ELSEVIER

Reactive Polymers 25 (1995) 101–109

Reactive polymers

Degradable biomaterials with elastomeric characteristics and drug-carrier function

B.I. Dahiyat, E.M. Posadas, S. Hirosue, E. Hostin, K.W. Leong *

Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD 21218, USA

Accepted April 10, 1994

Abstract

The design of drug-carrying elastomers based on poly(phosphoester-urethanes) (PPUs) is presented. Bis(2-hydroxyethyl)phosphite and bis(6-hydroxyhexyl)phosphite were used as the chain extenders and 1,4-butane diisocyanate was the basis of the hard segment. The labile phosphoester linkage in the backbone of the PPU confers biodegradability on the polymer. Using the reactive phosphite side chain in the PPUs, *p*-aminosalicylic acid and benzocaine were attached pendantly to the polymer with or without a spacer. In vitro release of both drugs was complete in several hours. In contrast, ethambutol incorporated into the backbone of the polymer was released in over 10 days. Preliminary cytotoxicity of the drug-carrier to a macrophage cell line was also assessed.

Keywords: Polyurethane; Poly(phosphoester); Biodegradable polymer; Controlled release; Pendant delivery

1. Introduction

Compared to metals and ceramics, polymers are more versatile and more amenable to custom design. An interesting feature of polymeric biomaterials is the possibility for biodegradability. Biodegradable polymeric devices suggest interesting routes of tissue repair and eliminate the need for surgical removal. Another unique characteristic of polymers is their viscoelastic properties, which have been taken advantage of in designs of artificial hearts, catheters, vascular grafts, artificial skin, pacemaker wire insulation, etc. In addition, the drug-carrier capacity and the structural support function of polymeric biomaterials can be readily combined. While there has been ample research on biodegradable polymers and elastomers, separately, there has been little work done on biodegradable elastomers. Such materials can find medical applications in balloon angioplasty, coating of implantable devices, sutures, and any temporary scaffolds which require elastomeric characteristics. The objective of this study is to explore the plausible design of biodegradable elastomers with controlled-release capability.

When one thinks of medical elastomers, polyurethanes come to mind [1]. Through variations of the soft and hard segments and the chain extenders, polyurethanes can exhibit diverse physicochemical properties. However, used in a large number of clinical applications, they are designed to be inert and stable in the body. Although recent studies show that polyurethanes,

* Corresponding author.

especially the aliphatic ones, undergo oxidative degradation in vivo [2,3], the urethane and ether bonds are quite stable in a nonenzymatic aqueous environment. We introduce biodegradability by incorporating a hydrolytically cleavable phosphoester bond into the backbone of the polyurethane.

Biodegradable polyurethanes have been proposed and studied before [4]. The difference in our study is the inclusion of a phosphoester linkage in the chain extender instead of a commonly used polyester component in the soft segment. We have previously reported the synthesis and characterization of these poly(phosphoester-urethanes) (PPUs) [5]. With 1,4-butane diisocyanate as the hard segment component and bis(2-hydroxyethyl)phosphite (BGP) as the chain extender, these PPUs exhibit elongation up to 80% and a tan δ near 0.15. The degradation rate of these polymers can be controlled by the content of the phosphoester diol BGP. A PPU with 12.5 mol% BGP loses 20% mass by 3 months while a PPU with twice the BGP content loses 40% mass in the same period.

Like any other biodegradable polymers, these PPUs can be used for drug delivery via a diffusion-controlled or matrix-degradation-controlled mechanism. To take advantage of the reactable side chain of BGP, however, in this paper we covalently coupled drugs to the PPU for a pendant delivery system. In addition, to take advantage of the facile reaction between an isocyanate and an alcohol, we incorporated a diol drug into the polyurethane backbone. After confirmation of the chemical structure of these polymer–drug conjugates, their release behavior was studied in vitro. Anticipating the use of these PPUs in microspheric form, we also screened their cytotoxicity with a macrophage cell line.

