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NOVEL DESIGNED POLYISOBUTYLENE-BASED BIOPOLYMERS: SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL TESTING OF AMPHIPHILIC CHAMELEON NETWORKS

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ABSTRACT

After a very brief introduction into the impact of living polymerization on preparative polymer chemistry and a look into the dearth of *de novo* polymer synthesis research for novel biopolymers, this presentation will focus on a new class of synthetic biopolymers: amphiphilic chameleon networks, i.e., biocomponent networks comprising random strands of hydrophobic and hydrophilic polymers, for blood contact application and mainly for use as narrow diameter (<4 mm) vascular grafts. First, the precision syntheses of amphiphilic networks will be outlined. Subsequently the surface and bulk characterization of these novel molecular composites by a battery of physicochemical methods will be highlighted. Finally, representative results of biological in-vitro and in-vivo testing will be summarized. We propose that for bio- or hemocompatibility to arise, it may be necessary to employ "smart" amphiphilic surfaces capable of rapid reversible hydrophobic/hydrophilic reorganization so as to present the most favorable lowest energy surface conformation to the medium. It appears that these surfaces are smooth, flexible, and of very low modulus, and that the bulk of these materials have cocontinuous phase-separated microarchitectures with random microdomains in the 10–100 Å diameter range. Contemporary macromolecular engineering can deliver materials exhibiting this combination of characteristics.

INTRODUCTION

While the synthetic power of living polymerization has been richly exploited for the preparation of engineering materials (witness, for example, the commercial production of styrenic thermoplastic elastomers, designed polyolefins), the potentialities of this methodology for the preparation of synthetic biopolymers have not yet been explored. (To coin a definition: A synthetic biopolymer is a macromolecule that is compatible with living tissues and has properties suitable to replace living tissue or augment their function.)

In view of the very high rate of progress in polymer synthesis toward well-defined tailored structures and molecular composites, surprisingly little *de novo* biopolymer design and synthesis is being carried out. This low level of research activity is even more astonishing since for biopolymer development cost is less critical than function: While the cost is all-important for engineering polymers, the deciding parameter for biopolymer development is less the cost than the projection that the contemplated product will save a life or increase the comfort of a patient.

In view of this, how come biopolymers are not designed but are still largely developed empirically by trial-and-error, and that most contemporary biopolymers were originally developed for engineering applications [consider, for example, poly(vinyl chloride), polyethylene, polyesters, nylons, silicon rubber, polyurethanes, etc.]? Part of the answer is that physiologists, biologists, and medical professionals are far from able to define their needs in materials science let alone polymer-chemical terms. Unless bridges are built between the biomaterial scientist on the one hand and the polymer chemist/synthesist on the other hand, and communication is improved between these disciplines, there is little hope for progress toward the development of novel designed biopolymers.

This author (remembering that a long time ago he received his Ph.D. in biochemistry) has devoted a considerable amount of his energies during the past decade for the synthesis by living carbocationic polymerization of novel biopolymers. Specifically, he designed, prepared, and reported on well-defined heretofore unsynthesizable macromolecules for use as intervertebral disks [1], impact resistant bone cements [2–5], delayed drug-release reservoirs [6–8], and, more recently, small diameter tubes for vascular grafts. Since the first three of these developments have already been adequately described in the scientific and patent literature (see above), this presentation will primarily focus on the fourth item: The design and synthesis of amphiphilic chameleon networks, and their physicochemical and physiological characterization, for narrow lumen (<4 mm) vascular grafts.

DESIGN AND SOME FUNDAMENTALS OF AMPHIPHILIC CHAMELEON NETWORKS

Hypothesis

There is a need for truly "blood-compatible" synthetic materials for small diameter (<4 mm) vascular grafts. As a result of several years of symbiotic research between our precision macromolecular design and synthesis group at The University of Akron and the pathophysiology group at Case Western Reserve University (Drs.

J. M. Anderson and N. P. Ziats), we have recently developed a hypothesis regarding the nature of blood-contacting synthetic surfaces.

Specifically, we propose that blood compatibility arises by the use of "smart" surfaces, that is, with soft low-modulus surfaces that are rapidly able to reorganize their hydrophilic/hydrophobic domain morphology as demanded by the molecules/cells/tissues/media they come in contact with. Such surfaces would constantly minimize their surface energetics as dictated by the surrounding milieu.

One group of structures that appears of particular interest for biopolymers is medium-responsive two-component amphiphilic networks, i.e., a random assembly of flexible hydrophilic/hydrophobic chains giving rise to a two-phase system of cocontinuous channels. The pore size of the channels can be controlled by controlling the M_n between crosslinking sites. Importantly, the external and internal surfaces (i.e., the micromorphology) of these networks change rapidly in response to the medium they are in contact with, so as to assume the thermodynamically most favorable chain conformations dictated by the medium. Amphiphilic networks are expected to exhibit a unique cocontinuous microheterogeneous domain morphology; for example, they swell *both* in water and hydrocarbon solvents. Figure 1 helps to visualize the microarchitecture of such a network system comprising hydrophilic and hydrophobic chains swollen by water, by a hydrocarbon solvent, and by an amphiphilic solvent, say THF, which is miscible with both water and hydrocarbons.

We have prepared a variety of such networks and have investigated their properties both by physicochemical characterization methods and by using biological techniques (see below).

Similar Research by Others and Some Unanswered Questions

A literature search revealed very limited activity aimed at the synthesis of bicomponent amphiphilic networks, and the associated characterization research was certainly insufficient, particularly in regard to biomaterials development.

