

BIODEGRADABLE POLYMERS WITH A PHOSPHORYL-CONTAINING BACKBONE: TISSUE ENGINEERING AND CONTROLLED DRUG DELIVERY APPLICATIONS*

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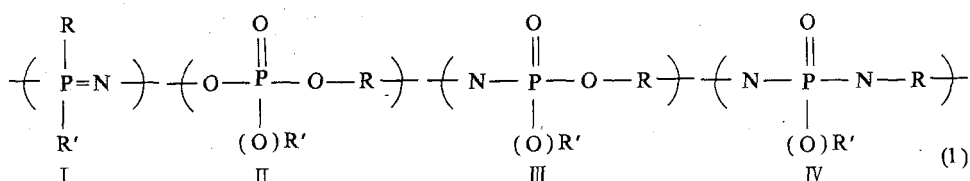
INTRODUCTION

Polymers enjoy wide spread use in medicine and continue to stimulate new designs of biomaterials for various biomedical application^[11,15]. As a special class of biomaterials, biodegradable polymers benefit from the obviation of surgical removal after they have fulfilled their mission. More than just convenience, biodegradability can also provide unique advantages. For example, a biodegradable polymeric controlled release system allows for greater flexibility in release rate and depends less on drug characteristics in comparison with diffusion-controlled systems^[19]. As osteosynthetic materials, biodegradable polymers possess the potential benefit of allowing gradual load transfer from the fixation device to the healing bone. This in principle might lead to a bone remodelling process more favorable than that effected by persistent rigid fixators^[18]. Many other medical devices and applications, such as nerve guides, vascular grafts, and tissue regeneration also favor a temporary scaffolding function^[15]. Not surprisingly,

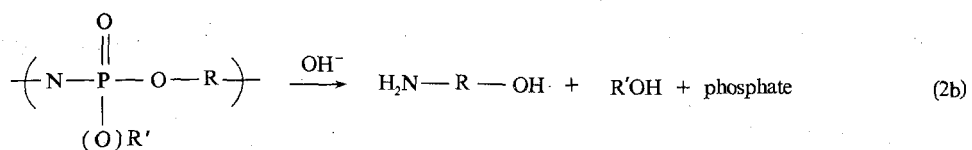
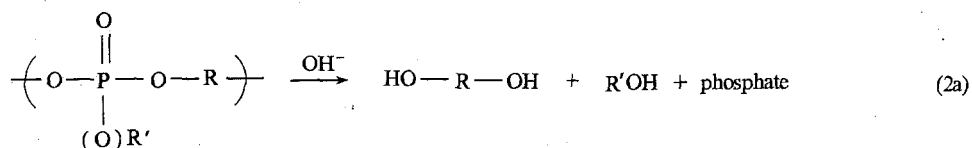
* This manuscript is submitted on the congratulatory occasion of the 80th birthday of Professor X. D. Feng, a leader in the research of polymer science and education of a generation of polymer chemists.

many classes of biodegradable polymers have been proposed for biomedical applications.

In the past decade, we have been developing a new class of degradable biomaterials with a phosphorus-containing backbone. Polymers containing a phosphorus atom in the backbone and are hydrolytically labile can be represented by structures I-IV as shown in Equation 1. Structure I is poly (phosphazene), a class of polymers developed by Allcock and have been evaluated recently as biomaterials^[1]; that is not what we studied. Our focus is on structures II-IV with a phosphoryl functional group in the backbone. Most studies have been focused on II. Depending on the nature of the side chain connected to the phosphorus atom in II, the polymers are called poly (phosphate) with the oxygen in the side chain, poly (phosphonate) without the extra oxygen, or poly (phosphite) with just the hydrogen.



Past interests in phosphorus-containing polymers have primarily been centered on their flame retardation property^[5]. Because of a combination of factors—high cost associated with the synthesis of these polymers in comparison to the carbon analogs, and the perceived hydrolytic instability as shown in Eq. 2—research interest faded since the 1960's.



These polymers are attractive to us because of the following considerations:

1) Versatility The versatility of these polymers comes from the versatility of the phosphorous atom, which is known for its multiplicity of reactions. Its bonding can involve the 3p orbitals or various 3s-3p hybrids; spd hybrids are also possible because of the accessible d orbitals. Looking at the general structure shown in Eq. 1, one can see that the physicochemical properties can be readily altered by varying either the R or R' group. An expansive variety of phosphorus-containing polymers therefore exists.

2) Processability It is known that the P-O-C group would confer the polymer solubility in common organic solvents. Although not immediately apparent, this is highly desirable since the polymers can be easily characterized and processed.

3) Biocompatibility Biocompatibility is difficult to predict. Poly (phosphate)s can be expected to break down into phosphates, alcohols and diols, all of which have the potential to be non-toxic (Eq. 2a). Similarly, structure III can be synthesized from amino acids and the breakdown products would then be amino acid, alcohols, and phosphates (Eq. 2b).

4) Pendant chain functionality The pentavalency of phosphorus allows the chemical linkage of drug molecules to the polymer. For instance, drugs with carboxyl groups may be coupled to the phosphorus atom via an ester bond, which is hydrolyzable. Pendant delivery system has the advantage of attaining a high drug loading level and allowing a sustained release for hydrophilic drugs. There are only a handful of pendant delivery systems available, which are based on a non-biodegradable carbon-carbon backbone. These phosphoryl-containing polymers are natural candidates for a completely biodegradable pendant delivery system.

Initially we synthesized these polymers for controlled drug delivery^[8,26]. The field of controlled release has been continuously fueled by the desire to improve the therapeutic index of many valuable pharmaceuticals. It has long been recognized that simple pills and injections may not be the best mode of drug administration. For example, many potent polypeptides derived from genetic engineering are poorly absorbed through the gastrointestinal tract and have short in vivo half-lives. Cancer chemotherapeutic drugs are also notorious for their narrow therapeutic ranges. Polymeric controlled release offers to maintain the drug concentration within the desirable therapeutic levels for a prolonged period of time. In addition, polymeric controlled drug delivery provides the potential

advantages of: (1) reduction of side effects because of less total drugs required, (2) increase of patient convenience and compliance in eliminating the need to adhere to rigid frequent dosing regimes, (3) protection of unstable drugs from decomposition, and (4) extension of expiring patents on existing drugs through controlled release formulation.

As the advantages of improved drug therapy realized by polymeric controlled drug therapy become more transparent, this technology has been increasingly applied to different biomedical applications, and the role of the polymeric drug-carriers becomes more elaborate. More than just a monolithic device to leach out the bioactive agents, the drug-carrier often possesses particular physicochemical properties to double as a facilitator of other biological processes. This review highlights our experience in designing these phosphoryl-containing polymers to integrate the controlled release feature with other biomaterial functions^[6-8, 12, 13, 20-23, 25, 26, 30, 31].

