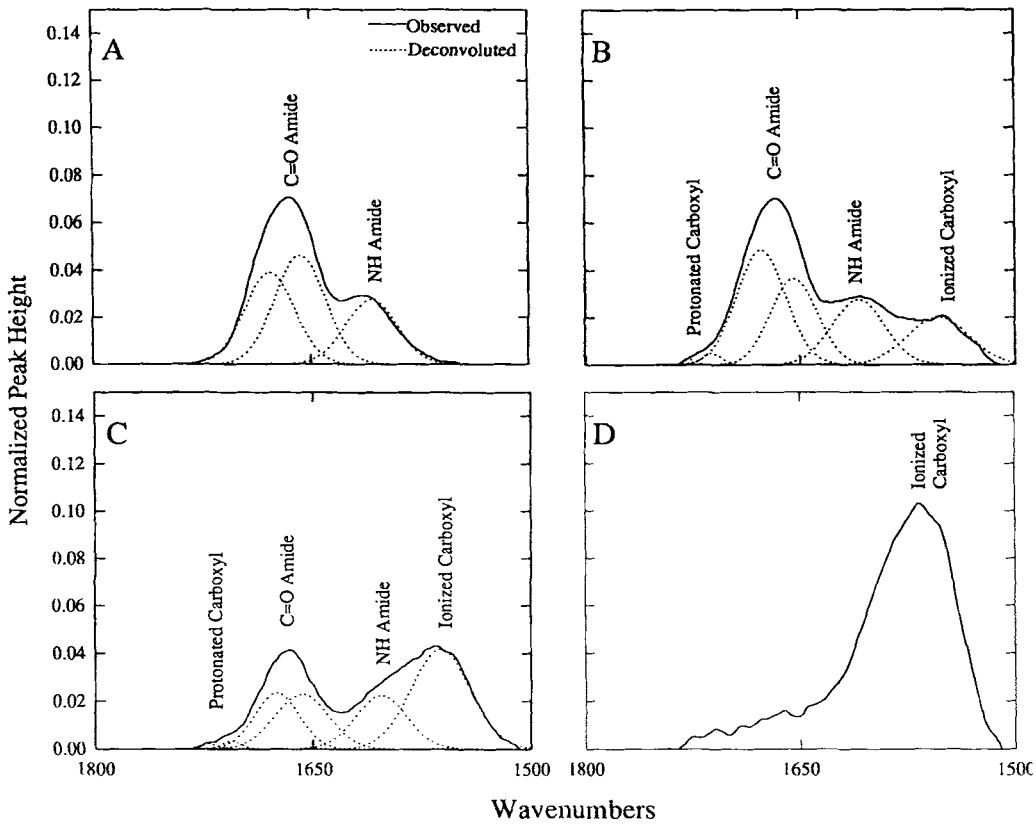


Figure 1 Infrared spectra at a pH of 7.1: (A) sample A1, (B) sample A2, (C) sample A3, and (D) sample A4. (—) Observed spectrum; (· · ·) deconvoluted spectrum.

faces exposed to different pHs were compared. Figure 2 shows the spectra of sample A4 collected at four pH levels: 0.15, 5.6, 7.1, and 12.0. The spectra show a large decrease in the peak of the ionized carboxyl (1550 cm^{-1}) and a corresponding increase in the protonated carboxyl peak (1709 cm^{-1}) between pH 7.1 and 5.6.

To estimate the pK_a of the carboxyl groups, the normalized peak height of the 1550 cm^{-1} absorbance was plotted as a function of pH for samples A2, A3, and A4 (Fig. 3). A sharp transition was observed in the ionization state of the carboxyl groups on A4 at a pH of 6.3. A transition was also evident at the same pH with A3 and possibly with A2, although this transition became less distinct as carboxyl content decreased (Fig. 3). Apparently, the presence of amide groups on samples A2 and A3 did not affect the pK_a of the carboxyl groups. It is probable that the amide and carboxyl groups are far enough apart that interactions between the functional groups are minimized. A pK_a value of 6.3 is consistent with the pK_a range (5.6–7.4) reported for polyacrylic acid.[17,18]