The first description of an amphiphilic network was by Good and Mueller in 1980 [9]: Poly(tetramethylene oxide)-α,ω-diol was reacted with an excess of diisocy-

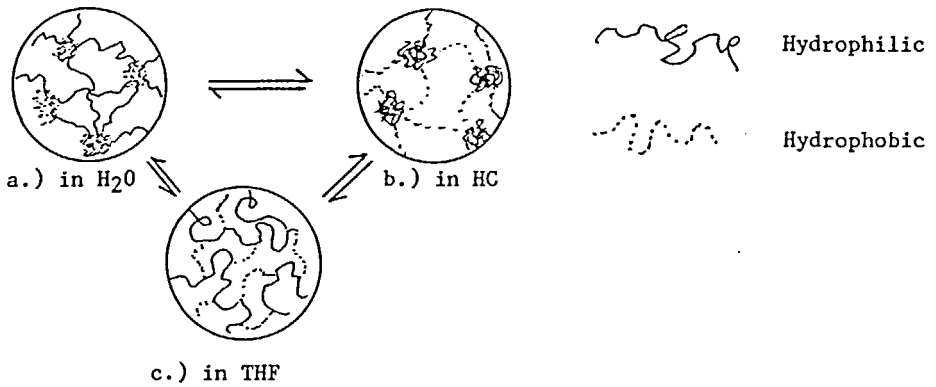


FIG. 1. Scheme of an amphiphilic network system comprising hydrophilic and hydrophobic chains swollen by water (a), hydrocarbon solvent (b), and THF (c).

anate to yield a chain-extended material with isocyanate end groups. The product was then dissolved in 2-hydroxyethyl methacrylate (HEMA) to give a macromonomer with methacrylate termini. A network of hydrophilic polyHEMA crosslinked by hydrophobic poly(tetramethylene oxide) chains was obtained. The materials were investigated as potential carriers for controlled drug release. Drug release rates were affected by both the crosslink density and the amount of hydrophobic component present in the network. The diffusion rate of a water-soluble drug, Phenformin-HCl, decreased with increasing crosslink density and hydrophobic component. The authors concluded that drug diffusion was mainly through the water-swollen areas of the amphiphilic network. Regrettably, this work was not followed up.

Another example of an amphiphilic network was reported by Keszler and Kennedy [10]. In this work, styryl-ended polyisobutylene (PIB) macromonomers were copolymerized with *N*-vinylpyrrolidone (NVP). The binding of methyl orange to the PIB/PNVP amphiphilic networks was investigated. Binding constants for these materials were considerably higher than those of purely hydrophilic gels. The results suggested that amphiphilic networks may be useful matrices for controlled drug release.

Weber and Stadler [11] crosslinked polybutadiene by 1,2,4-triazoline-3,5-dione telechelic poly(ethylene oxide) in THF. The materials swelled in THF, water, and cyclohexane. The maximum swelling in water was a function of crosslink density. Transmission electron microscopy indicated the existence of two bicontinuous phases. Itsuno and coworkers [12] copolymerized styrene with *p*-vinylbenzyl telechelic poly(ethylene oxide)s. We are not aware of any publications concerning the evaluation of biocompatibility of amphiphilic networks, or, specifically, studies in regard to blood compatibility with human whole blood under dynamic (flow) conditions. In particular, it is relevant to determine if these materials could act as better blood compatible devices for use in vascular graft prostheses, which for the most part are rather poor substitutes for native blood vessels.

The use of prosthetic devices such as vascular grafts to replace or bypass diseased vessels is a common procedure in surgery, and their use and application will increase in the future. Large diameter grafts have achieved better patency rates, reported at 80–90% at 5 to 10 years [13], than the small arterial reconstructions. Patency rates for femoral-popliteal and infrapopliteal bypass, after 3–5 years, range from 15 to 50% [13, 14]. Vieth et al. [15] reported that currently available small diameter expanded polytetrafluoroethylene (ePTFE) vascular prostheses are less efficacious than the use of autogenous veins for either femoral-popliteal or more distal bypasses. These clinical results show the need for new vascular graft materials in applications where small diameter or low blood flow is important.

The overall mechanism(s) of vascular graft failure in low flow (<200 mL/min), small diameter (<6 mm) systems is poorly understood, but evidence indicates that occlusion is primarily due to thrombosis. The differences between the efficacy of biologic versus synthetic prosthetic materials is likely due to biomechanical characteristics such as compliance, porosity, durability and flexibility, anastomotic and graft healing responses, as well as the surface thrombogenicity of the materials [14, 16–19].

Many questions remain unanswered regarding the blood and tissue compatibility of these synthetic materials. A major concern is the blood/materials interaction

involving protein and cellular interactions with the surface of the graft as well as the healing phase following implantation. The success of the graft and survival of the patient (or patient's limb) are the most important features to consider regarding the development of new graft materials. The development of a fibrous capsule surrounding the graft, luminal development of a pseudointima or neointima and, finally, the loss or proliferation of endothelial cells lining the graft are all aspects that may occur in these grafts either experimentally in animal models or in humans [20–26]. Therefore, the success or failure of grafts in humans and in experimental models is a complex problem related not only to the type of graft but also to the accumulation of proteins, platelets, and leukocytes derived from the blood as well as rheologic factors of the blood flow.

Amphiphilic materials may be most promising candidates for use in cardiovascular applications since they can be made soft, flexible, rubbery, smooth, pulsating, stable, of sufficient mechanical strength, of reduced protein adsorption, reduced monocyte adhesion, enhanced endothelial cell attachment and growth, and, most importantly thromboresistant.

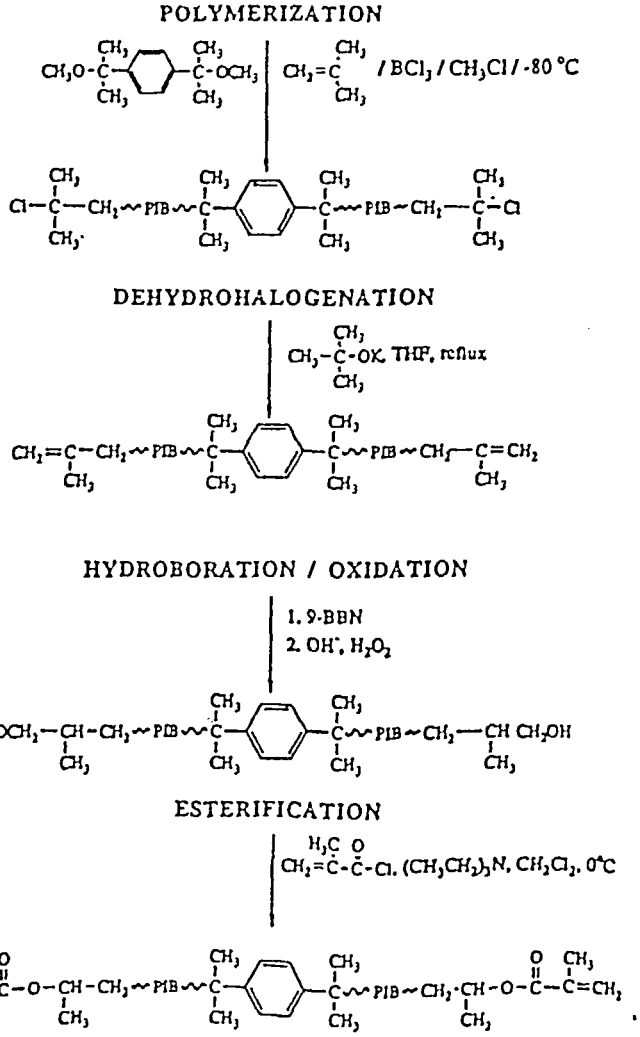
Synthesis, Characterization, and Biological Testing of Amphiphilic Networks

