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Preparation and biocompatibility of novel polar-nonpolar networks

I. Synthesis, characterization and histological-bacterial analysis of mixed polytetrahydrofuran-polyisobutylene networks

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ABSTRACT

Mixed polar-nonpolar urethane networks have been prepared by crosslinking various mixtures of polyisobutylene diols (HO-PIB-OH) and polar polytetrahydrofuran diols (HO-PTHF-OH) with stoichiometric quantities of a triisocyanate. Essentially complete crosslinking was demonstrated by very low (<4%) sol fractions in benzene and THF. The mixed networks were biocompatible and had less tissue response than pure PIB as evaluated by bacterial and histological analysis after 11 weeks of implantation at the following sites: dorsal neck muscle, abdominal cavity and near the abdominal aorta.

INTRODUCTION

Systematic research in our laboratories concerns the design and synthesis of unique materials exhibiting a combination of useful chemical-mechanical properties. Recent research resulted in the preparation of PIB-based urethane elastomers and foams by reacting hydroxy-telechelic PIBs with various isocyanates [1-7]. An extensive investigation of PIB-based polyurethanes indicated outstanding oxidative, thermal and hydrolytic stability [1,2,3]. Recently we have succeeded in the synthesis of novel amphiphilic networks, i.e., networks that comprise water-soluble and water-insoluble sequences, and found that within a certain composition range mixed PIB-poly-(dimethylamino ethyl acrylate) networks exhibit not only good mechanical properties and unusual membrane characteristics, but also good in vivo biocompatibility [8,9]. Encouraged by these findings we have now expanded research in this field and have prepared novel polar/nonpolar networks combining PIB and PTHF sequences and investigated their biocompatibility. PTHF-based urethanes have received recognition in recent years as biocompatible materials [10], e.g. as coatings of pacemaker leads, artificial heart bladder. This paper concerns synthesis and characterization details of mixed PIB-PTHF networks together with their biocompatibility as evaluated by histological and bacterial analysis. A future report will concern physical-mechanical properties.

EXPERIMENTAL

Materials

Narrow molecular weight distribution (MWD) α,ω-dihydroxy polyisobutylenes (HO-PIB-OH) were prepared from the corresponding α,ω-di-tert-chloro PIB, obtained by living polymerization

of isobutylene [11-13] followed by routine quantitative dehydrochlorination and hydroxylation [14]. The HO-PIB-OHs were purified by precipitation from CH_2Cl_2 solutions into CH_3OH/H_2O (80/20) mixtures. The HO-PIB-OHs thus obtained were characterized by GPC, and IR and ^1H NMR spectroscopy [15]. Number average molecular weights $\bar{M}_n$ and MWDs were determined by GPC. The instrument comprised a Waters Model M-6000 A pump, Waters μ-Styragel columns of 10^5, 10^4, and 10^3, 500, 100 A and a Waters Model 410 differential refractometer. The flow rate was 1 mL/min in tetrahydrofuran (THF). The calibration curve was constructed with narrow MWD ($\bar{M}_w/\bar{M}_n$ = 1.1) PIB samples of various molecular weights.

The PTHF-diols (HO-PTHF-OH) were obtained from E. I. DuPont (Terathane) and were used as received. Triphenylmethane triisocyanate (TTI, Desmodur R, Mobay Chemical) was received as a 20% solution in CH_2Cl_2. It was purified in a dry box under nitrogen by precipitating in dry n-pentane to remove insoluble oligomer, then distilled under high vacuum. The distillate was a yellow viscous liquid which on storage crystallized to a waxy solid. It was stored under dry nitrogen at -20°C. The purity of TTI was checked periodically by di-n-butylamine/HCl titration (ASTM D1638) and was found to be close to 100% over 2 years of storage. The THF was refluxed over Na-benzophenone overnight and distilled under dry nitrogen before use. N-pentane was refluxed over CaH_2 for 2 days, distilled, and stored under dry N_2. Reagent grade benzene and THF were used for swelling and extraction studies. Triethylene-diamine (DABCO), Aldrich Chemical Co.) was used as received and stored in the dry box under dry N_2.