Figure 4 shows the spectra of sample A3 collected at pH levels 0.15, 5.6, and 7.1. The spectra show a large increase in the peak of the ionized carboxyl (1550 cm^{-1}) and a decrease in the protonated carboxyl peak between pH 5.6 and 7.1. The pH treatment was found to be reversible and the intensities returned when the pH was subsequently decreased. The normalized peak height for the amide NH deformation (1602 cm^{-1}) is plotted as a function of pH for samples A1, A2, and A3 in Figure 5. This peak did not undergo a transition like that observed for the carboxyl groups. The amide groups can be expected to be unprotonated over the pH range investigated in this study.[19] As confirmation, IR bands characteristic of the protonated form were not observed.[20] With the experimentally determined pK_a of the carboxyl groups, the ratio of ionized to protonated carboxyl groups was calculated at the physiological pH of 7.1. The results, included in Table II, indicate that the carboxyl groups are primarily (85%) in the ionized state under physiological conditions.

In summary, the ATR-FTIR spectra indicate that the carboxyl groups on samples A2, A3, and A4 have a pK_a of approximately 6.3. Furthermore, the IR results suggest that the amide groups on A1, A2, and A3 are unprotonated in the pH range of 0.15–12.0. It is now possible to understand the contact angle

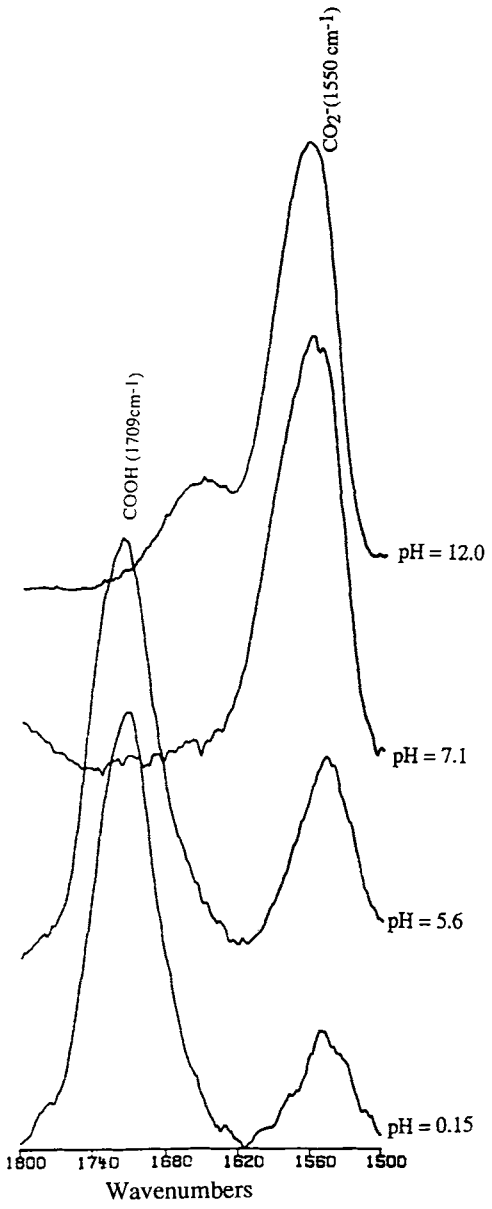


Figure 2 Infrared spectra of sample A4 at different pH levels.

data. The carboxyl groups did not impart a large increase in the hydrophilic character of the surfaces because they were protonated at the pH of the deionized distilled water used in the contact angle experiments (pH 5.6). However, at the physiological pH of 7.1, the carboxyl groups are largely ionized and hence there should be significant differences in the hydrophilicity of the surfaces.

Monocyte Adhesion and Activation

The results of the monocyte adhesion assays on fibrinogen and IgG preadsorbed surfaces are shown in Figure 6. This plot shows the mean monocyte adhesion as a function of the total carboxyl group content as determined from ESCA characterization. The measured cytokine (IL-1β, IL-6, and TNF-α) release was normalized to the mean number of monocytes adhered to the respective surface. Figure 7 shows cytokine released per cell as a function of the total carboxyl group content for both the fibrinogen and IgG preadsorbed surfaces.

On the fibrinogen preadsorbed surfaces monocyte adhesion was significantly higher on samples A2, A3, and A4 that have ionized carboxyl groups, compared to sample A1 that had only amide groups (Fig. 6). Additionally, cytokine release per cell was significantly lower on samples A2, A3, and A4, in contrast to sample A1 [Fig. 7(A)]. In other words, a larger number of monocytes adhered to the FEP modified surfaces with ionized carboxyl groups (A2, A3, and A4) and these cells were less activated. Conversely, on the FEP surface that had only amide groups (A1), fewer monocytes were adhered and these cells were more activated.