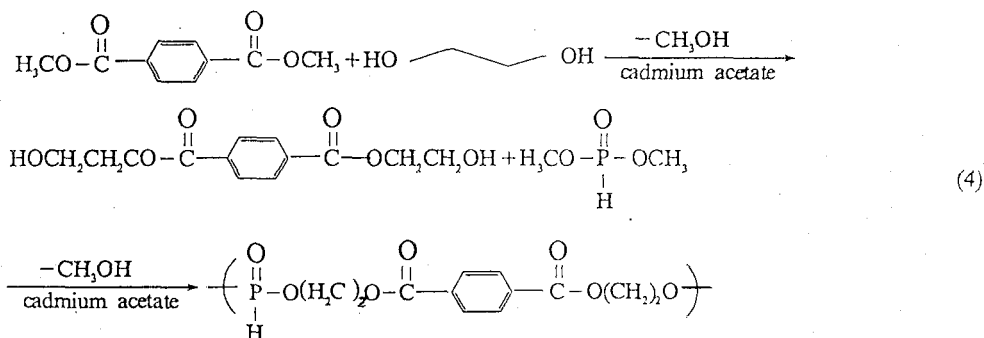
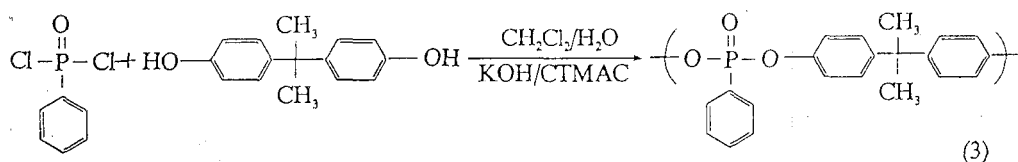
BONE GRAFT WITH CONTROLLED RELEASE CAPABILITY

Over 100,000 bone grafting procedures are performed each year in the United States for treating various kinds of bone defects. Autografts and allografts are usually the materials of choice. Although generally effective, autografting of large bony defects is limited by the availability of healthy graft material and donor site morbidity. Extra blood loss from the donor site is an additional disadvantage. As the principal alternative to autograft, allograft does not provide a viable osteogenic population of cells and its osteoinductive potential is uncertain, leading to a higher frequency of failure. Fresh allograft bone tissue which has not been MHC matched to the host elicits a marked immune response. Transmission of infectious diseases such as AIDS and hepatitis also reduces the appeal of allografts. All this highlights the need to develop an alternative to autograft and allografts.

A synthetic bone graft unlimited in supply, available in different physicochemical properties, and can be fabricated into complex shapes would be an attractive alternative. Ideally the synthetic bone graft would be osteoconductive based on controlled porous architecture, biodegradable to avoid any interference with complete regeneration of the bone tissue, and osteoinductive based on con-

trolled release of growth factors such as bone morphogenetic proteins (BMP)^[29].

Two poly (phosphoesters) were synthesized for this bone graft applications^[21, 22]. The polymer with a bisphenol-A backbone and a phenyl side chain (BPA/PP) was synthesized by interfacial polycondensation as shown in Eq. 3. A copolymer with an ethylene terephthalate backbone was synthesized as shown in Eq. 4. The poly (bis (2-ethoxy) phosphoryl terephthaloyl) structure (PPET) is an interesting structure. It can be viewed as the phosphorus and biodegradable version of poly (ethylene terephthalate) (PET). It plays the role of a fast degrading companion of BPA/PP. BPA/PP hydrolyses slowly because two aromatic rings are adjacent to the phosphoester bond. The high electron density environment repels the nucleophilic attack of the hydroxyl ion. In contrast, PPET has two methylene groups bordering the phosphoester bond and was shown to degrade two orders of magnitude faster in vitro. We hence synthesized co-poly (phosphoesters) from these two monomers for a range of degradation profiles.



Working with these poly (phosphoester) s we developed highly porous degradable foams with controlled release capability using a phase separation principle^[4, 21, 22]. Briefly the polymer was dissolved in molten naphthalene at 85°C. The polymer solution was then connected to an atomizer head previously heated to 80°C, atomized into fine particles, and deposited onto a cold mold causing solidification of the spray layer by layer. The naphthalene was subsequently

removed by sublimation, leaving behind a structure with relatively controlled porous architecture. Different foam porosities could be obtained by varying the polymer concentration in naphthalene. The unique advantage of this fabrication technique is that the porogen is not removed by the leaching-out method, but by sublimation. Drugs or proteins can hence be incorporated into the foam for sustained release.

To further improve the quality of the foams, the interaction of the poly (phosphoester)s with micronized hydroxyapatite (HA) powders and calcium ions were also studied. Hydroxyapatite (HA) ceramics have been a popular choice in reconstructive surgery since the materials mimic the natural bone mineral. Incorporation of synthetic HA into the degradable foams may render a good template for bone graft applications in that they would provide good bonding to the host bone and could also be resorbed to allow reunion of fractured bones^[9,10]. There is the added advantage that the presence of HA could improve handling and provide some initial structural stability to an otherwise soft and weak material. Phosphates are known to have a good affinity to calcium and other metal ions, it is possible that the phosphate groups of poly (phosphoester)s generated by hydrolysis of the side chain or the backbone could form a calcium phosphate complex on exposure to calcium ions. As an attempt to characterize the degradation behavior in the presence of calcium, the poly (phosphoester) foams were exposed to a solution high in calcium content. After 3 weeks, the samples were removed and washed at least four times in deionized water to remove any loosely adsorbed residual salts.

Foams made from the BPA-PP/naphthalene system exhibited a connected-bead structure (Fig. 1a). Oval shape pores were obtained if a solution composed of PP-PPET copolymer was used. By incorporation 10% HA into the copolymer foam (Fig. 1b), the pores became more circular in appearance. Foams made from polylactide are also shown for comparison. Typically these foams can have porosities over 70% and a pore surface area of over 5 mm²/mm³.

EDS-analysis indicated that foams soaked in calcium chloride solution for three weeks (Fig. 2) had a Ca/P ratio of 0.165 even after extensive washing with deionized water. Sufficient washing was confirmed by the fact that there was no detectable signal for chlorine in the EDS spectra. This result confirmed that calcium has a certain affinity to the poly (phosphoester)s.

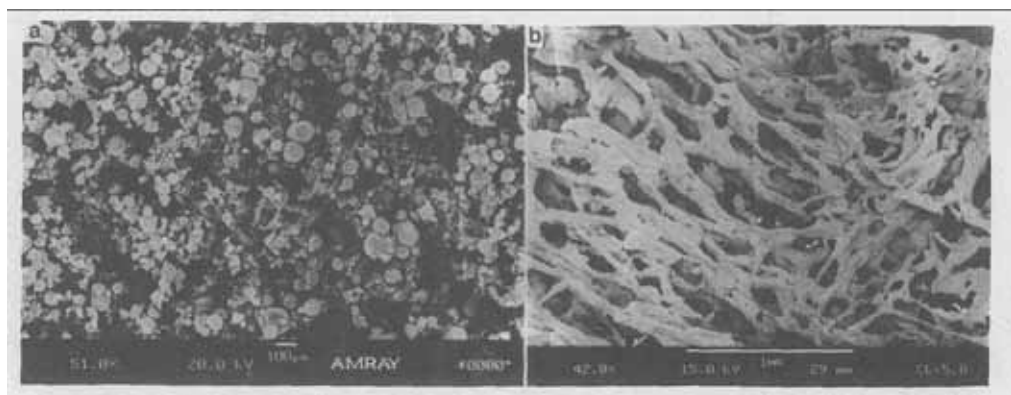


Fig. 1 Scanning electron micrographs of poly (phosphoester) foams fabricated by phase separation
 (a) BPA/PP foam showing a connected bead-like structure
 (b) a copolymer of BPA/PP and PPET foam with a layered network morphology