2. Materials and methods

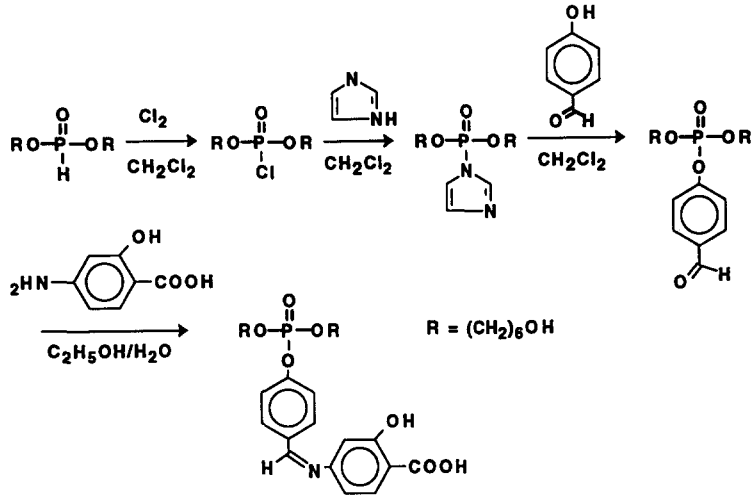
2.1. Monomers

All reagents were obtained from Aldrich unless otherwise noted. All solvents were dried over molecular sieves before use. Bis(2-hydroxyethyl)phosphite (BGP) and bis(2-hydroxyhexyl)phosphite (BHP) were synthesized as described previously [6]. Briefly, dimethylphosphite was reacted with ethylene glycol or 1,6-hexanediol in a 1 : 4 molar ratio, initially at 90°C and slowly rising to 120°C over 8 h. The reaction was then maintained at 120°C for 2 days at a house vacuum of 200 mm Hg to remove the methanol. The temperature was then raised to 140°C while a high vacuum was applied for 3 h to remove the unreacted diols and impurities. The products were colorless viscous liquids. 1,4-butane diisocyanate (BDI) was synthesized via a Curtius rearrangement [5,7]. Adipic dihydrazide was oxidized in aqueous nitrous acid forming the diacyl azide. Upon heating in benzene at 70°C overnight, the azido compound eliminated two equivalents of nitrogen resulting in BDI, which was purified by distillation at 65°C and 0.4 mm Hg.

Coupling of a spacer 4-hydroxybenzaldehyde (HBA) to BHP via a P–O bond was achieved by adapting the method of Penczek and colleagues [8], and attachment of *p*-aminosalicylic acid (PAS) to the spacer was described previously (Scheme 1) [5]. Briefly, BHP was chlorinated in methylene chloride, followed by reaction with a 2 M excess of imidazole at room temperature. After removal of the excess imidazole, HBA was added and allowed to react for 2 days. After isolation, the BHP–HBA complex was reacted with an equimolar amount of PAS in absolute ethanol for 30 min. The product was a bright orange viscous liquid readily soluble in polar solvents.

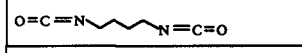
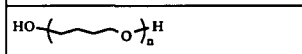
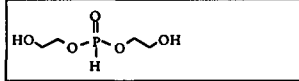
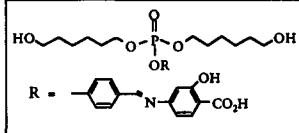
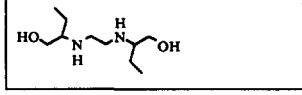
2.2. Polymers

The compositions of the PPUs synthesized are shown in Table 1. Polymerization was a two-step reaction under nitrogen atmosphere in dry dimethylacetamide (DMAc). Fifty mole percent polyol as soft segment was added to 1 g of BDI and heated to 90–110°C for 3 h. Either poly(tetramethylene oxide) MW 1000 (PTMO-1000) or poly(ethylene glycol) MW 400 (PEG-400) was used as soft segment after drying at



Scheme 1.

Table 1
Compositions of PPUs studied

Reactant	Composition of Polymers (mole ratio of reactants)			
	PPU-B1	PPU-B2	PPU-B5	PPU-H2
	4	2	4	2
	-	-	2	-
	2	1	-	1
	1	1	1	-
	-	-	-	1
	1	-	1	-

60°C in a vacuum oven overnight before use. A solution of chain extenders was then added containing the balance of diol needed to react with the diisocyanate and heating was continued for 6 h. The reaction mixture was quenched into ethyl ether, and the product was isolated as a solid by filtration or as a syrup by centrifugation.