On the IgG preadsorbed surfaces, Figure 6 clearly shows that monocyte adhesion was not significantly affected by the carboxyl group content. Similarly, Figure 7(B) does not suggest a trend in terms of cytokine release per cell as a function of the total carboxyl group content. It appears that the IgG protein is preadsorbed similarly on the FEP modified surfaces regardless of the carboxyl group content, and therefore these surfaces did not show any specific trends in monocyte adhesion or activation.

The results strongly suggest that on the fibrino-

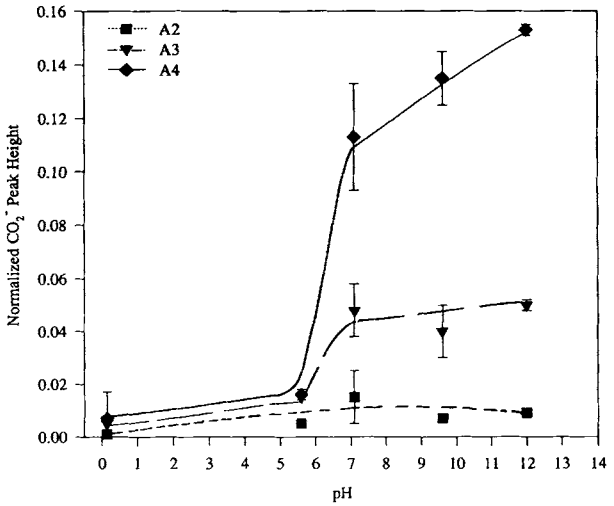


Figure 3 Normalized peak height (mean ± SD) of ionized carboxyl groups at 1550 cm^{-1} as a function of pH in samples A2, A3, and A4.

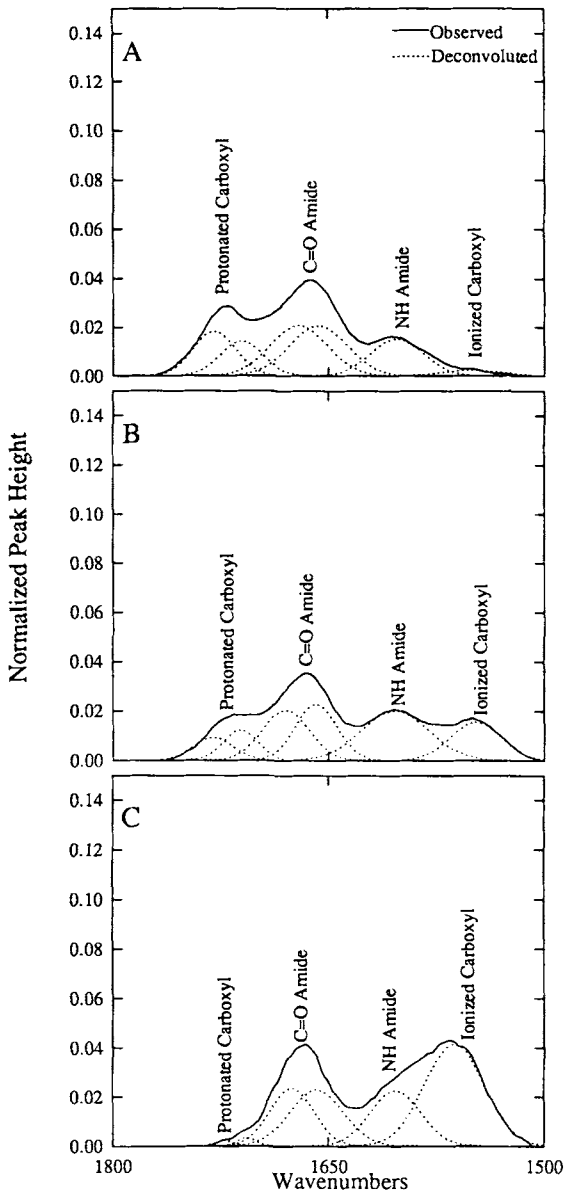


Figure 4 Infrared spectra of sample A3 at pH: (A) 0.15, (B) 5.6, and (C) 7.1. (—) Observed spectrum; (· · ·) deconvoluted spectrum.

gen preadsorbed surfaces, both monocyte adhesion and activation depend on the surface chemistry. It is possible that changes in the surface chemistry translate into differences in either the amount of fibrinogen preadsorbed or the conformation of the fibrinogen molecules preadsorbed on the surfaces. It has been reported that monocytes express receptors for fibrinogen. Therefore, differences in the amount or conformation of fibrinogen molecules preadsorbed on the surface may lead to differences in monocyte adhesion and activation.