Procedures

The networks were prepared under a dry N_2 atmosphere in a dry box. Preweighed mixtures of HO-PIB-OH and HO-PTHF-OH were prepared in 125 mL Erlenmeyer flasks and dried in a vacuum oven at 50°C until weight constancy. A stoichiometric quantity of TTI relative to the total -OH content was added to the diol mixture and the system was diluted with THF to 30-35 wt% solid content. A few crystals (∿0.005g) of DABCO were introduced to accelerate crosslinking. The system was stirred manually by rotating the flasks until it reached a honey like viscosity (30-45 min). The viscous liquid was poured into the cavity of an open Teflon mold (10 x 10 x 0.5 cm). The mixture should pour like honey prior to transferring it into the mold; premature transfer results in phase separation (opacity, patchy appearance during crosslinking).

The network films were allowed to cure for one day at room temperature in the dry box, then for one day at ambient in a vacuum oven, and finally for 3-4 days at 60°C in a vacuum oven. Some cracking was observed during film formation, particularly with the low $\bar{M}_n$ prepolymers, due to stresses that develop in these two-phase crosslinked systems.

The amount of soluble (sol) fractions were determined by extraction of two samples each with benzene and THF, both good solvents for PIB and PTHF. Thus two preweighed samples of known dimensions (∿1.0 x 0.5 x 0.04 cm) were immersed in benzene or THF at room temperature and the swollen samples were

blot dried and weighed every 24 hrs until weight-constancy was reached. Then the samples were dried in a vacuum oven at ambient temperature (weight constancy in about 2 days) and their dry weights determined. The $\bar{M}_c$ (number average molecular weight between crosslink sites) was calculated from the amounts of PIB and PTHF used for the synthesis and assuming complete crosslinking. $\bar{M}_c$ values could not be directly determined by the conventional Flory-Rehner method [16] since solvent interaction parameters for composite mixed networks have not been developed.

Biocompatibility

To test the biocompatibility of the polymers listed in Table I, a group (n=20) of 4-6 month old male spontaneously hypertensive rats (Harlan-Sprague Dawley, Indianapolis, IN) were implanted dorsally, intraperitoneally and next to the abdominal aorta with 1 of 3 polymers (Table I, n=4/group). The networks were sterilized in an autoclave and the surgery was performed using sterile techniques. After a barbiturate anesthetic (50 mg/kg, Brevital, I.P., Eli Lilly, Indianapolis, IN) was given, the dorsal implant (4 x 4 mm) was placed in the superficial neck muscles 1.5 cm from the base of the skull using 4-0 silk suture (Ethicon, Inc., Somerville, N.J.) to anchor the implant and close the incision. The peritoneal implant (4 x 4 x 1 mm) was inserted and anchored to the inside of the peritoneal wall approximately 3-4 cm superior to the penis using the same type of suture. The aortic implant (4 x 4 x 1 mm) was attached with 4-0 silk suture to the tissue surrounding the abdominal aorta at the level of the kidney. The control rats had 4-0 silk suture only implanted at the three sites. Although the suture could be a possible source of a sinus tract infection, it is unlikely this occurred since the control animals with surgery but no implant were found to have the same type of bacteria. Another group of animals (n=4) served as controls for blood analyses and had no implant. Body weight was measured at the time of surgery and 2 months later after implant removal. The animals were housed 6-8 to a cage (40 x 50 x 20 cm) with wood shavings for bedding changed once per week and Purina Lab Chow and water provided ad libitum. After 11 weeks the implants were surgically removed using sterile technique and without knowledge of polymer type. Gross morphological observation of the implant site was recorded at this time. Notation was made of fluid accumulation around the implant (0 = no fluid, +5 = maximum fluid); fibrosis and adhesion of the tissue to the implant (0 = no fibrosis or adhesion, +5 = maximum amount); and new vascularity directly around the implant (0 = none, +5 = maximum amount). Two 20-30 mg tissue samples were taken, one for histological analysis and one for bacterial culture. A heart puncture blood sample was taken for a differential blood count and bacterial culture at the time of termination.