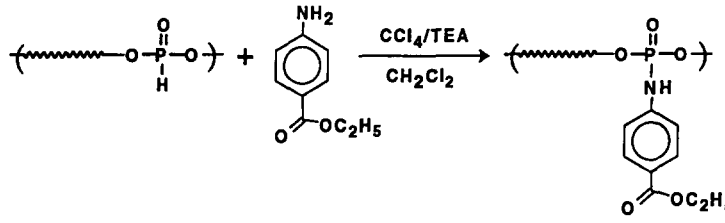
Benzocaine was coupled to PPU-B2 and PPU-B5 (Scheme 2) using the Todd reaction. For example, 67 mg of benzocaine, 760 mg of PPU-B5 and 42 mg of triethylamine were dissolved in 15 ml of CH_2Cl_2 and 10 ml CCl_4, and stirred overnight at room temperature. The solvent was evaporated and the product, a tacky, dark solid was washed exhaustively with ether to remove unconjugated benzocaine. The polymer–benzocaine conjugate was vacuum dried overnight prior to use.

2.3. Microparticle fabrication

Microparticles were formulated from PPU-H2 by a solvent evaporation process optimized to yield particles 3 μm or less in size. One hundred mg of polymer was dissolved in 10 ml $CHCl_3$, which was then emulsified with a 0.2% aqueous solution of Tween 80 in pH 7.4 phosphate buffer using a cell homogenizer for 30 s. The resulting oil in water emulsion was then placed in a rotary evaporator to remove the organic solvent. The resulting aqueous suspension of microparticles was lyophilized. Size distribution was assessed by scanning electron microscopy.

2.4. Characterization

Gel permeation chromatography (GPC) was performed on a Hewlett Packard (HP) 1090M



Scheme 2.

liquid chromatograph. DMF, at 1 ml/min flow rate and 60°C, was used as the mobile phase to elute the sample through two mixed pore size polystyrene gel columns (Polymer Laboratories) in series. The system was calibrated with monodisperse polystyrene standards (Polymer Laboratories). A HP 1037A Refractive Index detector and HP Chemstation software acquired and analyzed the chromatographs. Fourier transform infrared spectroscopy (FTIR) was performed on a Perkin–Elmer 1600 Series machine and samples were prepared by film casting on NaCl plates or using KBr pellets. Proton and ^{31}P NMR were performed on a Bruker AMX300 in $CDCl_3$, d6-DMSO or D2O.

2.5. In vitro drug release

Release kinetics of benzocaine from PPU-B5 was followed by placing 2 ml of a polymer–drug conjugate solution (10% in pH 7.4 phosphate buffer) in dialysis tubing that has a 6000 molecular weight cut off and dialyzing against 40 ml of pH 7.4 phosphate buffer at 37°C. The 40 ml of buffer was analyzed for drug by UV spectrophotometry (absorption max. 282 nm) periodically. Release of benzocaine from PPU-B2 was also followed by UV spectrophotometry, but on PPU-B2 films cast from chloroform on Teflon.

Microparticles of PPU-B1 were suspended in pH 7.4 phosphate buffer and dialyzed in a manner similar to the benzocaine releasing polymer, except the molecular weight cut-off the dialysis tubing was 12,000. Ethambutol was assayed as a chelate with Cu^{2+} by UV spectrophotometry (absorption max 414 nm).

Films of PPU-H2 were cast from 10 wt% chloroform onto Teflon. Samples weighing about 50 mg were cut from the film and placed in 10 ml of 0.1 M pH 7.4 phosphate buffer at 37°C. The buffer solutions were periodically changed and analyzed for drug concentration by HPLC. A HP 1090M liquid chromatograph was used and the column was a 15 × 7.5 mm Hypersil C8 (Alltech) running 70 : 30 acetonitrile : H_2O at 1 ml/min and 40°C. The detector was a diode array operating from 200 to 400 nm. The signal at 265 nm was used to analyze elution of *p*-aminosalicylic acid, 4-hydroxybenzaldehyde and the HBA–PAS conjugate.