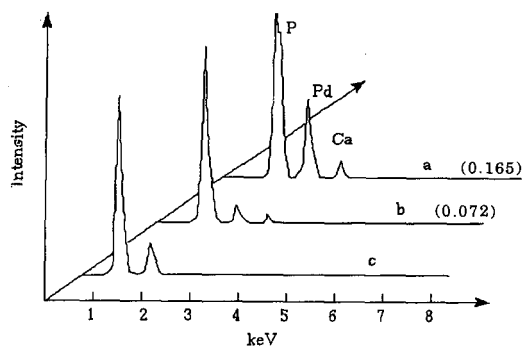


Fig. 2 Energy dispersive spectra of (a) PP-PPET copolymer foam after a 3-week immersion in 1mol/L pH 9.0 calcium chloride solution, (b) copolymer foam loaded with 10%HA, (c) copolymer foam without immersion in calcium chloride solution and showed the absence of the calcium peak. Calcium/phosphorus ratio is given in parentheses. The paladium peak in the spectra was a result of the sputter coating of the samples prior to EDS analysis

The stiffness and viscoelasticity of the foams were characterized by dynamic mechanical testing. This measurement provides a quantitative assessment of the quality of the foams other than microscopic observation. Samples were tested dried and under in vitro condition of 37°C in 0.1mol/L pH 7.4 phosphate buffer. Complex moduli (E^*) of the original copolymer foams have a value of 42 kPa and are the lowest among the foams studied here (Fig. 3). The complex moduli increased to 70 kPa when 10 wt% HA was incorporated. Interestingly,

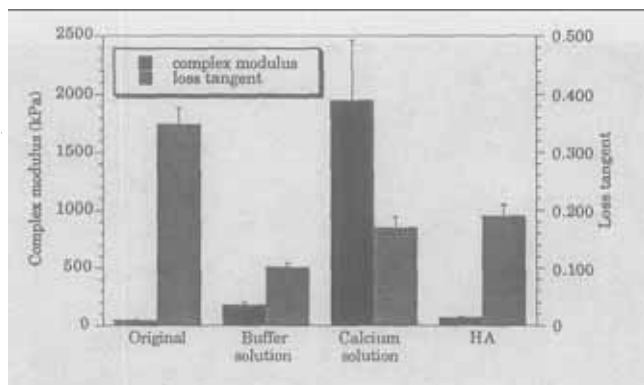


Fig. 3 Complex moduli and loss tangents of PP-PPET copolymer foams, copolymer foams immersed in 0.1mol/L pH 7.4 phosphate buffer for 3 weeks, copolymer foams immersed in 1mol/L pH 9.0 calcium chloride solution for 3 weeks, and copolymer foams reinforced with 10 wt% hydroxyapatite powders ($n=3$)

the complex moduli of the unloaded foams after soaking in phosphate buffer had a substantial improvement to 176 kPa. The improvement could be attributed to the loss of low molecular weight portions of the copolymer that leached out during the three-week period. The viscous element of the partially degraded foams, therefore, should have been reduced, as is evident by the decrease in loss tangent from 0.350 to 0.101. Greatest improvement was seen with samples immersed in calcium chloride solution for three weeks, when E^* increased to 1948 kPa, a 50 fold increase over the original copolymer foams. Loss tangent of these foams were more comparable to that of HA loaded foams, 0.170 and 0.190, respectively.

An unique advantage of the foams fabricated by this procedure is that bioactive agents can be incorporated into the foam for sustained release. Simulation of peptide and growth factor release from foams was studied on both the BPA/PP as well as the polylactide (PLLA) matrices loaded with sulforhodamine B (SD) and alkaline phosphatase (AP) respectively. SD with an aqueous solubility of 20 mg/ml and 600 daltons in size mimics peptides such as angiotensin and chemotactic agents. Phase contrast and confocal microscopy showed that the drug mostly reside on the periphery of the polymer matrix. The release profiles of SD in PLLA and BPA/PP foams are shown in Fig. 4. Foams with 10 wt% SD loading leached out the marker at a faster rate than the 1 wt% loaded foams, and have a higher burst of 20% in the first hour. The plateaus of these curves

indicate that a portion of the dye was embedded in the matrix, and that portion will be released when the matrix degrades. Plateau values of the two higher loading levels indicate that SD molecules have a higher partitioning into the PLLA foam matrices than into BPA/PP. Incongruity in the 1% loading cases can be explained by morphological differences between the two polymer foams. The thin walls of fibrous matrix in PLLA foams allow the SD that has been partitioned into the PLLA during foam fabrication to travel through the polymer and diffuse easily into the buffer medium. On the other hand, it is difficult for release of SD to occur from the BPA/PP foam matrix since it is embedded into the thick wall of the connected-ball structure of the BPA/PP foams.

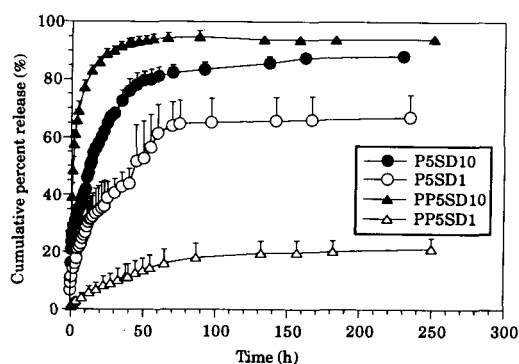


Fig. 4 In vitro release curves of sulforhodamine B (SD) from PLLA and BPA-PP foams in 0.1 mol/L pH 7.4 phosphate buffer at 37°C; 10 wt% loading foam made of 5% PLLA solution (P5SD10); 1 wt% loading foam made of 5% PLLA solution (P5SD1); 10 wt% loading foam made of 5% BPA-PP solution (PP5SD10); 1 wt% loading foam made of 5% BPA-PP solution (PP5SD1)

Therefore, the release study highlights the significant influence of drug distribution in the foam on the resulting release kinetics. The drug distribution is thermodynamically affected by the drug loading level and the affinity of the drug to the polymer. At 10% loading, release of SD was faster from BPA/PP than from PLLA foams because most of SD was just adsorbed on the surface of BPA-PP foam while more SD was imbedded in the PLLA matrix. At 1% loading, most SD was imbedded inside the BPA-PP matrix, as inferred from the fact that 1% of SD appeared to be trapped in the 10 wt% SD loaded BPA-PP (PP5SD10) foams after 250 hours. Release of SD in this case had to be diffusion

through the polymer phase, and probably because of a lower diffusivity in BPA/PP than in PLLA, a lower release rate from BPA/PP matrix was seen at the low loading level.