Tissues for bacterial culture were aseptically ground in sterile broth and inoculated onto tryptic soy agar, 5% sheep blood agar, chocolate agar(Difco) and brain heart infusion agar (Difco). Duplicate plates were incubated for 1-7 days in a regular aerobic incubator, a candle jar, a CampyPak jar (Becton Dickinson, BBL Microbiology System, Cockeysville, MD), and a

GasPak jar (BBL). The remaining fluid and tissue were inoculated into thioglycollate broth. Bacteria were identified by Gram stain, hemolysis, colonial morphology, and standard biochemical tests. The test performed for Staphylococcus were catalase, coagulase, novobiocin, and mannitol salt. Streptococcus was identified using catalase, CAMP test, optochin, bacitracin, bile esculin, and 6.5% NaCl. Gram negative rod identification included MacConkey, oxidase, citrate, indole, urease, motility, and triple sugar agar. Gram negative rods which could not be identified with these tests were inoculated into ApI strips (Analytab Products, Plainview, NY). Lymphocytes were quantified by Hematoxylin and Eosin stain (H and E stain) using the 40X objective of a light microscope. The following percentages refer to the number of lymphocytes observed divided by the total number of cells on the microscope slide studied: 0 = no lymphocyte infiltration, +1 = slight ($\sim$10%) lymphocyte infiltration, +1 to +3 = moderate (15-40%) lymphocyte infiltration, +4 = large ($\sim$50%) infiltration, and +5 = most of the tissue section ($\sim$75%) showing lymphocyte infiltration. Standard differential white blood cell counts of 100 white blood cells were performed using blood smears (two per animal) stained with Wright's stain. One month after implant all rats were placed on two different antibiotics for a week due to a respiratory infection in the entire rat colony.

Statistical evaluation was performed using one-way analysis of variance (ANOVA) followed by Tukey's test for multiple equal sample sizes of Scheffe's test for multiple comparisons of unequal sample sizes. Statistical significance was assumed if $p < 0.05$.

RESULTS AND DISCUSSION

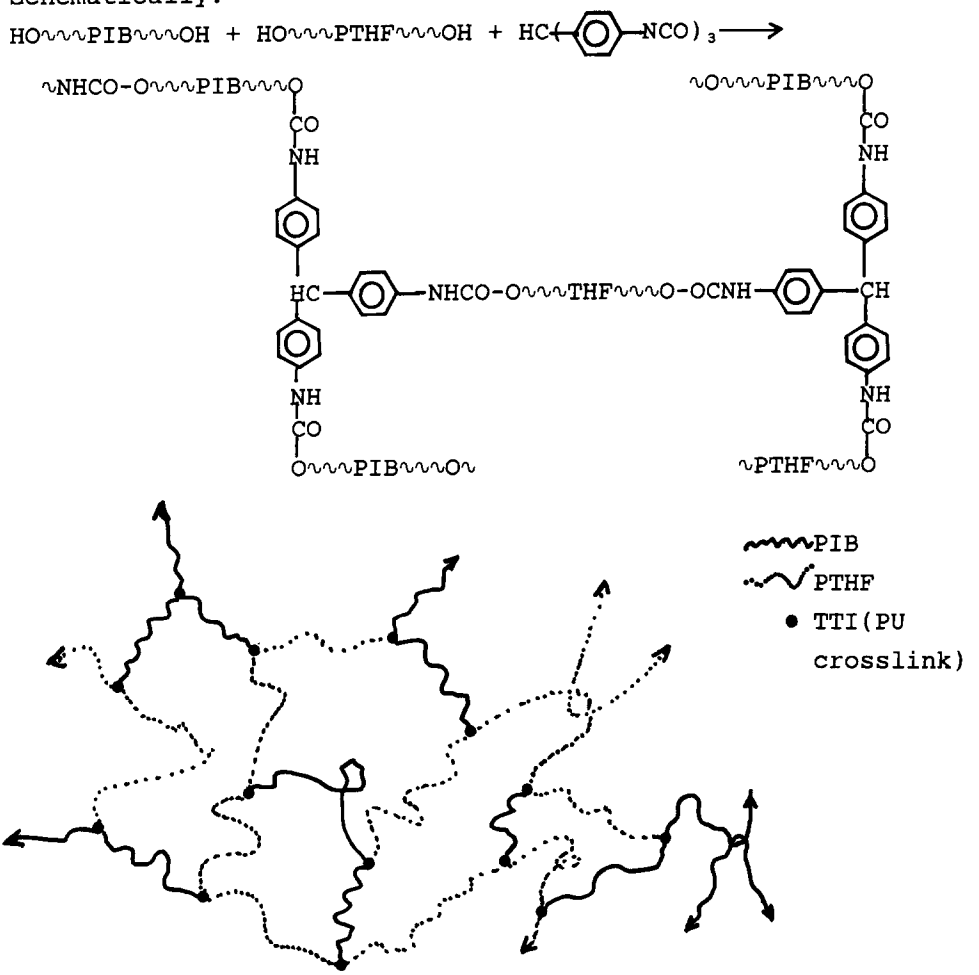
Both PIB and PTHF are well-known, respectively nonpolar and polar elastomers, FDA approved for certain biomedical applications. In view of their advantageous combination of physical-mechanical-biological properties it was of interest to prepare randomly crosslinked urethane networks of HO-PIB-OH and HO-PTHF-OH, and to explore their properties. We visualized that the synthesis could be readily accomplished by crosslinking various mixtures of HO-PIB-OH and HO-PTHF-OH by the use of stoichiometric amounts of a triisocyanate in the common good solvent THF in the presence of a small quantity of catalyst.