2.6. Culture with macrophages

U937 human histiocytic lymphoma cells, ATCC CRL 1593, were cultured in RPMI-1640 media with 10% fetal bovine serum, 2 mM L-glutamine, 10 mM pH 7.4 HEPES buffer, 50 U/ml penicillin and 50 μg/ml streptomycin. Culture conditions were 37°C and 5% CO_2 atmosphere. All cell culture reagents were supplied by Gibco BRL unless otherwise stated. The cells were activated in a suspension culture by incubating 105 cells/ml for 2 days with 10 nM phorbol myristate acetate diester (PMA). Adherent cells were washed with phosphate-buffered saline (PBS) and then incubated with PPU microparticles. Plain media and 0.325 mm poly(methylmethacrylate) (PMMA) microparticles (PolySciences) were used as controls.

Activated cells were incubated in Falcon 35 mm Petri dishes with PPU microparticles at 0.4 and 0.2 mg/ml for 3 h and then fixed with 2.5%

glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Cells were further prepared by fixing in 1% OsO_4 in cacodylate buffer followed by dehydration with a graded series of ethanol solutions (25%, 50%, 70%, 90% 100%). Cells were then embedded in EPON embedding resin with 1.5% DMP30 catalyst and then sectioned for transmission electron microscopy.

Scanning electron microscopy (SEM) was used to assess gross cell morphology. Cells were activated on Corning 18 mm^2 coverslips and incubated with 0.2 mg/ml PPU microparticles or 0.25 mg/ml PMMA microparticles for 1 h. After glutaraldehyde and OsO_4 fixation and followed by ethanol drying as described above, cells were critical-point dried and then sputter-coated with gold. Cells were examined on an Amray 1810.

Cell viability was measured by following incorporation of [Me-^{3}H]thymidine, (NEN Research Products). Activated cells were incubated for 6 h with 0.2 mg/ml PPU microparticles, media alone or 0.2 mg/ml PMMA microparticles. Cells were then washed with PBS and incubated with 5 μCi/ml of [Me-^{3}H]thymidine for 2 h. Fixation in 3:1 methanol:acetic acid followed by methanol and PBS washes preceded addition of trypsin and sodium dodecyl sulfate (SDS) which lysed cells in preparation for counting. The lysed cell mixture was diluted 1:5 with Packard OPTI-Fluor scintillation cocktail and counted on a Beckman LS 1801 liquid scintillation counter.

3. Results and discussion

3.1. Polymers

All 4 drug-loaded PPUs dissolved in chloroform and the more polar solvents such as dimethylformamide and dimethylacetamide. PPU-B5, which had PEG 400 as the soft segment, also dissolved in water. Polymers based on PTMO 1000 were soft and elastic, but the PEG 400 analogues were syrupy and tacky. FT-IR confirmed incorporation of phosphoester into the PPUs with a $P = O$ peak at 1237 cm^{-1}. In vitro degradation of PPU-B1 is shown in Fig. 1. The MW of PPU-B1 was reduced by a factor of three by 1 week, and by 6 weeks decomposition of high molecular weight chains was almost complete.

The molecular weight distribution of the polymers was typically broad, with polydispersities over 20. The high polydispersity was probably caused by the presence of impurities in the BGP, as the transesterification used to synthesize BGP was likely to produce a number of side products [6]. The NMR often showed more than one type of phosphite hydrogen around 6.8 ppm and JP-H of 694 Hz to suggest side reactions in the side chain. Also elemental analysis showed a phosphorus content of 20.2% as opposed to a theoretical value of 18.2%. Purification of BGP has proved difficult because of its high boiling point, which precludes vacuum distillation. Attempts using chromatographic techniques also failed as no suitable separation conditions could be identified.

One of the most common side reactions is probably cyclization between the terminal OH and the phosphite hydrogen [6]. In addition to providing a more hydrophobic chain extender, BHP was synthesized partly to circumvent this side reaction, since cyclization in the transesterification of dimethyl phosphite and hexanediol is minimal. The purity of BHP was confirmed by elemental analysis and proton NMR.