Next we examined the feasibility of incorporating proteins into the foams. After an initial burst, AP was released at an average rate of 0.32 ± 0.04 mg/day/g foam (Fig. 5). The burst indicates that approximately 70% of the protein

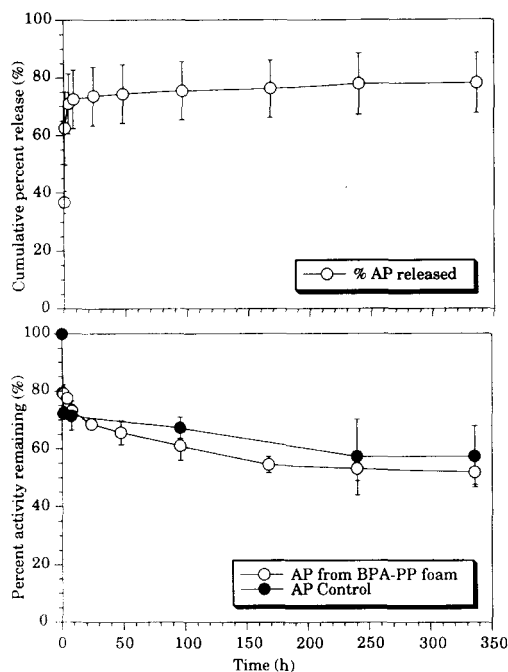


Fig. 5 (Upper curve) In vitro release curve of FITC-labelled alkaline phosphatase (FITC-AP) from 10 wt% (FITC-AP) loaded BPA-PP foams in 0.1 mol/L pH 7.4 phosphate buffer at 37°C. (Lower curves) Changes in bioactivity of FITC-AP after release from the foam and pure FITC-AP placed under the same conditions as a control

was merely adsorbed on the surface. About 25% of the enzymatic activity was lost during the fabrication. The bioactivity of AP released from the matrix was comparable to that of pure AP immersed in phosphate buffer for the same time period. This suggests that the protein was at least partially protected by the hydrophobic BPA-PP matrix until it was released into the medium.

These studies confirm our hypothesis that calcium might chelate with the partially degraded and negatively charged side chains of the poly (phosphoester)s. The calcium present throughout the polymeric structure acted as a reinforcing agent which consequently improved the mechanical properties of

the foams as indicated by the drastic improvement of their complex moduli. This improvement in strength may help the foams maintain their integrity and stability during the implantation process and in early stages *in vivo*. In summary, the studies so far support the hypothesis that the poly (phosphoester)s can be developed into a bone graft with osteoconductive and osteoinductive properties.

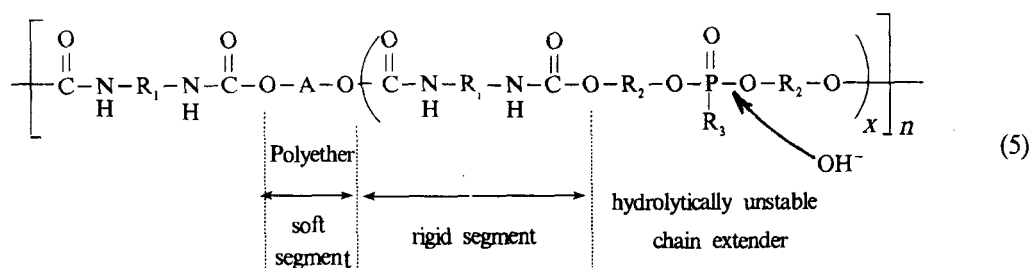
STENT COATING WITH CONTROLLED RELEASE CAPABILITY

Another design of the poly (phosphoester)s was in the configuring of a drug-eluting coating to combat restenosis^[6,7,20,33]. Ischemic heart disease (IHD) is the leading cause of mortality in the western world. The primary factor contributing to IHD is coronary arteriosclerosis, when the arterial wall thickens and hardens. When arteriosclerosis leads to significant narrowing of the coronary arteries, medical intervention is necessary. A common surgical procedure used to relieve the patients is percutaneous transluminal coronary angioplasty (PTCA). The procedure consists of percutaneous introduction of a dilating balloon catheter, via a brachial or femoral artery, into a stenosed coronary artery under fluoroscopic monitor. When placed properly the balloon is inflated to enlarge the coronary lumen. While PTCA is adequate for relieving acute symptoms, its overall success is hampered by restenosis. Between 20 to 30 percent of patients with initially successful procedure experienced return of angina within six months of the procedure. The former is caused by intimal dissection, and the latter by elastic recoil of the vessel wall and neointimal smooth muscle hyperplasia at the PTCA site. One of the methods to prevent restenosis is the placement of a metallic stent at the site of angioplasty. While metallic stents prevent vascular occlusion, they show little improvement in arresting post-PTCA restenosis. Systemic administration of anti-coagulants to prevent or reduce the incidence of restenosis has also been unsuccessful. Presumably systemic administration is unable to provide a high enough therapeutic concentration without causing systemic toxicity. Approaches have therefore been proposed to deliver drugs to the PTCA site through a drug-eluting coating on the metallic stent^[27].

The materials requirement for this coating therefore include viscoelastic characteristics, biodegradability, biocompatibility, and controlled release capability. We proposed to synthesize polyurethanes with a biodegradable phos-

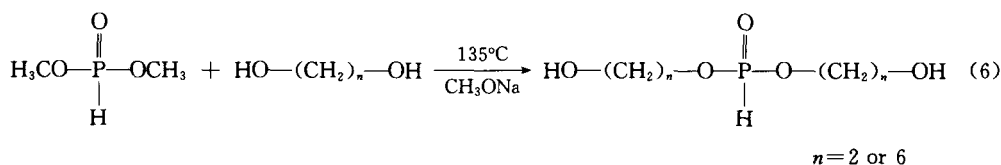
phoester chain extender for this purpose.

Polyurethanes have been widely used to construct blood-contacting biomedical materials^[17]. The unique and excellent mechanical properties of polyurethane come from its two-phase microstructure, consisting alternating blocks of hard and soft segments. Used in a large number of clinical applications, polyurethanes are designed to be inert and stable in the body. Using a bis (2-hydroxyethyl)phosphite as the chain extender, we introduced biodegradability by incorporating a phosphoester bond into the backbone of the polyurethane. The chemical structure of the repeating unit is shown in Eq. 5.

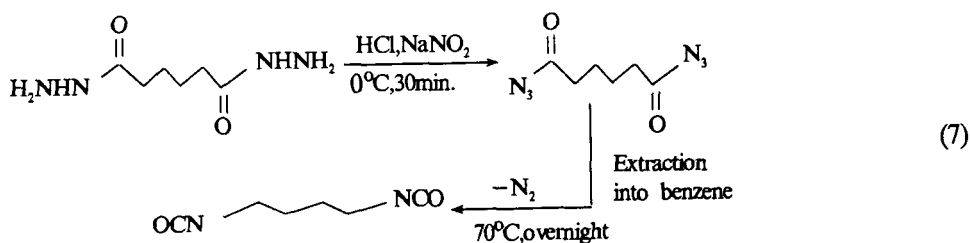


The other challenge to the development of these poly (phosphoester-urethane)s (PPU) is to ensure the polymers would breakdown into non-toxic residues. Intended to be stable, current biomedical polyurethanes are commonly synthesized from aromatic diisocyanates such as toluene diisocyanate (TDI) and methylene diphenyl isocyanate (MDI). When rendered biodegradable, however, polyurethanes derived from such diisocyanates might yield aromatic amines that are toxic. A plausible scenario is that when the polyurethane hydrolyzes, the bio-material disintegrates into particulates, which would be phagocytosed. Under potent biochemical attack in the endolysosomes, the urethane bond would be decomposed to yield an amine. Efforts were then made to synthesize diisocyanates, which after polyurethane formation, would decompose into non-toxic compounds. To achieve this, 1,4-diaminobutane and L-lysine were used. The former is an intermediate in the urea cycle and goes by the biochemical name of putrescine; L-lysine is one of the 20 basic amino acids. We converted these diamines to diisocyanates, expecting that after biochemical decomposition, the polyurethane would generate the starting diamines. To achieve controlled released in a thin film configuration, drugs were coupled to the phosphorus side chains as a pendant delivery system.