Synthesis and Overall Composition

Our objective was to develop convenient syntheses for tailored amphiphilic networks with controlled composition, M_n, molecular weight distribution (MWD), and hydrophilic/hydrophobic ratio.

We have selected polyisobutylene ($-[-CH_2C(CH_3)_2-]-_n$ (PIB)) as the hydrophobic segment for a variety of reasons: PIB is a fully saturated soft, chemically, and oxidatively stable aliphatic hydrocarbon rubber. PIB has FDA approval for food-contact applications, chewing gum base, medicinal adhesives, stoppers, etc. In its pure form PIB is nonmutagenic and noncytotoxic. It is cheap, and many thousand of tons of PIB are used commercially in the form of inner tubes, inner liners, adhesives, insulating materials, etc.

The recent discovery of living carbocationic polymerization of isobutylene led to the synthesis of PIBs with designed molecular weight, narrow molecular weight distribution, and controlled end functionalities [27–30]. We prepared *t*-Cl-telechelic PIBs by living carbocationic polymerization and the Cl-endgroups were quantitatively converted to methacrylate termini. The synthesis route is shown in Scheme 1 [27]. A series of well-characterized (M_n, MWD, end functionality = 2.0 ± 0.1) methacrylate–ditelechelic PIBs (MA–PIB–MA) have been copolymerized by a free radical initiator (AIBN) in THF solution with hydrophilic monomers, e.g., 2-hydroxyethyl methacrylate (HEMA), *N*,*N*-dimethyl acrylamide (DMAAm), and dimethylaminoethyl methacrylate (DMAEMA) to amphiphilic networks: PHEMA-*l*-PIB or H networks, PDMAAm-*l*-PIB or A networks, PDAEMA-*l*-PIB or D networks (where *l* signifies "linked by"). The above hydrophilic segments were selected because of their low cost, commercial availability, and because a large amount of biomaterials research has already been carried out with these polymers. In regard to the abbreviations used, our code indicates the particular network (H, or A, or D),



SCHEME 1.

the M_n of the MA–PIB–MA hydrophobic moiety (×1000), and the wt% PIB in the network; for example H-4.5-50 stands for a PHEMA-*l*-PIB network with $M_{n,PIB}$ = 4500, containing 50% PIB. Network compositions were determined by solvent extraction studies, IR spectroscopy, and elemental analysis [7, 8].

Table 1 shows select networks studied and some bulk characteristics. The molecular weight of the hydrophilic segments (i.e., the molecular weight between crosslink points, M_c) was estimated by

$$M_c = (W_h M_n)/(2W_{PIB})$$

where W_h is the weight fraction of the hydrophilic polymer, M_n is the number-average molecular weight of the PIB moiety (which is, in fact, the M_c for the

TABLE 1. Characterization of D, A, and H Networks

D network				A network				H network			
Sample	M_c, PDMAEMA	PIB phase T_g, °C	PDMAEMA phase T_g, °C	Sample	M_c, PDMAAm	PIB phase T_g, °C	PDMAAm phase T_g, °C	Sample	M_c, PHEMA	PIB phase T_g, °C	PHEMA phase T_g, °C
D-4-31	4,400	−56	−6	A-4-26	5,600	−61	104	H-4-29	4,850	−61	107
D-4-39	3,100	−54	−7	A-4-45	2,400	−54	102	H-4-37	3,350	−59	106
D-4-50	2,000	−54	−7	A-4-56	1,550	−54	94	H-4-49	2,050	−57	104
D-4-59	1,400	−51	−8	A-4-59	1,400	−53	90	H-4-57	1,500	−55	98
D-4-71	800	−64	−7	A-4-77	600	−52	N.D.	H-4-68	950	−54	100
D-9.5-33	9,650	−61	−9	A-9.5-31	10,600	−63	115	H-9.5-38	7,750	−61	111
D-9.5-39	7,450	−59	−9	A-9.5-40	7,150	−62	110	H-9.5-46	5,600	−60	111
D-9.5-51	4,550	−59	−8	A-9.5-49	4,950	−60	108	H-9.5-54	4,050	−58	107
D-9.5-63	2,880	−57	−9	A-9.5-59	3,300	−59	95	H-9.5-64	2,650	−57	107
D-9.5-72	1,850	−59	−9	A-9.5-76	1,500	−58	N.D.	H-9.5-75	1,600	−58	105

hydrophobic component), and W_{PIB} is the weight fraction of PIB incorporated into the network. The M_c of the hydrophilic segments and the M_n of the PIB linked segments give insight into the molecular architecture. The concentration and the M_n of the MA–PIB–MA controls the M_c of the hydrophilic segments.

Surface and Bulk Characterization

The surfaces of our amphiphilic networks were characterized by dynamic contact angles (DCA by Wilhelmy plate balance), x-ray photoelectron spectroscopy (XPS), transmission and scanning electron microscopy (TEM and SEM), and atomic force microscopy (AFM). Bulk characterization included T_g determination by differential scanning calorimetry (DSC), swelling and diffusion studies, and studying the equilibrium water uptake as a function of pH.

Table 2 shows advancing and receding water contact angles of select dry and hydrated H networks. The contact angle hysteresis can be explained by proposing that in air (a very hydrophobic medium) the hydrophobic PIB segments dominate the surfaces, while in water the hydrophilic chains are dominant. Analysis of dynamic contact angles provides insight into the conformational mobility of the surfaces in different environments. The large contact angle hysteresis observed is most likely due to the conformational rearrangement of surface chains and their side groups, or to surface roughness or heterogenity.