Attachment of the spacer *p*-hydroxybenzaldehyde and the drug *p*-aminosalicylic acid to BHP reduced the yield of the polymer from approximately 75 to 40%. The molecular weight averages of the drug-attached polymer were also lower because of the higher percentage of the low molecular fractions. Nevertheless MN and MW in the range of 100–300k were routinely obtained. Attachment of benzocaine to the polymer had no effects on the molecular weight distribution, indicating that the coupling was mild enough to not cleave the backbone.

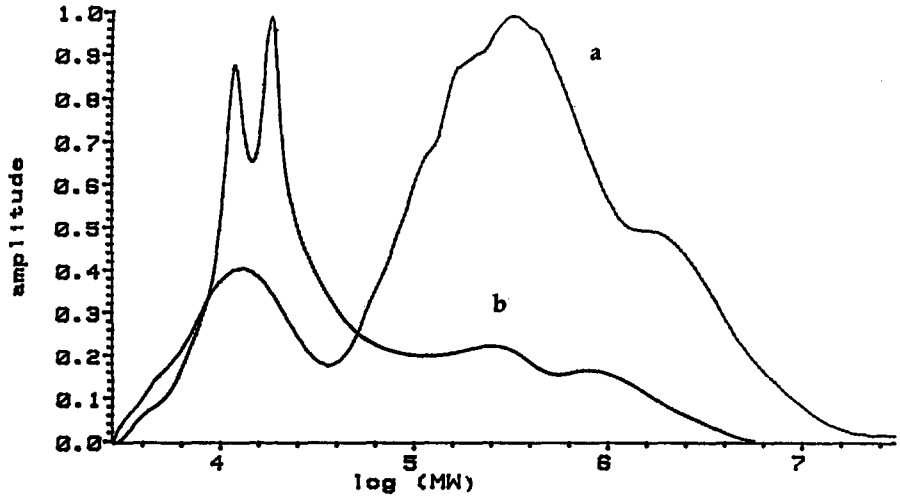


Fig. 1. Molecular weight distributions of PPU-B1 (*a*) prior to and (*b*) after 6 weeks of in vitro degradation.

3.2. Drug release

Two approaches of linking drugs to the side chains of PPU have been demonstrated. Benzocaine was attached after polymerization, while PAS was coupled to the chain extender before polymerization. The drug release kinetics of these two drugs is shown in Fig. 2. In both cases, release was complete within hours. Complete release of PAS could be visualized as the samples changed from orange to colorless. The fast release of benzocaine from PPU-B2 and PPU-B5 indicates the lability of the phosphoramidate bond (Scheme 3). Interestingly there is little difference between benzocaine released from the soluble PPU-B5 and the solid PPU-B2 films. Chemical integrity of the released benzocaine was confirmed by UV spectroscopy.

For PAS release from PPU-H2, the labile imine bond is responsible for the rapid release. Analysis by HPLC shows that only free PAS was present in the buffer. Release of PAS could be realized through different pathways (Scheme 3), for instance, cleavage of the polymer–spacer bond followed by detachment of the drug from the spacer, or even backbone scission preceding the side chain disconnection. However, neither the spacer nor the spacer–drug conjugate was observed during the release experiment. The result attests to the higher hydrolytic reactivity of the imine compared to the phosphoester linkage, but may also reflect the role of steric hindrance in protecting the spacer from cleavage.

Each of the attachment schemes has its advantages and disadvantages. Coupling after polymerization is applicable to a wider range of drugs and is done on polymers having proven satisfactory properties. However, reaction of polymers is seldom complete and it is difficult to control and predict the extent of drug attachment. Starting with a drug-attached monomer can, in principle, better control the final drug loading level. However, one must be careful that the drug has no functional groups that will interfere with the polymerization. This precludes aliphatic hydroxyl and primary amino groups (other than the one attached to the spacer of the phosphoester diol) in the drug. Since no interference was observed in the polymerization of PAS-attached monomer, this study suggests that aromatic hydroxyl and carboxylic acid groups may not pose a problem, which is consistent with the knowledge that their reaction rates with isocyanate are much lower than those of the aliphatic hydroxyl group [9].

