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Conditioning Polytetrafluoroethylene Surfaces for Use in Vascular Prostheses*

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Synopsis

Polytetrafluoroethylene surfaces have been treated to reduce thrombogenicity in order to make them suitable for use in prosthetic devices that come in contact with blood. This was done by first etching the surface with potassium in liquid ammonia to produce double bonds and then using these double bonds as sites for grafting on polyacids or as sites for chemical reactions. Tubes so treated were tested for thrombogenetic activity by implantation in the thoracic aorta or inferior vena cava of dogs. These tests showed that the thrombogenicity of a polytetrafluoroethylene surface can be reduced by attaching negatively charged groups provided the surface concentration of these groups is not too high (order of 1–2 $\times$ 10^{-6} equivalent per cm^2 geometric area) and provided the distribution of these groups is uniform. Sulfonic acid groups obtained by chlorosulfonation and carboxyl groups attached by grafting *tert*-butyl crotonate and hydrolyzing to crotonic acid were effective. Long chains of poly(acrylic acid), poly(ethylenesulfonic acid), and poly(vinyl alcohol) sulfate were less effective.

INTRODUCTION

Modern surgical techniques have advanced to the point where surgeons can replace many damaged or diseased portions of the human body provided that there are suitable materials to use as replacements. Polymers are the most promising materials for tissue replacement, but polymers intended for this use must meet strict requirements. These polymers must withstand long contact with the enzymes in tissue without deterioration. They must not cause irritation or abnormal growth at the site of implantation or at more remote sites and they must not induce allergic reactions. Even though a polymer meets these requirements for implantation in the body, it is not suitable for use in vascular surgery if it causes blood clotting or surface thrombosis. A number of polymers are reasonably well tolerated by the human body but they tend to be thrombogenic.

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There are two approaches to the selection of a polymer for use in vascular surgery. One approach is to accept the fact that polymers are normally thrombogenic and to live with this fact.[1-3] The other approach is to treat the surface chemically to eliminate thrombogenicity. We have taken the second approach.

Almost all workers who have modified the surface of polymers to reduce thrombogenicity have attached heparin molecules to the surface.[4-6] We believe that it is not necessary to attach the whole heparin molecule to a surface to attain nonthrombogenicity but that a certain critical portion of the molecule will suffice. We know that a nonthrombogenic surface has a negative charge. This has been amply demonstrated.[7,8] However, a negative charge is not the sole requirement for nonthrombogenicity, since glass, which is negatively charged in aqueous media, is strongly thrombogenic.

EXPERIMENTAL

Our first series of experiments used electrons from a Van de Graaff accelerator to graft poly(acrylic acid), crotonic acid, and poly(ethylenesulfonic acid) to the surface of polytetrafluoroethylene, a polymer that, except for its thrombogenicity, is reasonably well tolerated by the animal body. In order to increase the amount of grafting, we pretreated the polymer with a solution of potassium in liquid ammonia. This treatment increased the surface area 10,000-fold (as measured by nitrogen adsorption using the Brunaur, Emmett, and Teller method) and introduced conjugated double bonds turning the surface deep brown. The number of double bonds introduced in this way was shown by bromine addition at room temperature to be 2×10^{-6} moles of double bond per cm^2 geometric area. This is a lower limit to the unsaturation since bromine absorption gives low values when double bonds are conjugated. Poly(acrylic acid) was attached by first grafting poly(methyl acrylate) to the surface and then hydrolyzing. This indirect method was used because poly(acrylic acid) is degraded by radiation. Crotonic acid was likewise attached by grafting *tert*-butyl crotonate followed by hydrolysis. Ethylenesulfonic acid was used as a 10% aqueous solution. The number of acid groups attached to the surface was determined to $\pm 5\%$ by microtitration. In preparations with poly(acrylic acid), this treatment added an average of from 1.4 to 25×10^{-6} equivalents of acid per cm^2 geometric area.