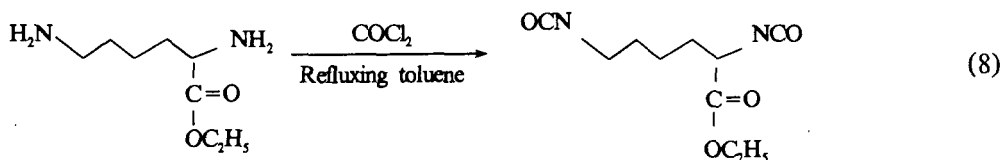
The biodegradable chain extenders bis (2-hydroxyethyl)phosphite (BGP) and bis (6-hydroxyhexyl)phosphite (BHP) were synthesized by adopting the procedures described by Borisov and Troev (Eq. 6)^[3]. The commonly used polyethers, PEO and PTMO were used as the soft segments.



The diisocyanate (BDI) derived from 1,4 diaminobutane was synthesized via a Curtius rearrangement. 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 to generate BDI. The compound could be readily purified by vacuum distillation (Eq. 7).



The synthesis of the diisocyanate (LDI) derived from lysine was achieved by phosgenation of lysine ethyl ester in anhydrous toluene at 135°C (Eq. 8)^[33].



Using the standard two-step polymerization, the poly (phosphoester-urethane)s (PPU) typically have polydispersities over 20 and bimodal molecular weight distributions, with one peak corresponding to chains in the 20,000 Dalton range and the other consisting of very high molecular weight polymer that often extended beyond the 3 million Dalton exclusion limit of the GPC columns used. PTMO1000 based PPU were generally light colored, soft and flexible while PEG400 based PPU were hard, brittle and tacky. All of the PEG400

polymers dissolved readily in water, in addition to DMF, DMAc, alcohols and chloroform. The PTMO1000 polymers generally dissolved in DMF, DMAc and chloroform but, as expected from the hydrophobic nature of the polyol, were insoluble in alcohols or water. The ready solubility of these PPU in chlorinated solvents can likely be attributed to the solubilizing effect of the phosphoester group in the backbone. These polymers can be cast into clear resilient films, and can be molded readily at 70°C.

The PTMO-based PPU have a $\tan \delta$ ranging from 0.07 to 0.21, depending on chain extender composition, while the magnitude of the complex dynamic modulus, E^* , varied from 1.6 MPa to 10.2 MPa. The BHP based polymers were usually softer, with higher E^* and $\tan \delta$ than their BGP analogs, as can be expected from the longer hexamethylene chains in the monomer.

The ultimate tensile strengths (UTS) of BDI and PTMO-based PPU were in the 2 to 3 MPa range and were quite similar for various compositions. The elongations at break, ranging from 36 to 84%, were more varied, and the trends could be correlated to the chain extender structures. In comparison, the PPU derived from LDI were all weaker and softer, having a lower UTS and higher elongation at break. The lower mechanical properties of these PPU may be due to the asymmetric nature of lysine. This asymmetry of the hard segment might reduce the crystallizability of the polymer, and render the final phase separation irregular and incomplete.

The effect of in vitro degradation on PPU-B2, PPU-B3 and PPU-B4, polymers with 25.0 and 12.5 mol% BGP respectively, was assessed. The thin, 300 μm , films reached swelling equilibrium in less than 3 days, with PPU-B2 and PPU-B4 swelling about 45% and remaining nearly constant for 110 days. PPU-B3, without the phosphoester chain extender, swelled only about 10% and remained clear and translucent throughout the study in contrast to the other polymers which turned opaque. Mass loss of the BDI-based PPU is shown in Fig. 6. While PPU-B3 showed no significant degradation for over 3 months, a rapid initial weight loss was seen for PPU-B2 and PPU-B4, of approximately 20% and 5% respectively. The mass loss then leveled off for about 75 days before accelerating again. Degradation was still occurring during the plateau phase as verified by GPC. As expected, PPU-B3, which contained no biodegradable chain extender, did not show any detectable change in molecular weight distributions.

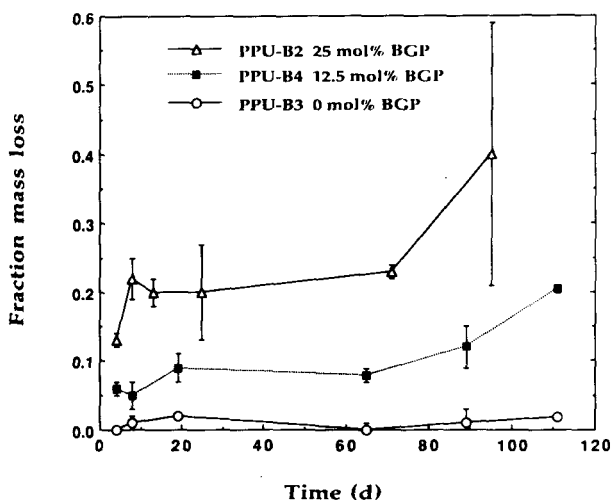


Fig. 6 In vitro mass loss of the PPU as a function of the biodegradable BGP content in the backbone. PPU-B2 (25 mole % BGP), PPU-B3 (0%), PPU-B4 (12.5%). All points are averages $\pm$ SEM ($n=3$)

Changes in dynamic moduli during degradation are shown in Fig. 7. PPU-B2 and PPU-B4 showed initial increases in E^* followed by declines at later time points. PPU-B2 dropped off steeply and was too soft to perform the testing after 75 days while PPU-B4 showed a more deliberate softening and PPU-B3 remained unchanged. The time course of E^* matched that of the mass loss and chain cleavage discussed above, which suggests a possible explanation. The initial loss of low molecular weight oligomers, effectively raising the molecular weight, would be expected to stiffen the polymer, then, as high molecular weight polymer degraded, the modulus would be expected to drop. PPU-B2 showed a greater effect than PPU-B4 for both E^* and mass loss, which can be attributed to the difference in phosphoester contents, while the control PPU-B3 remained unchanged. $\tan \delta$ showed similar trends, but the structural features responsible for energy dissipation are not as apparent as those effecting modulus. Two approaches of linking drugs, either before or after polymerization, to the side chains of PPU were investigated. Benzocaine was attached after polymerization, while p-amino salicylic acid (PAS) was coupled to the chain extender before polymerization. A spacer was also sometimes used to generalize the coupling chemistry. Coupling of a spacer 4-hydroxybenzaldehyde (HBA) to BHP via a P—O bond was achieved by adapting the method of Penczek^[28], and attachment of (PAS) to the spacer was achieved as described in Eq. 9.