In view of microdomain separation inherited from the synthesis, surface conformational rearrangements and surface heterogenity are proposed to be mainly responsible for the observed contact angle hysteresis. Since all polymer samples were optically clear and SEM showed featureless smooth surfaces, surface roughness is conceivably of limited significance. The hydrophobic PIB will show little reduction in advancing contact angle upon hydration in water while the amphiphilic networks show large decreases in the advancing angle because they can conformationally rearrange and thus minimize their interfacial energy.

The surface composition and surface roughness of select A and H networks were studied by XPS and AFM, respectively. According to ESCA spectroscopy, the surface and bulk elemental compositions (O/C and N/C%) of these networks show significant differences, see Fig. 2.

TABLE 2. Contact Angles of Water Measured on Dry and Wet Amphiphilic Networks

	Contact angles, θ, dry		Contact angles, θ, wet	
Sample	Advancing	Receding	Advancing	Receding
PHEMA	92.7	54.6	72.4	49.5
H-3.8-40	99.2	32.7	79.2	26.1
H-3.8-50	103.3	28.9	82.2	29.4
H-3.8-60	103.8	33.2	85.3	31.0
A-3.8-50	115.5	17.4	96.9	34.2
D-3.8-50	102.8	9.2	81.6	33.7
PIB	109.8	36.3	106.5	33.6

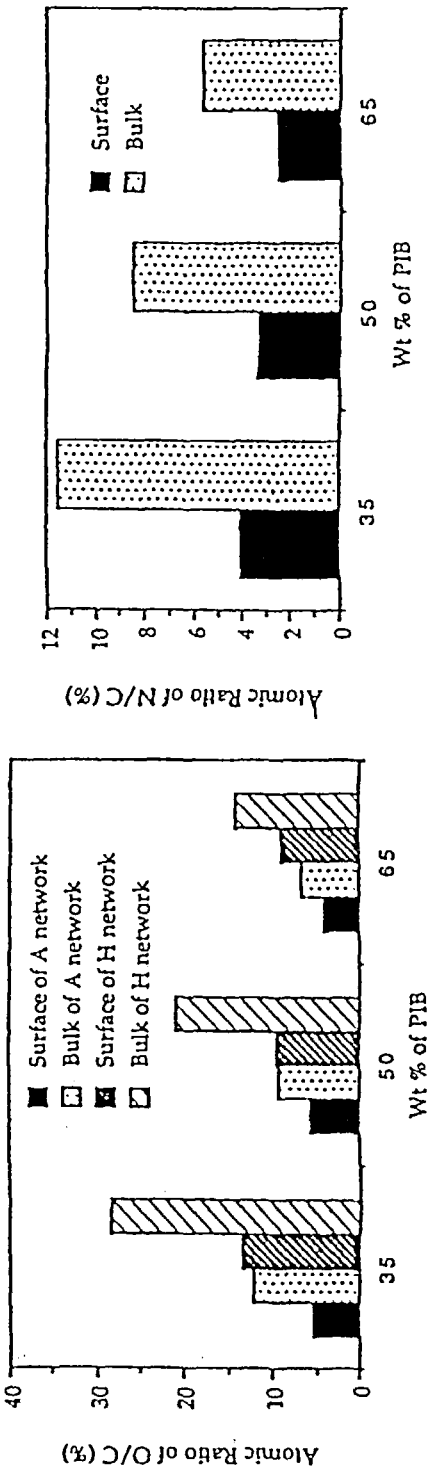


FIG. 2. XPS C1s study of networks. Left: Comparison of O/C ratios between surface and bulk for different compositions of amphiphilic networks; calculated from XPS C1s spectra. Right: Comparison of N/C ratios between surface and bulk for different compositions of A network; calculated from XPS C1s spectra.

TEM and AFM studies also indicated phase separation (see, for example, Fig. 3). Apparently both the hydrophilic and hydrophobic phases form cocontinuous microdomains, suggesting a system of cocontinuous intersecting channels. In the dry state the diameter of both the hydrophilic and hydrophobic domains are in the 10-60 Å range. These conclusions are supported by the results of T_g, swelling and diffusion (transport) experiments.

Table 1 shows T_g data. The DSC traces consistently showed two T_gs, indicating microphase separation and an effect of M_n on T_g (the T_gs increased to a limit with increasing M_n) [7, 8].

The networks were also characterized by dynamic and equilibrium swelling behavior. The attainment of equilibrium swelling in water or heptane can be visually ascertained by noting the disappearance of advancing swelling fronts clearly observable during swelling. The fact that both solvents (heptane *and* water) are able to travel from the surfaces through the bulk to the core also indicates that both the hydrophilic and hydrophobic phases are cocontinuous [7, 8].

The A and H networks displayed clearly visible swelling fronts during swelling, which suggests relaxation-controlled swelling processes. In contrast, the D networks showed no swelling front during swelling, which suggests that swelling is controlled by penetrant diffusion. Figure 4 shows the equilibrium water and heptane content versus composition of four types of networks. Networks with similar composition but higher $M_{n,PIB}$ exhibited higher equilibrium water contents and higher swelling rates due to their lower crosslink density. The degree of swelling for networks of equal overall composition and $M_{n,PIB}$ decreased in the order PDMAEMA ~ PDMAAm >> PHEMA [6].

Diffusion characteristics of our networks were studied by the use of model compounds and dyes. Table 3, for example, shows the amount of theophylline (a sparingly water soluble, 0.83 wt% at 25°C, smooth muscle relaxant) loaded into various networks, the release time, the release exponent n, and the release constant k.

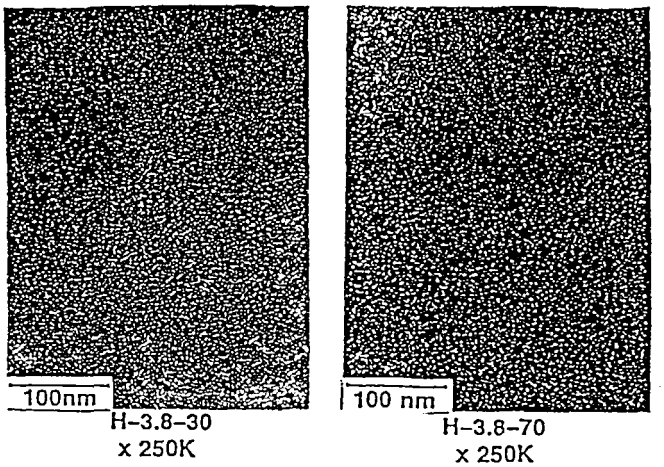


FIG. 3. TEM micrographs of amphiphilic networks.