The efficiency of grafting was estimated in a number of preparations by measuring the molecular weights of the ungrafted homopolymer formed during grafting. Combining this figure for chain length with the titration value permitted calculation of the number of grafted chains. This number was used to estimate the efficiency of grafting based on a surface concentration of double bonds of 2×10^{-6} moles per cm^2 geometric area. Results of these tests are given in Table I. The efficiencies in the last column may be thought of as weight-average efficiencies since all molecular weights except that of poly(*tert*-butyl crotonate) were estimated viscometrically. The

TABLE I
Efficiency of Grafting to Etched Polytetrafluoroethylene Using Electrons from a Van de Graaff Accelerator

Monomer	Radiation dose, Mrad	Degree of polymerization[a]	Surface conc. of acid, (Eq/cm²) × 10^6	Number of chains, ×10^9	Efficiency, %[b]
Methyl acrylate	1	670	1.71	2.5	0.12
Methyl acrylate	2	1080	2.59	2.4	0.12
Methyl acrylate	3	2560	4.89	1.9	0.10
Methyl acrylate	4	2560	6.59	2.6	0.13
Ethylenesulfonic acid	2	465	2.5	5.4	0.27
tert-Butyl crotonate	2	25[c]	1.7	68	3.3[c] 1.3[d]

[a] Viscosity average.
[b] Based on 2 × 10^{-6} moles of double bond per cm².
[c] Number average.
[d] Estimated for weight average.

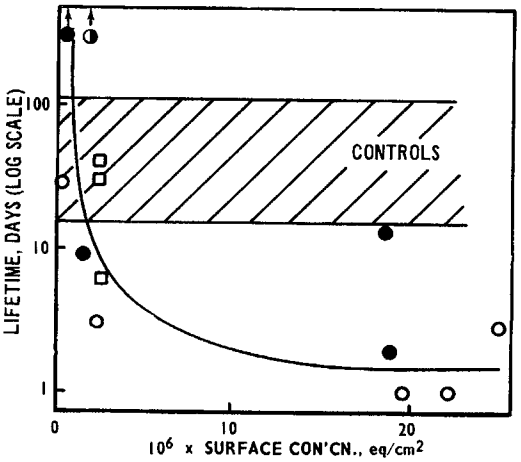


Fig. 1. Relationship between lifetime of dogs and surface concentration of acid, series I: (□) poly(ethylenesulfonic acid); (○) poly(acrylic acid); (●) isolated carboxyls; (◑) poly(crotonic acid).

molecular weight of poly(*tert*-butyl crotonate) was estimated microisopiestically and converted to an approximate weight average by multiplying by the arbitrary factor 2.5. Notice that no matter what the radiation dose, only 0.1% of the available double bonds was used in grafting methyl acrylate. Efficiencies were greater with ethylenesulfonic acid and with *tert*-butyl crotonate but the surface concentrations were lower with these monomers because the chains were shorter.

Treated polytetrafluoroethylene tubes, 5 cm long and 0.9 cm i.d., were tested for thrombogenicity in the Department of Surgery of the Downstate Medical Center of the State University of New York. Before testing, the acid groups on the surface of the tubes were converted to sodium salt by treatment with 0.2*N* sodium hydroxide, washed well, and sterilized by autoclaving in normal salt solution. Tubes were inserted in the thoracic aorta or the inferior vena cava of dogs and the condition of the tubes was examined when the dogs died or were sacrificed. Details of these in vivo tests are reported elsewhere.[9] The results of the initial series of tests were at first disappointing. A number of the dogs died after one to four days as a result of thrombus formation. Furthermore, some of our "best" tubes, i.e., those with the highest surface concentration of acid, gave the poorest results. However, closer inspection of the data lead us to conclude that we were attaching too many acidic groups to the surface. This idea is supported by Figure 1, which is a semilogarithmic plot of the lifetimes of dogs versus the surface concentration of acidic groups on the tubes. Notice that some of the poorest results occurred with tubes having the highest surface concentrations and that the best results occurred with tubes having a relatively low surface concentration. The two points at the far left of the plot are marked with upward arrows to indicate that the dogs might