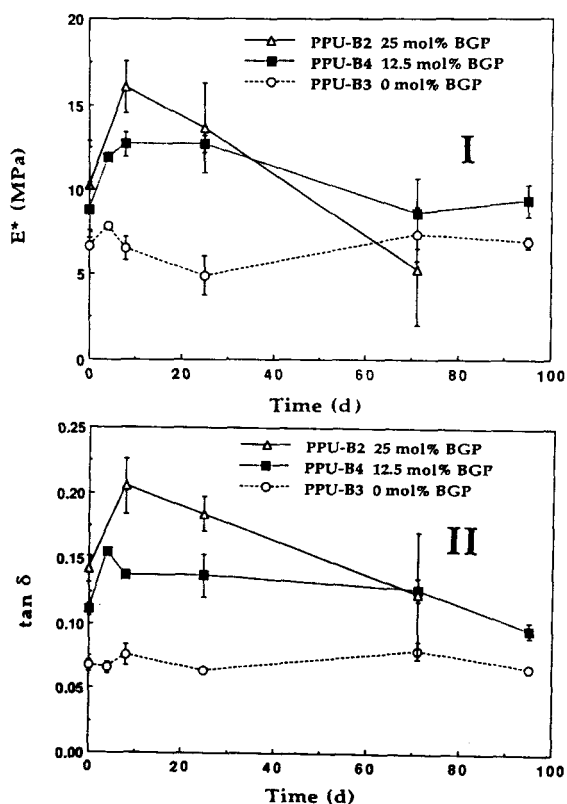
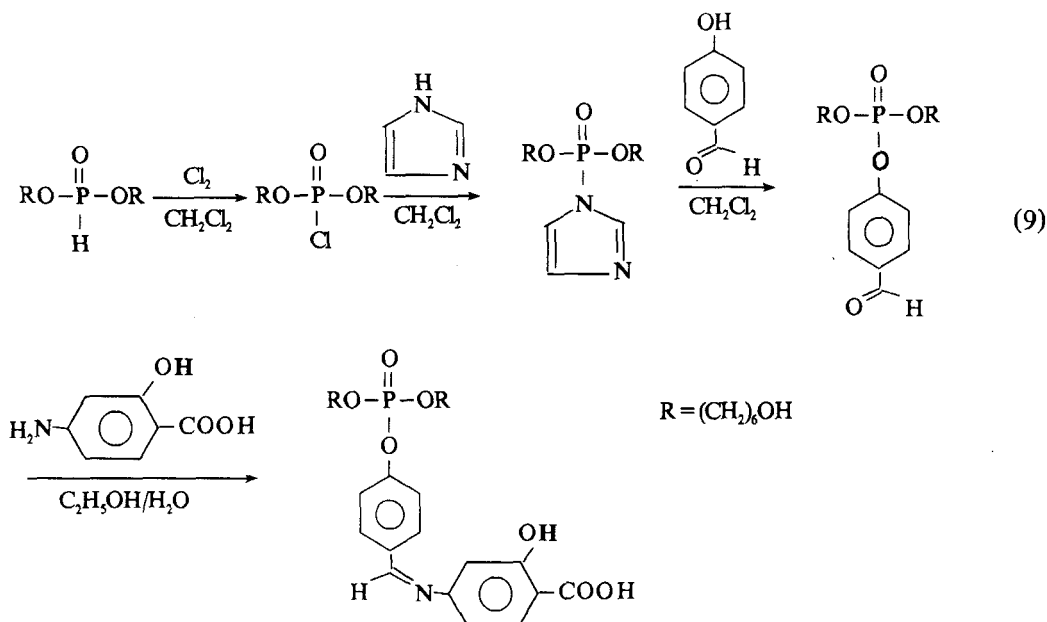
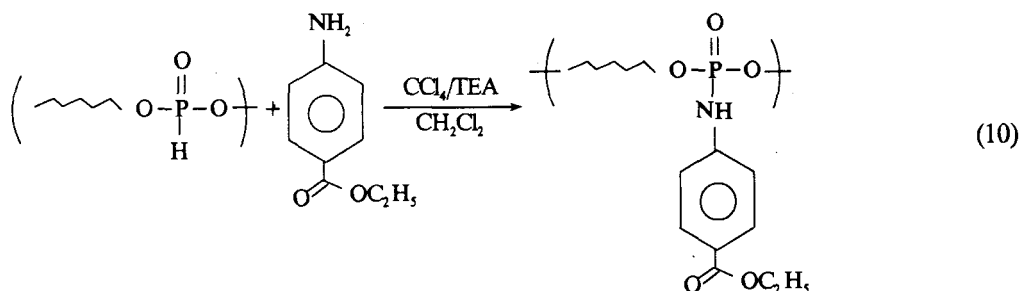


Fig. 7 Effect of in vitro degradation on the complex modulus and $\tan \delta$ of PPU. PPU-B2 (25 mole % BGP), PPU-3 (0%), PPU-4 (12.5%). All points are averages $\pm$ SEM ($n=3$)



Benzocaine was coupled to the PPU side chain (Eq. 10) using the Todd reaction.



The drug release kinetics of these two drugs is shown in Fig. 8. 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 indicates the lability of the phosphoramidate bond. Chemical integrity of the released benzocaine was confirmed by UV spectroscopy.

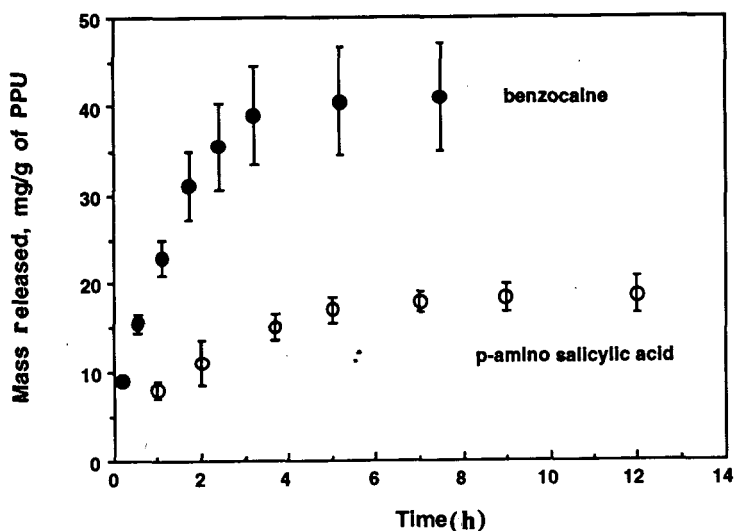
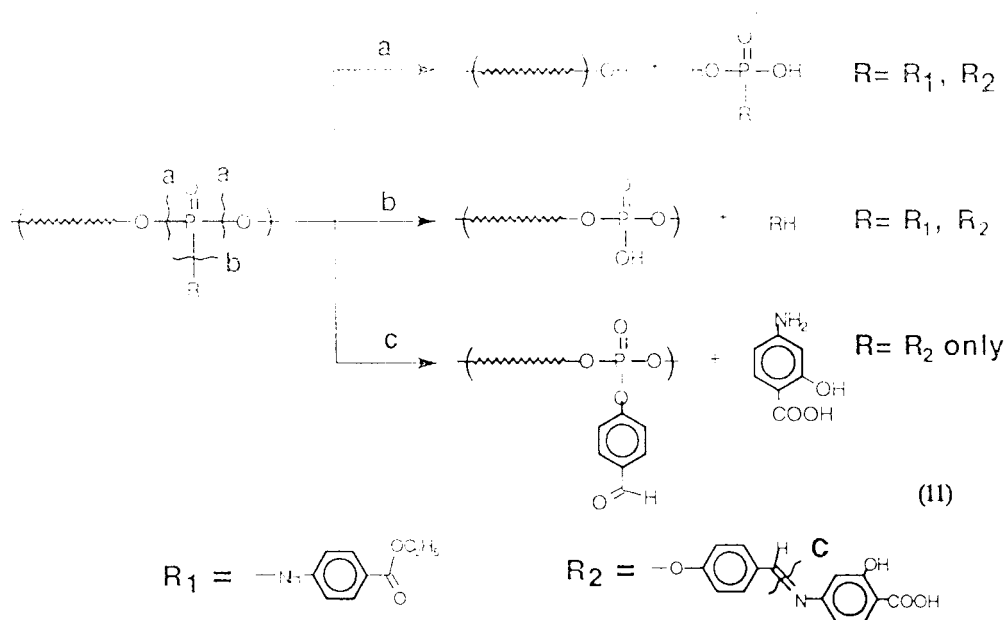