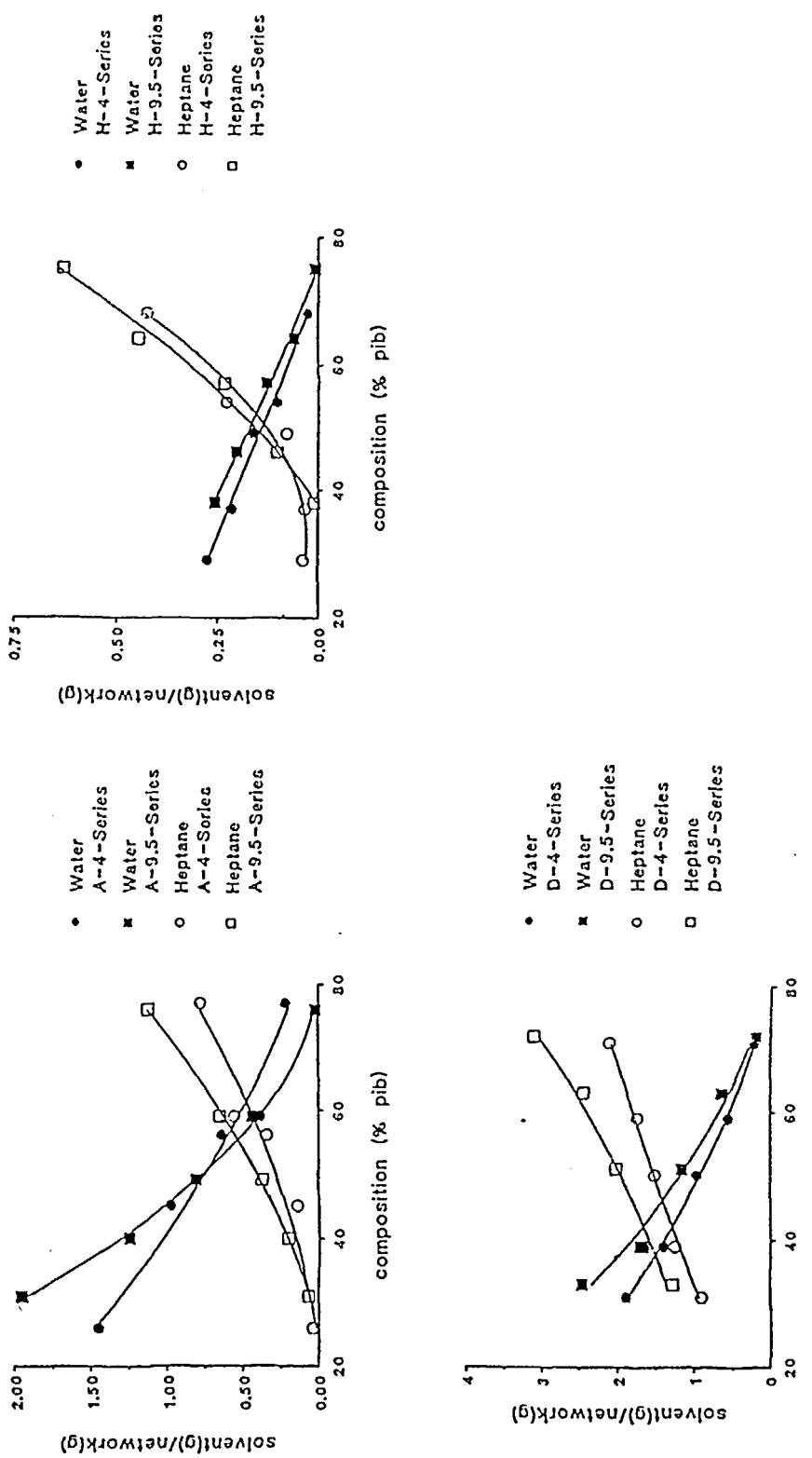


FIG. 4. Equilibrium swelling characteristics of selected amphiphilic networks.

TABLE 3. Kinetic Parameters of Theophylline Release Experiments

Sample	% Loaded	Release time, hours	Release exponent n
A-9.5-31	0.866	6.8	0.532 ± 0.015
A-9.5-40	0.483	16.5	0.779 ± 0.031
A-9.5-49	0.442	23.0	0.753 ± 0.024
A-4-26	0.690	15.0	0.831 ± 0.016
A-4-45	0.588	24.0	0.867 ± 0.027
A-4-56	0.326	68.5	0.744 ± 0.024
H-4-30	0.693	256.5	0.715 ± 0.011
H-4-37	0.443	169.0	0.738 ± 0.006
H-4-49	0.310	9.0	0.518 ± 0.009
D-4-31	2.295	14.0	0.522 ± 0.002
D-4-39	1.797	31.0	0.500 ± 0.001
D-4-50	1.411	35.5	0.056 ± 0.004

The loading levels reflect the swelling behavior of the networks. The swollen networks contain fluctuating water-filled microchannels or pores created by the segmental mobility of the swollen chains through which solute may diffuse. The size and fluctuation of pores are related to the degree of swelling and crosslink density. Samples which contain more hydrophilic component swell more, contain more water, and therefore imbibe more drug. Conversely, by increasing the crosslink density, water swelling and segmental mobility decrease, which in turn decreases the diffusivity and the amount of drug imbibed.

The effect of increasing crosslink density is demonstrated with the A networks: Crosslink densities in the $M_n = 9500$ series are lower than those in the $M_n = 4000$ series, therefore the former networks swell more and are able to imbibe more drug.

The release mechanism has been studied by M_t/M_∞ versus t plots, and the release exponent, n, was determined by

$$\log M_t/M_\infty = \log k + n \log t$$

When $n = 0.5$, diffusion is Fickian; in this case polymer relaxation is fast relative to the influx of the penetrant and transport is controlled by the latter. When $n = 1$, polymer relaxation is slower than the influx of the penetrant and transport is controlled by the rate of polymer relaxation (so-called Case II transport or diffusion); in other words, $n = 1$ indicates zero-order release. When $0.5 < n < 1$, anomalous diffusion exists and the rates of polymer relaxation and penetrant diffusion are comparable.