TABLE II
Equivalents of Acid Per Gram of Etched, Sulfonated Polytetrafluoroethylene Powder and Film

Sample	Treatment	Sulfonic acid, (Eq/g) × 10^5
Film	Usual wash	3.4
	Additional long wash	3.7
Powder	0.3*N* NaOH, 0.3*N* HCl, water vacuum dry 55°C	4.3
	Retreat 0.1*N* NaOH, 0.3*N* HCl, water, vacuum dry 55°C	4.4

have lived longer if they had not been sacrificed after 333 and 340 days. The tube in one of these dogs had crotonic acid units on its surface and the other had isolated carboxyl groups which were attached to the surface by exposure to carbon dioxide during etching.

As a result of the conclusion that we were attaching too many acidic groups to the surface, we discontinued the use of the Van de Graaff accelerator, which was chosen initially to produce a high level of grafting and, in the second series of preparations, attached ionic groups by grafting in polymerizations initiated by free radical initiators or by chemical treatment of the etched polymer. A type of chemical treatment that we found effective consisted in heating the etched polymer at 80°–90°C in chlorosulfonic acid. This treatment added 2×10^{-6} Eq/cm^2 of sulfonic acid to the surface in 1 hr and twice this number of equivalents in 6 hr. These groups were not removed by intensive washing or by treatment with sodium hydroxide, as shown in Table II. Therefore we conclude that the acid groups attached by chlorosulfonation are bound to the surface.

Experiments with radioactive silver ions were carried out to study the distribution of sulfonate and carboxyl groups on the surface of treated polytetrafluoroethylene strips. Strips (5 × 1 × 0.1 cm) having four surface concentrations of carboxyl groups (1.6, 2.5, 5.7, and 12.6×10^{-6} Eq/cm^2) prepared by irradiation, and strips having three surface concentrations of sulfonate (0.6, 1.7, and 3.6×10^{-6} Eq/cm^2), prepared by treatment with chlorosulfonic acid, were examined. The strips were soaked in a solution containing radiotagged silver ions which are strongly bound to poly(carboxylic acids) and to poly(sulfonic acids). Strips which had been exposed to radioactive silver solutions (0.1*N* $AgNO_3$-Ag-110m in 0.04*N* HNO_3, specific activity 0.1 millicurie Ag-110 per ml) followed by rigorous washing were examined for bound silver by autoradiography. Autoradiographs showed that the surface distribution of bound silver ions was very uneven on samples having a high concentration of carboxyl groups (5.7 and 12.6×10^{-6} Eq/cm^2) grafted by use of a Van de Graaff accelerator. Samples with lower concentrations of carboxyl and samples sulfonated by treatment with chlorosulfonic acid had much more even distributions of bound silver.

TABLE III
Monomers Grafted to Etched Polytetrafluoroethylene
by Free Radical Initiators

Monomer	Concentration, (Eq/cm²) × 10⁶
Acrylic acid	0.8–1.1
Methacrylic acid	0.8–1.6
Itaconic acid	1.5
Maleic anhydride	0.9[a]
Acrylamide	0.9–3.0[a]
Vinyl acetate	0.5–2.1[a]
N-Vinylphthalimide	0.8[a]
N-Vinylsuccinimide	1.0[a]
tert-Butyl crotonate	0.5
Ethylene sulfonic acid	1.3

[a] Measured on a derivative.