Fig. 8 In vitro release of p-amino salicylic acid and benzocaine from PPU-B7 and PPU-B2 films respectively. The drugs were covalently coupled to the side chain of PPU according to Equations 9 and 10, respectively

For PAS release, the labile imine bond is responsible for the rapid release. Analysis by HPLC showed that only free PAS was present in the buffer. Release of PAS could be realized through different pathways (Eq. 11), 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 or 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 that of aliphatic hydroxyl group.

We have chosen to couple PAS to the polymer 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.

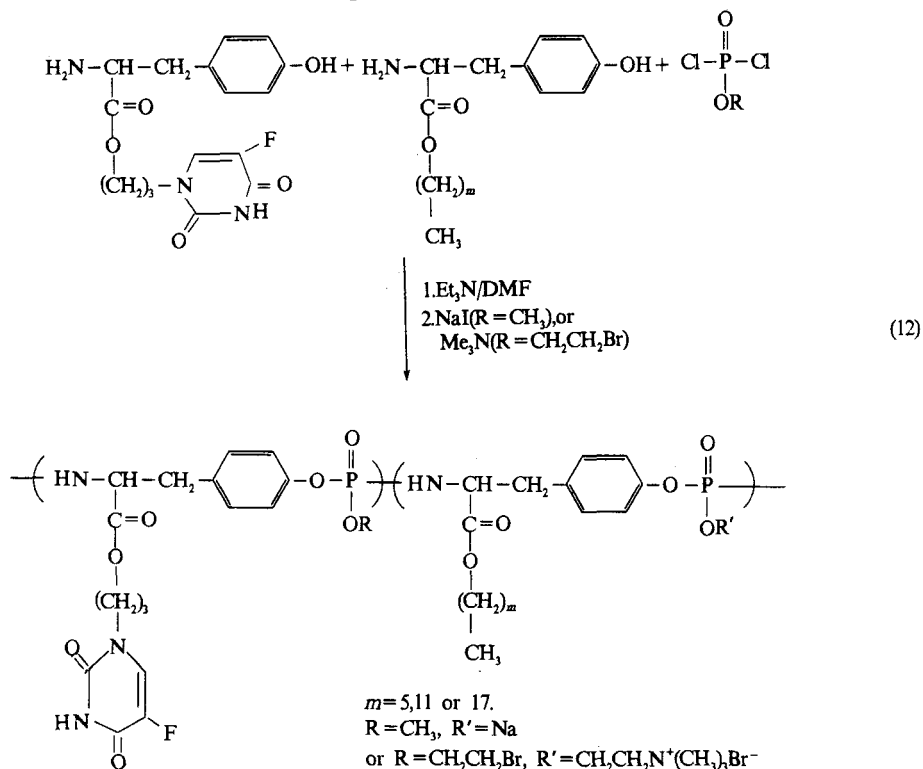
With regards to the original objectives of coating applications, the PPU show promise of meeting the expectations. As with any other new biomaterials considerations, biocompatibility is the biggest unknown. We have designed the polymers from non-toxic monomers, but to predict biocompatibility strictly from chemical structures is impossible. One should also remember that polyurethane chemistry is extremely complicated. Other than the definite urethane, ether, and phosphoester bonds, there are also likely urea, allophanate, biuret, and acylurea bonds in these PPU. The toxicology of these monomers and possible intermediates can only be evaluated *in vivo*. In summary, given the versatility of polyurethanes, with high degrees of freedom to obtain the desired physicochemical properties through manipulations of the soft segment and the chain extender, if the PPU can pass the biocompatibility scrutiny, they will make an interesting class of degradable and elastomeric biomaterials.

DRUG-CARRIER WITH CELL FUSION PROPERTY

A desirable goal of drug therapy is to deliver the drug not only to the target tissue space, but to the target cells. We reasoned that if a polymeric carrier can be effectively internalized by cells, it may carry with it the covalently bound drug and enhance the efficacy of the drug. The commonly used anti-neoplastic agent, 5-fluorouracil, was studied to test this concept^[23,26]

As shown in Eq. 12, 5-fluorouracil (5-FU) was first derivatized to N¹-3'-bromopropyl-5-fluorouracil according to published procedures and then coupled to the carboxylic group of tyrosine via dehalogenation. Separately the tyrosine was esterified to different alkyl chain lengths. These two co-monomers were then polycondensed with phosphorodichloridate in anhydrous DMF at room temperature with triethylamine as the acid acceptor. To obtain a negatively charged polymer, the methyl side chain was converted to a sodium phosphate structure by

refluxing with sodium iodide in acetone. Similarly, a net positive charge was obtained by reacting the bromoethyl group with trimethylamine. We hypothesized that these amphiphilic poly(phosphoramidate)s would form micelles in aqueous solution, facilitating their interaction with cellular membrane and subsequently non-specific endocytosis. To perform a systematic study, the lipophilicity of the polymer was varied by adjusting the ratio of the neutral L-tyrosine 3' (N'-5-fluo-



rouracyl) propyl ester to the L-tyrosine alkyl ester and the alkyl chain length (m).