Table I shows the $\bar{M}_n$ of the starting prepolymers, the overall composition of the mixed networks, the calculated $\bar{M}_c$, and the amount of the sol fractions obtained by benzene and THF extractions. The sol fractions were invariably very low in both solvents. In view of the very low amounts of extractables (<4%) the networks are regarded essentially completely crosslinked (minor amounts of dangling chain ends).

Biocompatibility

Table II shows the differential white blood cell count for each of the groups. Analysis of variance (ANOVA) showed no significant differences between groups for any of the white blood cells. Thus the implants did not promote infection or elicit any significant inflammation response as compared to controls. Table III shows the gross morphological and

Schematically:



Scheme I. Randomly mixed urethane network of HO-PIB-OH and HO-PTHF-HO-PTHF-OH Crosslinked by TTI

histological results of each group. There was no fluid accumulation or new gross vascular growth at any of the sites or within any group. However, ANOVA suggested differences between polymer groups with regard to fibrosis and adhesions in the abdominal and dorsal implant sites ($p < .01$). Post-hoc significance tests (Scheffe's) showed that PIB produced significantly more fibrosis than the controls ($p < .05$) in both the abdominal and dorsal sites. This may be due to the nonpolar hydrophobic nature of PIB. All three materials at the dorsal site showed a mild increase in fibrosis compared to the controls ($p < .05$). This mild form of fibrosis is to be expected with any implanted material and no real biological differences appeared between the three materials. There were no significant differences in fibrosis between groups at the aorta site.

TABLE I

Network	Prepolymers $\bar{M}_n$ ($\bar{M}_w/\bar{M}_n$)		Mixed PIB/PTHF Networks		$\bar{M}_c$ g/mL	Sol. Fraction(%)*	
	PIB	PTHF	wt.%	mole%		C H.	THF
** 1	2,700(1.6)	650	40/60	14/86	940	2.7	3.3
2	2,700(1.6)	2900	50/50	50/50	2,800	1.6	2.3
** 3	5,300(1.5)	650	50/50	10/90	1,100	3.2	3.9
4	5,300(1.5)	2900	50/50	35/65	3,700	3.8	4.1
5	1,500(1.15)	2900	50/50	66/34	1,980	1.6	1.9
6	1,500(1.15)	2000	50/50	56/44	1,720	1.6	1.7
7	1,500(1.15)	1000	50/50	39/61	1,200	0.6	0.8
8	1,500(1.15)	650	50/50	30/70	900	1.0	1.1

* Average of two extractions

** Networks implanted in rats, see Tables II-IV

TABLE II

INFECTION INDICATORS: WHITE BLOOD CELL COUNT (PERCENTAGE)

Group	Eosinophils	Monocytes	Lymphocytes	Bands	Neutrophils
Controls	2.6 ±0.7	15.5 ± 3.2	39.0 ± 2.5	2.6 ±0.8	40.3 ± 1.9
Polymer 1	4.6 ±3.9	19.0 ± 2.4	26.0 ± 9.7	1.1 ±0.5	49.3 ± 7.8
Polymer 3	0.6 ±0.1	23.3 ± 5.0	39.0 ±10.0	1.1 ±0.5	36.0 ± 9.0
PIB control	1.6 ±0.6	28.3 ± 3.4	32.0 ±.6.0	2.8 ±0.7	35.3 ± 3.3
ANOVA					
DF	16	16	16	16	16
F	0.74	2.49	0.78	1.67	1.08
P value	1.0 N.S.	0.11 N.S.	1.0 N.S.	0.22 N.S.	0.39 N.S.