We have chosen to couple PAS to the polymer

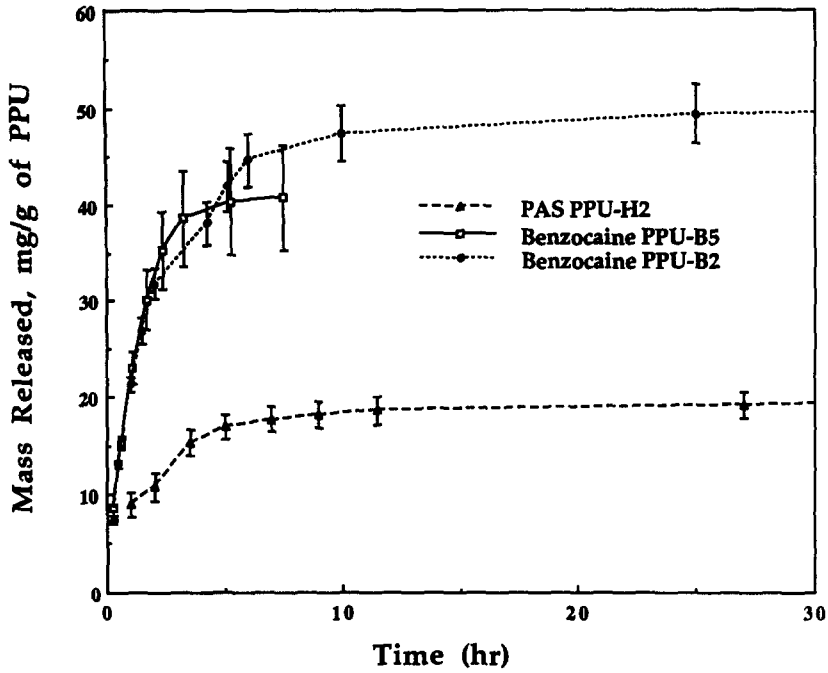
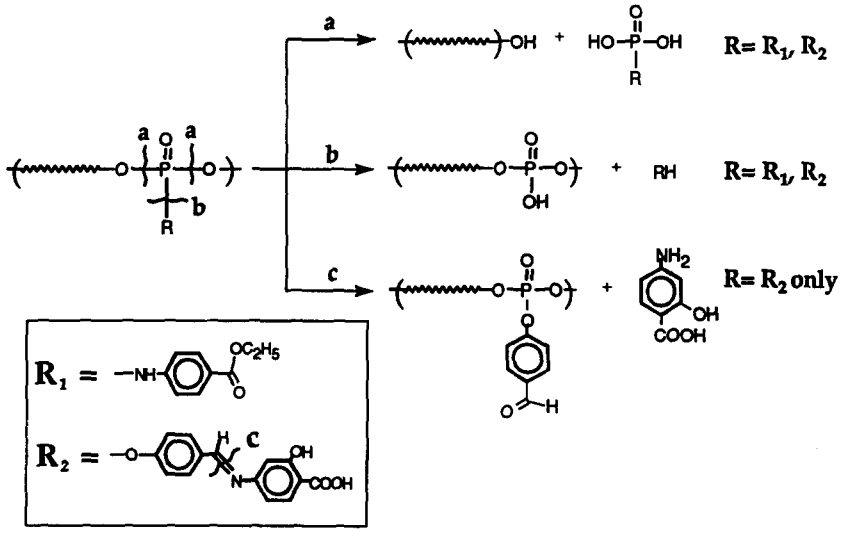


Fig. 2. In vitro release of benzocaine from PPU-B2 and PPU-B5, and release of *p*-aminosalicylic acid from PPU-H2. Values are averages ± SEM, $n = 3$.



Scheme 3.

via *p*-hydroxybenzaldehyde for two reasons. One is to include a spacer to facilitate the release. The other is to broaden the coupling chemistry available. The Todd reaction that links primary amino groups to the phosphite hydrogen has to be performed in chlorinated organic solvents, and hence is only applicable to lipophilic drugs. Attachment via the spacer HBA allows the use of more hydrophilic drugs as long as they are soluble in alcohols.