The D networks yielded $n = 0.500$–0.556, indicating close to Fickian diffusion. These networks did not exhibit the characteristic swelling front which is clearly visible when swelling is controlled by polymer relaxation. The relatively low T_g of PDMAEMA (−6 to −7°C) and high hydrophilicity of this moiety would lead one to expect fast relaxation (i.e., diffusion control by the penetrant diffusion) and Fickian kinetics. Interestingly, the $M_{n,PIB} = 9500$ and 4000 series of the A networks

yielded $n = 0.744$–0.831, indicating so-called anomalous diffusion, i.e., comparable rates of polymer relaxation and penetrant diffusion.

The release exponents obtained for the H networks showed an unexpected trend: while H-4-30 and H-4-37 yielded $n = 0.715$ and 0.738, respectively, indicating anomalous diffusion, H-4-49 gave $n = 0.518$, indicating simple Fickian release. Additional data and a discussion of diffusion phenomena have recently been published [6]. It is clear that the release mechanism of amphiphilic networks is influenced by the composition, crosslink density, and molecular weight of the PIB moiety in the networks.

Scouting experiments have been carried out to study equilibrium water swelling of various networks as a function of pH over the 1 to 14 pH range. Surprisingly, while water uptake by the A-4.5-50 network remained largely unchanged (100 ± 15%) over the entire pH range, that of a S-4.5-50 network exhibited a very large effect [in S networks the hydrophilic moiety is poly(2-sulfoethyl methacrylate) (SEMA)]: water uptake at pH 7 was 340 ± 5%, while at pH 2 and 14 it was 220 ± 5% and 160 ± 5%, respectively. Evidently S networks shrink significantly in both acid and alkaline media.

Mechanical Properties

The tensile strength, modulus and elongation, and hardness of select networks are being investigated. Figure 5 shows stress–strain traces of representative dry H, A, and AS networks (in the AS network the hydrophilic moiety is a random copolymer of DMAAm and SEMA units). The pronounced yield point diminishes with decreasing hydrophilic (increasing PIB) content, i.e., the failure mechanism changes with overall network composition. Not surprisingly, hardness decreases with increasing PIB content, see Fig. 6.

Biocompatibility Studies: Soft-Tissue Compatibility

The biocompatibility of five different D networks was investigated in vivo by implanting into rats dorsally or ventrally [30]. For comparison, four animals had polyethylene (PE) tubing implanted dorsally ($n = 2$) or ventrally ($n = 4$). Another group of animals ($n = 4$) served as controls and had surgery performed and sutures, but no implant. Network D-10-56 showed good tissue integration, with minimal bacterial contamination which can be confounded by sutures.

Table 4 shows gross morphological observations (scores) of the tissue around the implant and the histological scores recorded by light microscopy. The D-10-71 network showed the highest fluid accumulation. The D-10-49 network exhibited minor fluid accumulation but was not significantly different from the PE. Networks D-10-53, D-10-56, and D-10-58 did not exhibit any fluid accumulation.

Networks D-10-49, D-10-53, and D-10-56 showed the least fibrosis and adhesions (even less than PE tubing). Network group D-10-49 showed moderate to heavy lymphocyte infiltration (histological observations) around the implant site as compared to controls or to PE tubing.

Table 4 presents results of the differential white blood cell count and bacterial types present in the blood and implant tissue. The only significant differences (ANOVA) were the low number of monocytes in a network group D-10-49 and low number of lymphocytes in network group D-10-71. However, individual Scheffe's

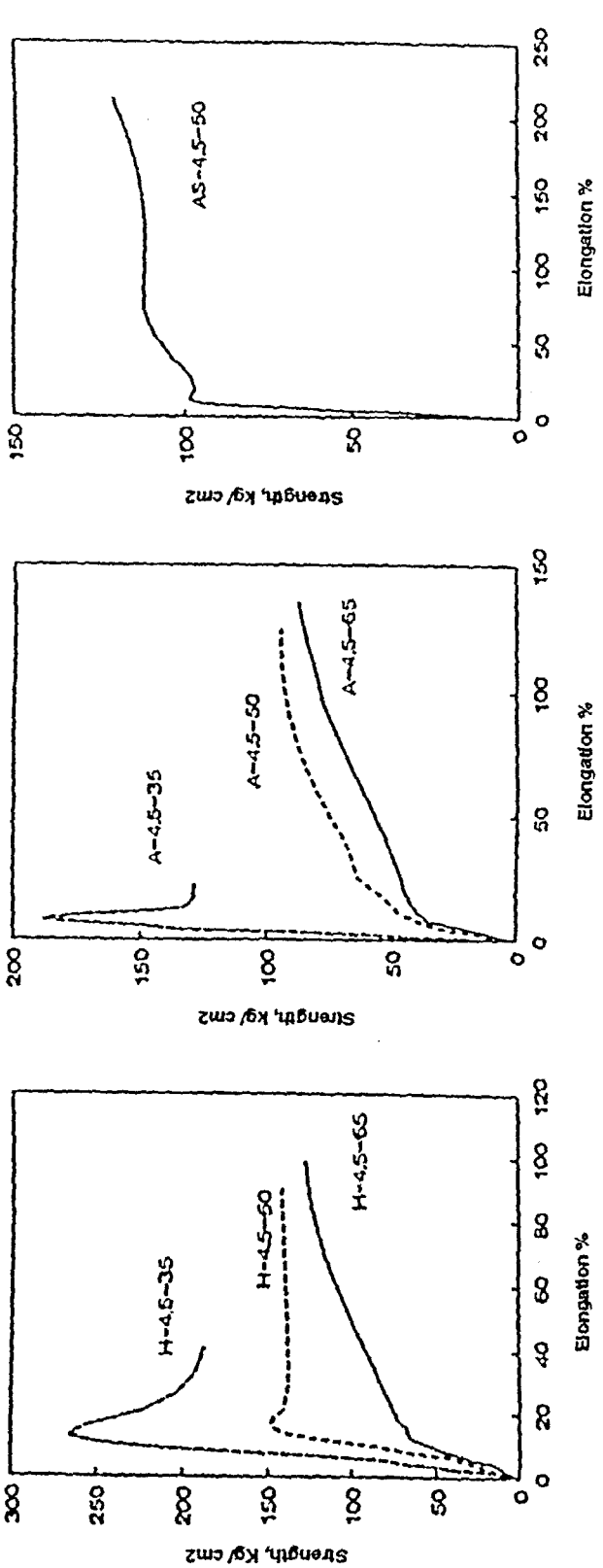


FIG. 5. Stress-strain curves of networks.