Several authors have investigated fibrinogen adsorption and platelet adhesion on biomaterial surfaces.[11,12,21-25] Noishiki and Boffa et al. have shown that more fibrinogen is adsorbed on hydrophobic materials compared to hydrophilic materials.[21,22] Specifically, the results of Boffa et al. indicate that fibrinogen adsorption is higher on poly(vinylpyrrolidone) grafted poly(tetrafluoroethylene) (PTFE) compared to untreated PTFE.[22] Investigators have also examined conformational changes in fibrinogen adsorbed on polymer surfaces. In a study where thermally denatured fibrinogen was preadsorbed on PE, a reduction in platelet adhesion was obtained compared to PE surfaces that were coated with undenatured fibrinogen.[23] This suggests that the conformation of the fibrinogen molecules

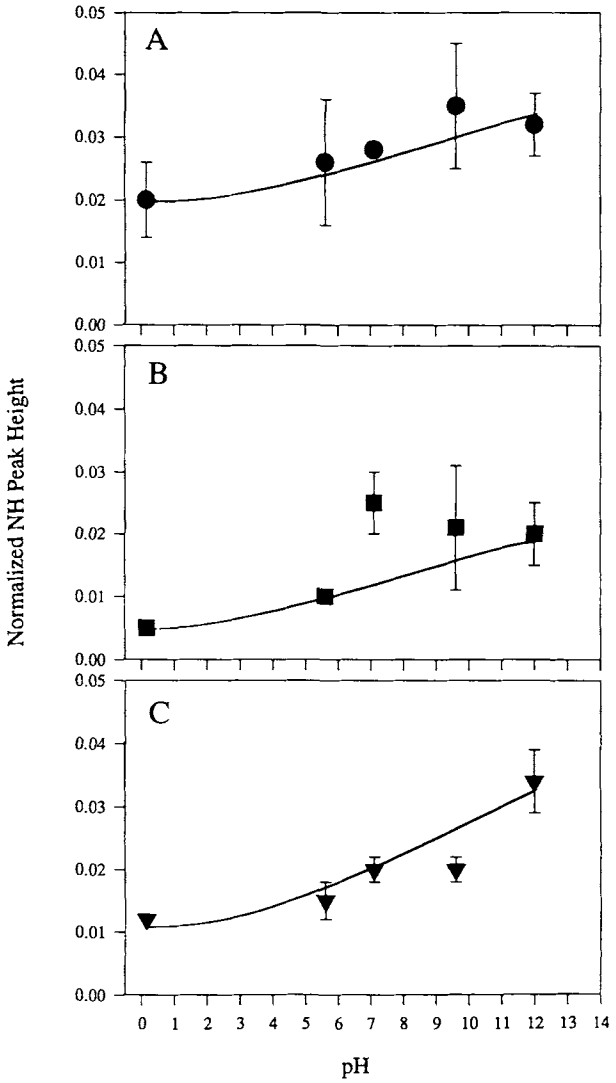


Figure 5 Normalized peak height (mean ± SD) of NH deformation at 1602 cm^{-1} as a function of pH: (A) sample A1, (B) sample A2, and (C) sample A3.

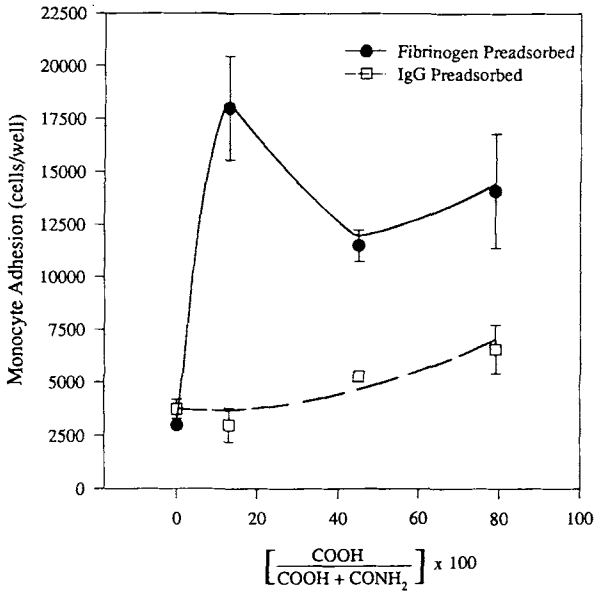


Figure 6 Monocyte adhesion (mean ± SEM) as a function of total carboxyl group content on fibrinogen and immunoglobulin-G preadsorbed surfaces.