Grafting initiated by free radical initiators was done by the general methods used for bulk polymerization of the particular monomers but larger amounts of initiator (0.5% to 2%) were used. Whenever a new preparation was attempted, preliminary experiments were run on small strips of etched polytetrafluoroethylene (5 × 1 × 0.1 cm electrical grade from Industrial Plastics Supply Co.) to make sure that grafting actually occurred. The presence of the desired compound on the surface was verified by examination with attenuated total-reflectance infrared spectrometry. Table III lists monomers that were grafted to the surface of etched polytetrafluoroethylene by the use of free radical initiators. The quantities of monomer grafted were determined by microtitration of the base taken up by the polymer on the grafted strip or, when indicated by an asterisk, by a derivative of that polymer. The efficiency of grafting with free radical initiators was determined on a number of preparations, which are listed in

TABLE IV
Efficiency of Grafting to Etched Polytetrafluoroethylene
Initiated by Free Radical Initiators

Monomer	Degree of polymerization[a]	Surface conc. of acid, (Eq/cm²) × 10⁶	Number of chains, ×10⁹	Efficiency, %[b]
Acrylic acid	600	1.1	1.8	0.09
Acrylic acid	850	0.8	0.94	0.045
Methacrylic acid	114	0.8	7	0.35
	114	0.8	7	0.35
Acrylamide	5500	2.96 (66% hydrolyzed)	1.2	0.06
Itaconic acid	154	0.85	5.5	0.20
Maleic anhydride	230	0.43	1.85	0.09

[a] Viscosity average.

[b] Based on 2 × 10^{-6} moles of double bond per cm².

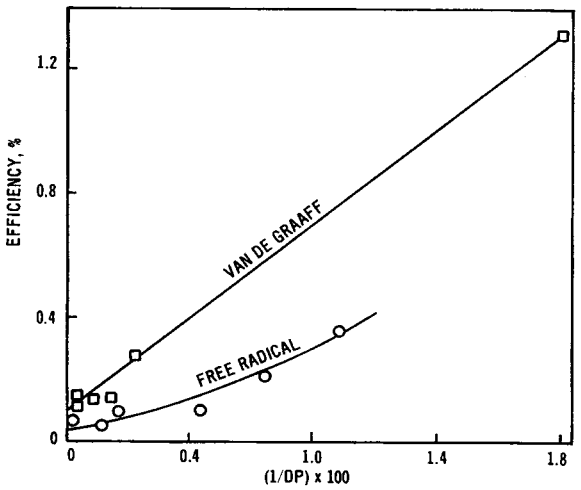


Fig. 2. Relationship between grafting efficiency and reciprocal of chain length, $1/DP$.

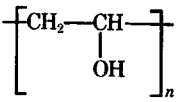
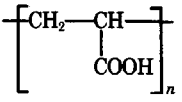
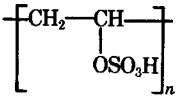
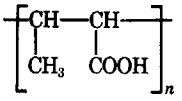
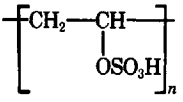
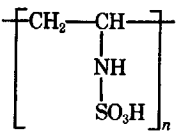
Table IV. These efficiencies vary considerably from monomer to monomer. However, examination of these data and the data in Table I shows that grafting efficiency is greater the less readily the monomer homopolymerizes. This relationship is seen in the plots of grafting efficiency versus reciprocal of chain length, DP, in Figure 2. This behavior is reasonable because surface double bonds compete with monomer for growing chain ends.

The polymer initially grafted to the surface need not be the desired one. Once attached, the polymer can be subjected to various reactions such as hydrolysis, esterification, sulfation, etc. Polymers which we have prepared by treating polymers already grafted to the surface are poly(vinyl alcohol), poly(vinyl alcohol sulfate), polyvinylamine, sulfated polyvinylamine, and poly(N-vinylsuccinamic acid). The quantity of poly(vinyl acetate) or of derived poly(vinyl alcohol) grafted to the surface could not be measured directly. However, infrared examination prior to hydrolysis showed the presence of significant quantities of poly(vinyl acetate) on the surface of strips and titration of the derived sulfate ester of poly(vinyl alcohol) showed concentrations of sulfate groups in various preparations of from 0.5 to 2.1 $\times$ 10^{-6} Eq/cm^2, indicating that the surface concentration of the untreated poly(vinyl alcohol) was at least that large.