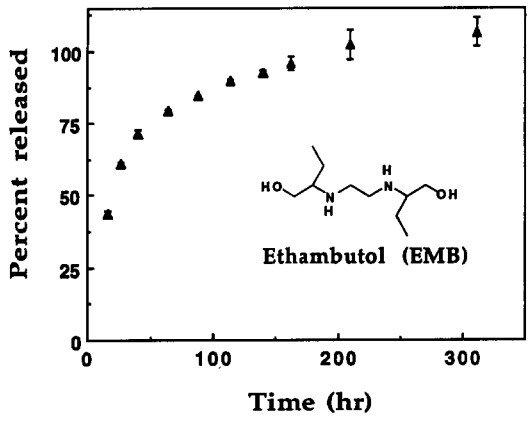


Fig. 3. In vitro release of ethambutol from PPU-B1. Values are averages ± SEM, $n = 3$.

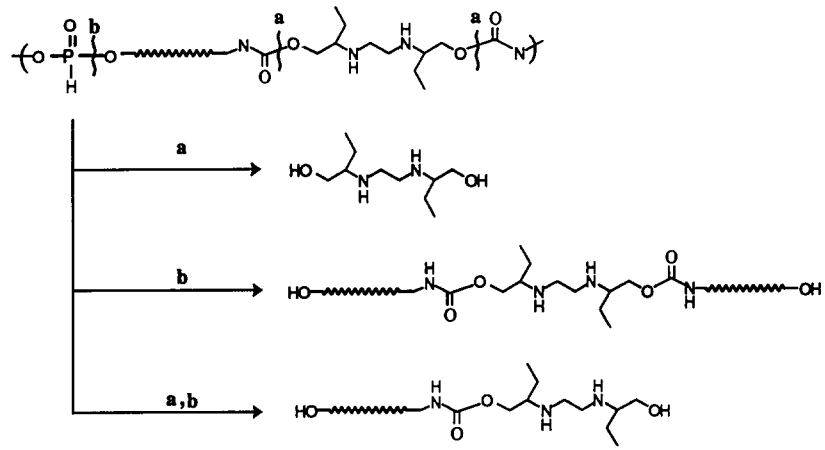
Release of ethambutol from PPU-B1 as determined by chemical assay is shown in Fig. 3. Verification of the purity of the released ethambutol using HPLC and other chemical assays has been unsuccessful. The current copper chelation assay does not distinguish free drug from drug still conjugated to polymer fragments. As suggested by Scheme 4, Fig. 3 probably reflects the release of a combination of free drug and drug–oligomer conjugates.

3.3. Cell culture

Microparticles were isolated in greater than 80% yield as a lyophilized powder. SEM analysis of PPU-H2 particles showed sizes ranging from 1 to 5 μm. Transmission electron micrographs of U937 cells incubated with PPU-H2 microparticles confirmed uptake of particles by cells. In addition, examination of cell and organelle appearance reveals no difference between controls incubated with media and PPU incubated cells, suggesting that the test cells are healthy.

Scanning electron micrographs of PPU-H2 incubated cells show the ruffling and finger-like processes expected from normally functioning activated cells (Fig. 4). These processes are the pseudopodia responsible for cell motion and particle engulfment. In contrast, cells incubated with PMMA particles, a known toxin to this cell line [10], were smooth and exhibited less spreading on the glass coverslip, suggesting impaired cell function and poor health. This difference was confirmed by the DNA synthesis assay. Cells incubated with PMMA showed no detectable incorporation of thymidine, suggesting severe cell damage, while cells incubated with PPU microparticles proliferated at 70 + 28% of control values.

U937 cells are macrophages that express phagocytic function in vitro when exposed to an activating compound such as phorbol myristate acetate. One of the appeals of a pendant delivery system is the ease of formulating it into microspheric form. This type of cell culture would be a sensitive and meaningful screening assay for toxicity. While a more detailed study on the dose



Scheme 4.