affects cell adhesion behavior. Furthermore, Lindon et al. reported that platelet adhesion increases with the amount of fibrinogen that maintains its native state on the surface of polyalkyl acrylates and polyalkyl methacrylates.[24] According to Horbett et al. it may be that the apolar, hydrophobic surfaces cause unfolding of the fibrinogen molecules to improve binding with the hydrophobic portions of the protein.[25] This suggests that on apolar surfaces, the adsorbed fibrinogen molecules may not be in their native state, resulting in lower cell adhesion.[25] It is possible that on the FEP surface with only amide groups (A1), the adsorbed fibrinogen molecules are not in their native state; this results in lower monocyte adhesion compared to FEP surfaces with ionized carboxyl groups (A2, A3, and A4).

CONCLUSIONS

The ionic state of amide and carboxyl groups present on a series of surface modified FEP copolymers were analyzed, and *in vitro* monocyte adhesion and activation behavior was observed when a protein was preadsorbed on these surfaces. The proteins considered were fibrinogen and IgG. From the results obtained in this investigation, the following conclusions can be drawn:

1. When ATR-FTIR was used to investigate the ionic state of carboxyl groups on FEP surfaces that contained both carboxyl and amide groups, the pK_a of carboxyl groups was found to be approximately 6.3. This pK_a value did not appear to change with increasing amide group content.
2. Fibrinogen preadsorption on FEP copolymers with both carboxyl and amide groups present on the surface resulted in higher monocyte adhesion and lower monocyte activation compared to FEP surfaces that were characterized only by amide groups. This was attributed to differences in the amount of protein adsorbed onto the surface and/or to differences in the conformation of the protein molecules adsorbed on the surfaces.
3. Monocyte adhesion and activation on IgG preadsorbed, surface modified FEP copolymers did not appear to be affected by the type of functional groups present on the surface.

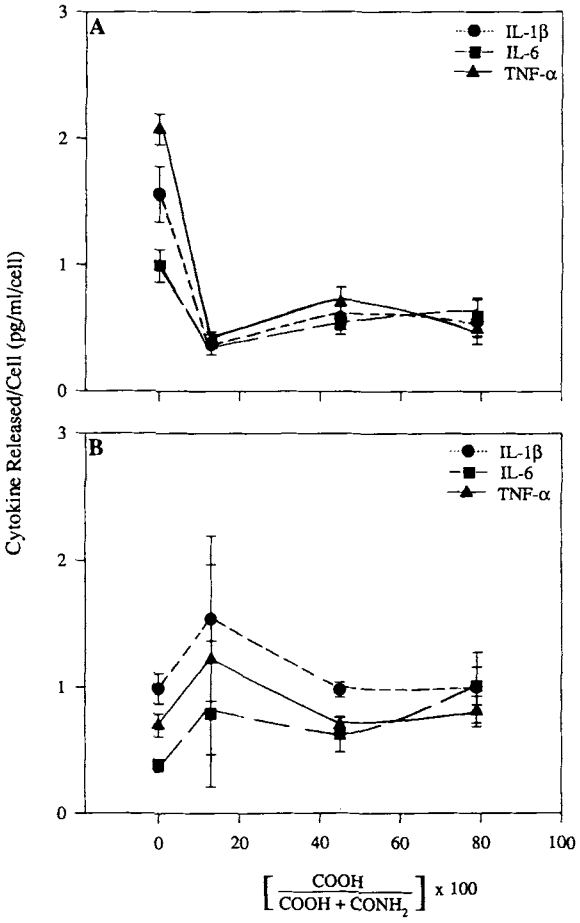


Figure 7 Cytokines (IL-1β, IL-6, and TNF-α) released per cell (mean ± SEM) as a function of total carboxyl group content: (A) fibrinogen preadsorbed and (B) immunoglobulin-G preadsorbed.