A surface having amine groups was prepared by attaching polyvinylphthalimide to the surface and hydrolyzing, first with hydrazine hydrate[10] and second with 10N sodium hydroxide.[11] The treatment with hydrazine hydrate converted 50% of the imide to amine and the following treatment with sodium hydroxide raised this figure to between 80% and 85%. Subsequent sulfation of amine was 100% effective.

Since dogs are expensive and in vivo tests are long and difficult, samples to be tested for thrombogenicity had to be carefully selected. We were guided in selecting samples for in vivo testing by noting the nature of the

TABLE V
Groups on Surfaces of Tubes Tested for Thrombogenicity, all as Sodium Salts

(a)		(b)	
Group	Conc.,[a] (Eq/cm²) × 10^6	Group	Conc.,[a] (Eq/cm²) × 10^6
Control	0.03		0.04
	11		1.4
	1.3	$-SO_3H$	1.23
	1.5	$-SO_3H$	0.2-0.6
—COOH	0.3		0.4[b]

[a] Based on geometric area.

[b] Also 0.08 × 10^{-6} Eq/cm² poly(vinylphthalmic acid) and 0.05 × 10^{-6} Eq/cm² carboxyl.

groups on active antithrombic agents such as heparin. These groups are: hydroxyl, carboxyl, sulfated hydroxyl, and sulfated amine. The groups which we attached to polytetrafluoroethylene surfaces for in vivo testing are listed in Tables Va and Vb. The results of tests on tubes having the surface groups listed in Table Va are shown in Figure 1.

The results[9] of in vivo tests on tubes having on the surface the groups listed in Table Vb are summarized in Table VI. Column 1 shows the composition of the surface, column 2 the number of days the tubes remained implanted before the dog died or was sacrificed. The last column gives an estimate of the percentage of the lumen of the tube that was filled by clot. In about 40% of the tubes thrombi formed at the junction of tube and blood vessel. Table VI shows that tubes with hydroxylated surfaces are unsatisfactory; all caused severe clotting within the tube. Tubes whose surfaces bore sulfated hydroxyl groups were better than hydroxylated surfaces alone, but they too were unsatisfactory. Tubes with sulfated amine groups on the surface (not listed in the table) were also unsatisfactory. Sulfonated surfaces performed the best of any of the surfaces tested. The surfaces having the higher concentration of sulfonation were better than those with the lower concentration of sulfonic acid groups.

From this second set of in vivo tests we conclude, first, that the thrombogenicity of polytetraflouroethylene can be reduced by sulfonation and,

TABLE VI
Results of in vivo Tests on Tubes in Second Series

Surface	Days	Dead (D) or sacrificed (S)	Occlusion, %
Hydroxyl	39	D	90
	58	S	60
	35	D	90
	136	S	20
	5	D	90
Sulfated hydroxyl	120	S	—
	130	S	—
	56	S	—
	1	D	50
	3	D	30
Sulfonate, 1.23×10^{-6} Eq/cm^2	163	S	—
	122	S	—
	163	S	—
	163	S	—
Sulfonate, $0.2\text{–}0.6 \times 10^{-6}$ Eq/cm^2	125	S	—
	120	S	—
	63	S	—
	53	D	50
	110	S	50

second, that an even distribution of negatively charged groups on the surface is important. Better results were obtained with tubes having crotonic acid or isolated carboxyls on the surface and with tubes that were chlorosulfonated than with tubes bearing poly(acrylic acid) or poly(ethylenesulfonic acid). Thus it appears that an even distribution of single acid groups or short polymer chains is more effective in clot prevention than is an equal concentration of acid in the form of long polymer chains.

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