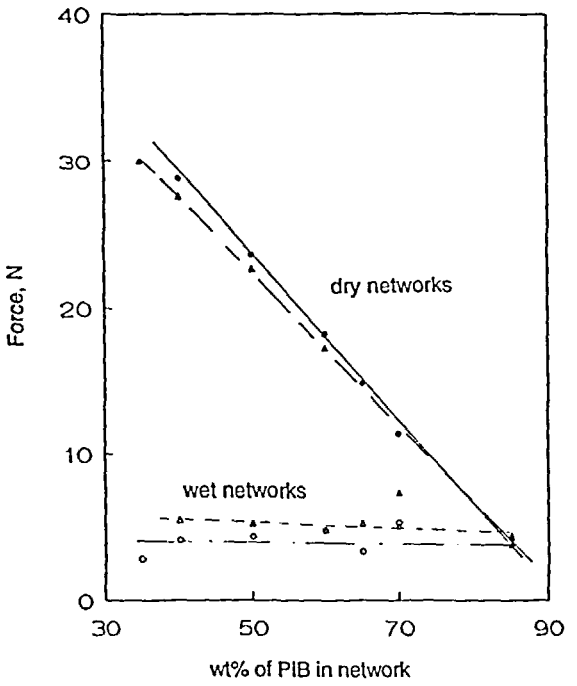


FIG. 6. Hardness as a function of network composition: (●, ○) A networks, (▲, △) H networks.

tests showed no difference between groups. Similarly, there were no significant blood-borne bacterial-type differences between the control of PE tubing and the experimental networks. Also, the tissue did not show any major bacterial-type differences, although the PE tubing and the networks did have more staphylococcus than the controls.

On the basis of both gross morphological and histological analyses, it appears that networks D-10-49 and D-10-71 were less well accepted than networks D-10-53, D-10-56, and D-10-58. The latter three networks exhibited better biocompatibility than PE tubing. The most biocompatible material was network D-10-56, which possesses a balance of hydrophilic and hydrophobic properties [30].

Protein Adsorption under Static Conditions: In Vitro Studies

Protein adsorption from human plasma (100% and 1%) was evaluated on hydrated A and H networks and on the reference materials glass, PE, and silicone rubber (PDMS) using radioimmunoassay [31]. The amphiphilic networks adsorbed less fibrinogen and albumin than glass, PE, and PDMS from 100% plasma (Fig. 7A). Significantly less fibrinogen, Hageman factor (factor XII), and albumin were adsorbed to A and H networks from 1% plasma (Fig. 7B) than to the reference surfaces. The extent of adsorption of factor VIII on A and H networks and glass was very similar. Controls to determine nonspecific adsorption of primary antibodies (AP) and labeled ^{125}I antibody (PBS) exhibited low background binding, indicat-

TABLE 4. Gross Morphological and Histological Scores, and White Blood Cell and Bacterial Count (means, ±SEM) at the Implant Site [30]

Polymer type	Fluid accumulation score	Fibrosis and adhesions score	Vascularity score	Histology score (lymphocyte infiltration)	White blood cells, %					Bacterial type	
					Ensoniphils	Monocytes	Lymphcytes	Bands	Neutrophils	Blood[a]	Tissue[b]
Controls (no implant)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 0.3	15 ± 3	61 ± 3	0.8 ± 0.5	19 ± 2	SE,GNR	GNR + 1
Polyethylene tubing (PE)	0.75 ± 0.5	2.0 ± 0.6	1.5 ± 0.4	0.3 ± 0.3	1.4 ± 0.5	13 ± 2	66 ± 2	0.7 ± 0.3	19 ± 4	Se,SS,GNR	SE + 1 to 13
D-10-19	1.0 ± 0	1.0 ± 0.4	10 ± 0.4	3.5**[t] ± 0.8	2.0 ± 0.6	3.5 ± 1	66 ± 0.5	0 ± 0	2.8 ± 0.8	SS,SE,GNR	GNR + 1
D-10-53	0 ± 0	1.0 ± 0.6	1.5 ± 0.9	0.5 ± 0.3	0.8 ± 0.5	13 ± 2	56 ± 9	0.3 ± 0.3	31 ± 0.8	SE,GNR,SS	SE +1 to +4
D-10-56	0 ± 0	1.0 ± 0.6	0.5 ± 0.3	0 ± 0	1.0 ± 0.4	12 ± 1	62 ± 4	0.8 ± 0.5	25 ± 4	SS,P	P +1 to 13
D-10-58	0 ± 0	2.0 ± 0.6	1.5 ± 0.3	0 ± 0	1.3 ± 0.5	17 ± 5	55 ± 4	0.8 ± 0.5	27 ± 4	SS,P	SE 12 C +1
D-10-71	3.0* ± 0.1	4.0** ± 0.4	2.0* ± 0.4	0.5 ± 0.3	0.3 ± 0.3	21 ± 1	19 ± 1	1.5 ± 0.5	29 ± 1	SE,GNR	SE 11 to +4
ANOVA-F ratio	37.36	5.5	2.5	11.4	1.11	3.51	2.85	1.44	2.25		SE +1 to +4
DF	27	27	27	27	31	31	31	31	34		GNR +1
Probability	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.01$	N.S.	.012	.029	N.S.	N.S.		SE +1

*$p < .05$, **$p < 0.1$ compared to controls, [t]$p < .05$, [t]$p < .05$ compared to PE tubing group, means, ±SEM.

[a]Bacterial abbreviations: SE = *Staphylococcus epidermidis*, SS = *Staphalococcus saprophylicus*, P = *Pseudomonas*, GNR = gram negative rod.