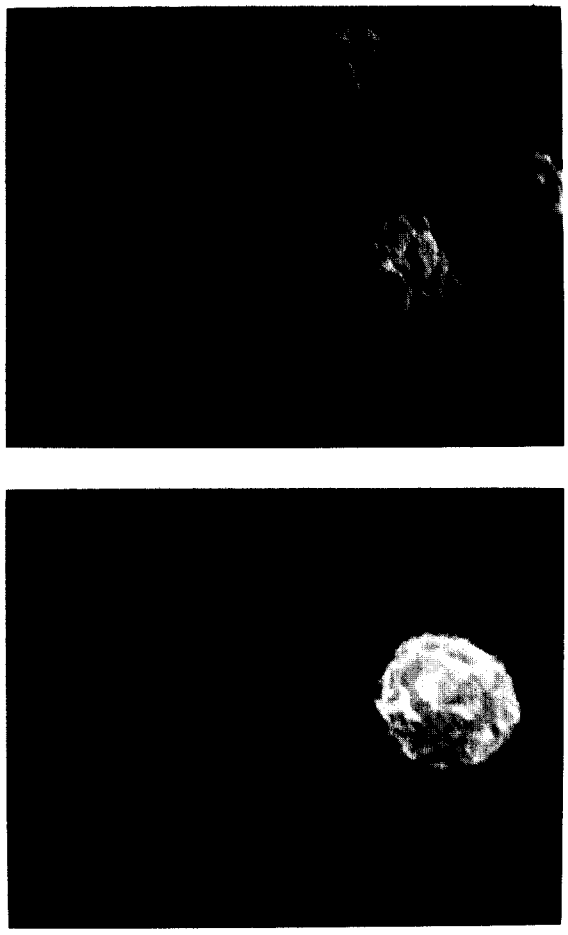


Fig. 4. SEMs of macrophages incubated with PPU or PMMA microparticles.

response is needed, this preliminary finding on PPU is encouraging.

4. Conclusion

Biodegradable elastomers were synthesized by introducing a hydrolytically labile phosphoester diol as a chain extender in the polyurethane backbone. A pendant delivery system can be developed from these polymers by taking advantage of the pentavalency of the phosphorus atom. Benzocaine was directly attached to phosphorus via a phosphoramidate bond, while *p*-aminosalicylic acid was coupled via a spacer, *p*-hydroxybenzaldehyde, to the polymer. The drug attachment could be achieved before or after polymerization. Release of both drugs was rapid, reflecting the hydrolytic lability of the phosphoramidate and imine bonds, respectively. Release could be much more sustained if the drug was introduced into the backbone. Ethambutol, with two primary hydroxyl groups, could be readily incorporated as part of the chain extender. However, while there can be no question of the chemical integrity of the drugs released pendantly, drugs released from the backbone are likely to be associated with oligomers because of the relative stability of the urethane bond. Preliminary culture assay with U937 cells indicated that cell morphology and cell viability were not adversely affected by the PPU microparticles.

Acknowledgements

The authors would like to acknowledge support from the American Foundation for AIDS Research. B.I.D was supported by a National Defense Science and Engineering Graduate Fellowship.

References

[1] M.D. Lelah and S.L. Copper, Polyurethanes in Medicine, CRC Press, Boca Raton, FL, 1986.
[2] Q. Zhao, N. Topham, J.M. Anderson, A. Hiltner, G. Lodoen and C.R. Payet, J. Biomed. Mater. Res., 25 (1991) 177.
[3] R.R. Smith, D.F. Williams and C.J. Oliver, J. Biomed. Mater. Res., 21 (1987) 1149.
[4] P. Bruin, P.J. Smedinga, A.J. Pennings and M.F. Jonkman, Biomaterials, 11 (1990) 291.
[5] B.I. Dahiyat, E. Hostin, E.M. Posadas and K.W. Leong, J. Biomaterials Sci., 4 (1993) 529.
[6] G. Borisov and K. Troev, Eur. Pol. J., 9 (1973) 1077.
[7] P. Eckert and E. Herr, Chem. Abs., (1944) 1564.
[8] J. Pretula, K. Kaluzynski and S. Penczek, Macromolecules, 19 (1986) 1797.
[9] J.H. Saunders and K.C. Frisch, Polyurethanes: Chemistry and Technology, Interscience Publishers, New York, 1962.
[10] S.M. Horowitz, C.G. Frondoza and D.W. Lennox, J. Orth. Res., 6 (1988) 827.