TABLE III

GROSS MORPHOLOGICAL AND HISTOLOGICAL SCORES AT THE IMPLANT SITE

Polymer Type	Fibrosis Adhesions Score			Histology Score (lymphocyte infiltration)		
	Abdominal	Dorsal	Aorta	Abdominal	Dorsal	Aorta
Suture Controls	0.10 ±0.10	0.2 ±0.10	0 ±0	0.50 ±0.29	0.13 ±0.10	0.38 ±0.20
Polymer 1	0.5 ±0.5	1.5* ±0.5	2.3 ±0.3	1.75 ±0.5	1.13 ±0.6	2.33 ±0.3
Polymer 3	2.0 ±1.2	2.0* ±0	1.75 ±1.0	1.5 ±0.6	0.63 ±0.2	1.25 ±0.5
PIB control	4.3* ±0.5	1.67* ±0.3	2.0 ±1.6	1.13 ±0.6	1.0 ±0.5	1.25 ±1.3
ANOVA F-Ratio	8.46	8.61	1.95	1.10	1.13	2.73
DF	16	15	15	15	14	14
Probability	p <.01	p <.01	N.S.	N.S.	N.S.	N.S.

* = $p < .05$ compared to controls

The histology score which examined lymphocyte infiltration at the implant site did not show any significant differences between groups or implant sites. The only notable difference between the three polymers appeared to be the increased fibrosis scores of the PIB implant. The two mixed networks appeared about equal in terms of tissue responses. Also body weight gain was similar between all groups (4.7 - 6.4 gms/week).

Table IV shows the implant sites which were contaminated with bacteria. One rat (#2) receiving mixed network 1 (see Table I) developed an infection at the abdominal and aorta sites. Rat #8, which received mixed network 2 (see Table II) had infections in the three implant sites. However, the rats had no visual signs of infection and their WBC differentials were not significantly different than that of the uninfected rats. This indicates that the infections were being contained and would probably have been eliminated if the animals had not been terminated. It seems that none of the three polymers promote infections. Interactions of biomaterials with bacteria and tissue cells are directed by specific receptors, surface molecules, atomic geometry and electronic state of the biomaterial surface (16). It appears that both of the implanted networks with 40% or 50% PIB allowed good tissue integration with minimal bacterial contamination.

TABLE IV

Bacterial Isolates[A]

Polymer	Rat	Abdominal Tissue	Aorta Tissue	Dorsal Tissue
Polymer 1	1	1+ Enterococcus[B]	N D [C]	NG[D]
	2	2+ Staphylococcus epidermidis	1+ Enterococcus	
		1+ diphtheroid[E]	1+ E. coli, 1+ GNR[F]	NG
	10	NG	Few Pseudomonas aeruginosa	
			NG	1+ Staph epidermidis
Polymer 3	7	NG	NG	1+ Staph epidermidis
	8	3+ E. coli	2+ E. coli	2+ Enterococcus
		3+ Alpha Streptococcus, not Group D		
	3	2+ GNR	NG	2+ Staph epidermidis
PIB control	16	2+ Gamma Streptococcus, not Group D	NG	NG
	17	NG	NG	N.F. [G]
Control[H]	11	NG	NG	NG

A Only includes isolates which were isolated in a concentration greater than 1+ on solid medium
B Enterococcus = Alpha, Beta or Gamma Streptococcus, Group D (enterococcus)
C N.D. = not done
D NG = no growth or growth less than 1+
E diphtheroid = nonpathogenic Gram positive rod
F GNR = Gram negative rod, not Pseudomonas or Enteric
G N.F. = Polymer not found
H Control = sutures implanted only

CONCLUSION

Polymer networks containing 40% or 50% PIB were equally biocompatible in rat muscle, abdominal and vascular tissues after 11 weeks. These networks elicit less tissue reaction in the form of fibrosis than pure PIB. It appears that a balance of hydrophilic/hydrophobic properties may be required for satisfactory biocompatibility to arise.

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