[b]Scoring system: +1 = 25% plate growth, +2 = 50%, +3 = 75%, +4 = 100%.

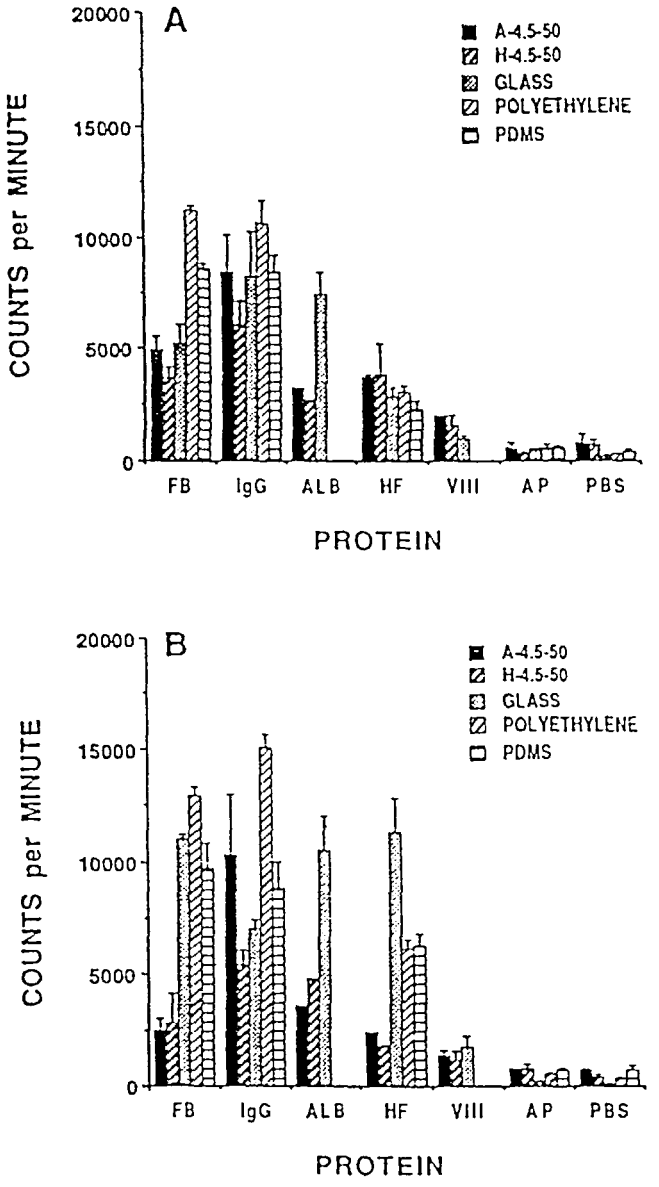


FIG. 7. Protein adsorption for 100% human plasma (A) or 1% human plasma (B) after 60 minutes to polymer surfaces using antibodies to human proteins: fibrinogen (FB), immunoglobulin G (IgG), albumin (ALB), Hageman factor or factor XII (HF) and factor VIII/von Willebrand factor (VIII). Controls include alphafetoprotein (AP) and phosphate-buffered saline (PBS). Results are expressed as surface counts per minute ± standard deviation of two separate experiments with duplicate polymers evaluated for each protein.

ing specificity and sensitivity of the assay. The detection of IgG from either 100% or 1% plasma on A network was appreciable: greater or equal to than glass, less than PE, and equivalent to PDMS. Network H showed less adsorption of IgG than one of the other materials. Overall, these data are significant since reduced protein adsorption to the amphiphilic networks is a desirable property, particularly with reduced adsorption of coagulation factors fibrinogen and Hageman factor. These proteins are important in thrombosis, with fibrinogen being particularly important as an adhesive protein for blood leukocytes and platelets. Thus, reduced adsorption of these proteins to biomaterials such as these amphiphilic networks may significantly reduce thrombus formation.

Human Monocyte Adhesion

Another index of biocompatibility is the evaluation of cell adhesion or antiadhesion. Thus, we have determined the adhesion of one circulating blood cell type, the human monocyte (MC). Peripheral blood MC were isolated by a nonadherent method [31–34]. In some experiments, culture wells were adsorbed with plasma or serum, washed twice to remove unadsorbed components, and MC were then added. As an alternative approach which more closely simulated blood contacting biomaterials upon implantation, MC were added in the presence of various proteins. Adhesion was measured spectrophotometrically by Giemsa staining [31] of adherent MC following an appropriate incubation period and removal of nonadherent cells by washing. According to the results shown in Fig. 8, there was significant inhibition of monocyte adhesion after 4 hours to networks A and H and to glass as compared to a positive adhesive surface, tissue culture polystyrene (TCPS). These data further support the biocompatibility of these amphiphilic materials at blood and tissue interfaces since monocyte adhesion and activation may lead to the production of

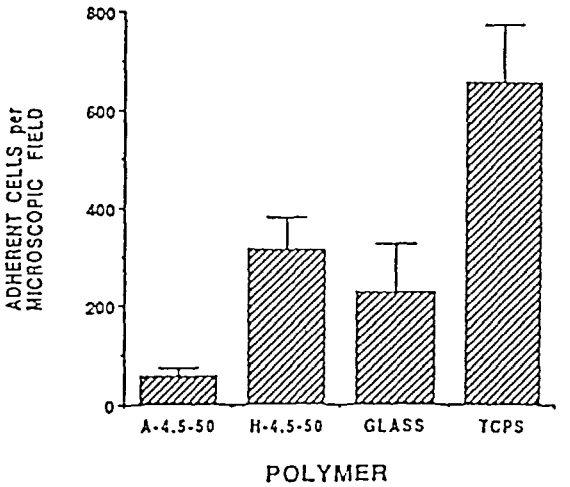


FIG. 8. Human blood monocyte adhesion to polymer surfaces after 4 hours incubation in RPMI medium with 1% BSA. Initial monocyte concentration was 2×10^5/mL. Bar graphs depict mean number of adherent cells per microscopic field (1 field = 0.43 mm^2) ± SD of two separate experiments.

cytokines and resultant fibrous tissue formation [32–35]. Our new amphiphilic networks may provide for less adsorption of proteins, which will ultimately lead to reduced adhesion of these inflammatory cells.

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This research could not have been accomplished without a grant by the NSF (DMR-89-20826).

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