The authors would like to thank the National Science Foundation (Grant EEC 9320054) and the National Institute of Health (Grants HL 33849 and HL 48771) for their generous financial support. We would also like to thank John Kao and Mark Schubert of the Department of Macromolecular Science at CWRU for their technical insights and helpful discussions.

REFERENCES

1. N. P. Ziats, K. M. Miller, and J. M. Anderson, *Biomaterials,* **9,** 5 (1988).
2. K. Smetana, J. Vacik, D. Souckova, Z. Krcova, and J. Sulc, *J. Biomed. Mater. Res.,* **24,** 463 (1990).
3. P. B. van Wachem, A. H. Hogt, T. Beugeling, J. Feijen, A. Bantjes, J. P. Detmers, and W. G. van Aken, *Biomaterials,* **8,** 323 (1987).
4. A. Kishida, H. Iwata, Y. Tamada, and Y. Ikada, *Biomaterials,* **12,** 786 (1991).
5. P. B. van Wachem, T. Beugeling, J. Feijen, A. Bantjes, J. P. Detmers, and W. G. van Aken, *Biomaterials,* **6,** 403 (1985).
6. A. Bruil, J. G. A. Terlingen, T. Beugeling, W. G. van Aken, and J. Feijen, *Biomaterials,* **13,** 915 (1992).
7. S. R. Holmes-Farley, R. H. Reamey, T. J. McCarthy, J. Deutch, and G. M. Whitesides, *Langmuir,* **1,** 725 (1985).
8. S. R. Holmes-Farley, C. D. Bain, and G. M. Whitesides, *Langmuir,* **4,** 921 (1988).
9. J. M. Anderson, *Cardiovascular Pathology,* **2,** 33s (1993).
10. E. Shiba, J. N. Lindon, L. Kushner, G. R. Matsueda, J. Hawiger, M. Kloczewiak, B. Kudryk, and E. W. Salzman, *Am. J. Physiol.,* **260,** C965 (1991).
11. S. M. Slack, J. L. Bohnert, and T. A. Horbett, *Ann. NY Acad. Sci.,* **516,** 223 (1987).
12. R. J. Rapoza and T. A. Horbett, *J. Biomed. Mater. Res.,* **24,** 1263 (1990).
13. S. Cheng, K. K. Chittur, C. N. Sukenik, L. A. Culp, and K. Lewandowska, *J. Colloid Interface Sci.,* **162,** 135 (1994).
14. T. L. Bonfield, R. E. Marchant, and J. M. Anderson, *J. Biomed. Mater. Res.,* **26,** 837 (1992).
15. R. C. Bening and T. J. McCarthy, *Macromolecules,* **23,** 2648 (1990).
16. J. K. Yun, K. DeFife, E. Colton, S. Stack, A. Azeez, L. Cahalan, M. Verhoevan, P. Cahalan, and J. M. Anderson, *J. Biomed. Mater. Res.,* **29,** 257 (1995).
17. H. Park and J. R. Robinson, *Pharmaceut. Res.,* **4,** 457 (1987).
18. J. W. Nemec and W. Bauer, in *Encyclopedia of Polymer Science and Engineering,* Vol. 1, J. I. Kroschwitz, Ed., Wiley, New York, 1985, p. 228.
19. J. March, *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure,* Wiley, New York, 1992, p. 251.
20. G. Socrates, *Infrared Characteristic Group Frequencies,* Wiley, Chichester, 1980, p. 5.
21. Y. Noishiki, *J. Biomed. Mater. Res.,* **16,** 359 (1982).
22. G. A. Boffa, N. Lucien, A. Faure, and M. C. Boffa, *J. Biomed. Mater. Res.,* **11,** 317 (1977).
23. F. Rubins, J. Brash, J. Weitz, and R. Kinlough-Rathbone, *J. Biomed. Mater. Res.,* **26,** 1651 (1992).
24. J. N. Lindon, G. McManama, L. Kushner, E. W. Merril, and E. W. Salzman, *Blood,* **68,** 355 (1986).
25. T. A. Horbett, C. M. Cheng, B. D. Ratner, and A. S. Hoffman, *J. Biomed. Mater. Res.,* **20,** 739 (1986).

Received March 30, 1995
Accepted May 11, 1995