Typically these polymers have a degree of polymerization around 10 as estimated by vapor pressure osmometry. Under the polymerization conditions adopted, three types of linkages, phosphate, phosphoroamidate, and a hybrid of the two (structures II-IV of Eq. 1), were possible in the backbone, and their presence was confirmed by ^{31}P -NMR. The electrically neutral polymers were readily soluble in common organic solvents such as methanol and dichloromethane, but were insoluble in water. The charged amphiphilic polymers in contrast formed stable colloidal solutions in phosphate buffered saline, yielding micelles with a size distribution averaging from 60 to 260 nm, depending on the length of the side

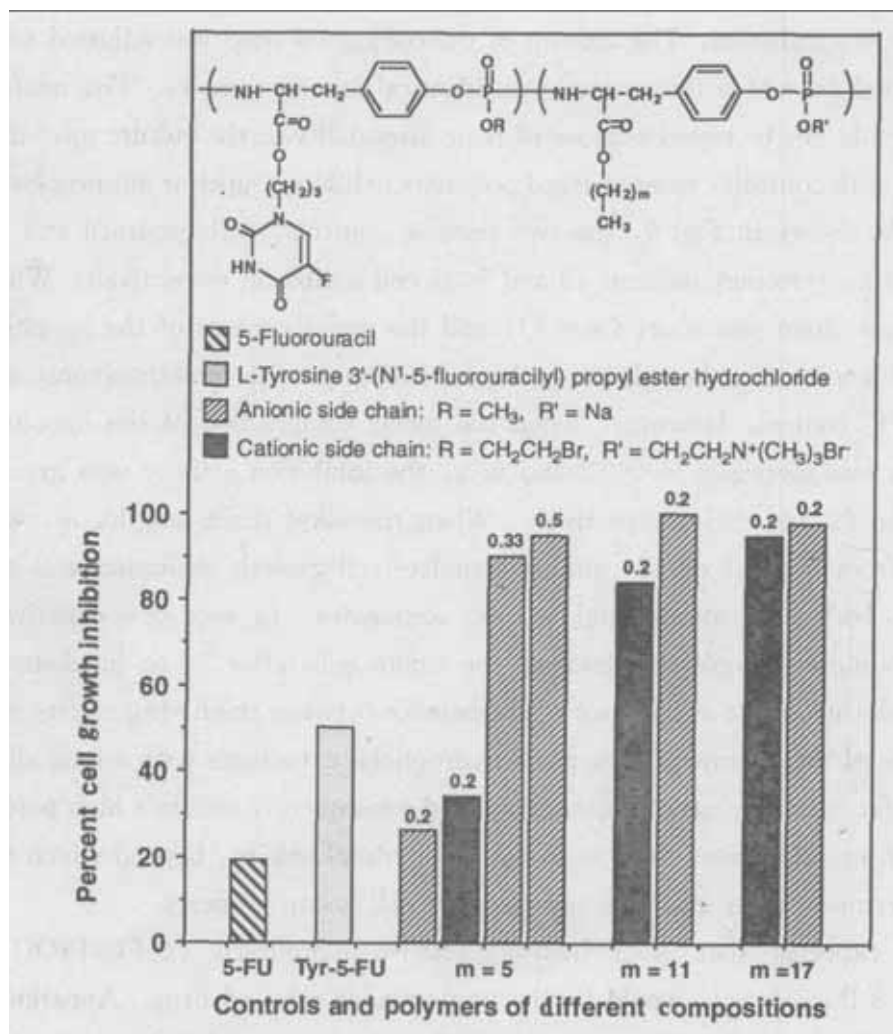


Fig. 9 In vitro antitumor activities of the amphiphilic poly (phosphoramidate)s against EAC murine tumor cells. Tyr-5-FU is L-tyrosine 3'-(N¹-5-fluorouracilyl) propyl ester hydrochloride. Polymers were tested at equivalents of 1.5 mg of 5-U/ml, whereas 5-FU and Tyr-5FU were tested at 5 and 2.5 mg/ml respectively. In reference to the polymeric structure shown on top, the polymers were varied with respect to the alkyl side chain length (m) and molar ratio of the lipophilic component in the backbone (represented by numbers shown on top of the bars)

chain, as measured by laser scattering measurement. Only semi-transparent at room temperature, the solution became clear once heated beyond 75°C. The transition was reversible.

The antitumor activity of the conjugates was tested against Ehrlich ascites carcinoma cells (EAC) by measuring the percent inhibition of cell proliferation

after 24 hr of incubation. The amount of the conjugates used was adjusted such that the total dose of 5-fluorouracil was identical among samples. The neutral polymers could not be tested because of their insolubility in the culture medium. Compared with controls, most charged polymers exhibited superior antineoplastic activity. As shown in Fig. 9, the two positive controls, 5-fluorouracil and its conjugate with tyrosine, induced 19 and 50% cell inhibition respectively. When the alkyl side chain was short ($m=5$), and the molar content of the lipophilic component low (0.2), the polymers achieved similar growth inhibition compared to the 5-FU control. However, when the molar composition of the lipophilic component was increased to 0.33 and 0.5, the inhibition activity was greatly improved to 90 and 95% respectively. When the alkyl chain length, m , was increased from 5 to 11 or 17, almost complete cell growth inhibition was observed for both the cationic and anionic conjugates. In fact one negatively charged conjugate completely dissolved the tumor cells after 24 hr incubation. These results stress the importance of the balance between the hydrophilicity and lipophilicity of the polymers. The more hydrophobic structures with a long alkyl side chain facilitate the intracellular uptake and consequently lead to a high potency of the drug. However, there is an optimal hydrophobicity, beyond which the polymer becomes water insoluble and loses the cell fusion property.

It is expected that N¹-3'-hydroxypropyl-5-fluorouracil (5-FU-PrOH), instead of 5-fluorouracil, would be the predominant released drug. Apparently the antineoplasticity of 5-FU was not significantly impaired by this derivatization.

These results confirmed our hypothesis that these biodegradable amphiphilic poly (phosphoramidate)s would constitute an interesting class of drug-carriers through their interaction with cell membranes. While cellular uptake may not be the limiting step for the therapeutic efficacy of 5-FU, these drug-carriers would be particularly appealing in dealing with drugs whose potency is greatly curbed by access to the intracellular compartment. Naturally not all drugs are suitable for this delivery design since it requires the presence of a reactable hydroxyl group for the coupling to the monomer, and the absence of other reactive groups that might interfere with the polymerization. With ingenuity, however, there are other chemistries that can circumvent these limitations.

DRUG-CARRIER WITH IMMUNOADJUVANTICITY

Rapid advances in immunology, molecular biology and genetic engineering have led to the identification and synthesis of many new antigens. Many antigens, particularly small peptides, however, are poor immunogens. Adjuvants often have to be administered in conjunction with the antigens to elicit the desired immune response. These adjuvants function by promoting a nonspecific inflammatory response, with recruitment of mononuclear phagocytes to the site of immunization, that lead to an enhanced antigen presentation.

It has been hypothesized that antigens in particulate form facilitates uptake into antigen-presenting cells with subsequent access to the MHC class I processing pathway^[16]. For instance, staphylococcal enterotoxin B imbedded in poly (lactide-co-glycolide) microspheres as a toxoid vaccine induced concurrent circulating and disseminated mucosal antibody responses^[32]. In another study, tetanus toxoid was significantly more immunogenic in mice than fluid toxoid^[2]. Sustained release of the antigen is also believed to be beneficial in enhancing an immune response. Thus a recent trend in designing adjuvants for immunopotential is to incorporate a controlled release feature such that a single administration of the antigens can induce prolonged immunity^[14,24].

The fact that many hydrophobic and surface active substances can augment an immune response suggests that compounds with a hydrophobic or amphiphilic structure tend to have immunoadjuvanticity. In view of the known adjuvanticity of L-tyrosine derivatives, we therefore synthesized a series of polyphosphates with L-Tyr-L-Tyr or L-Ser-L-Tyr dipeptide as the recurring unit and evaluate their potential as polymeric adjuvant^[23,25].

The polymers have molecular weights (M_w) ranging from 10,000 to 20,000 and are readily soluble in polar organic solvents but not water, ether, nor hydrocarbons. The in vitro degradation rate of the polymers is a function of both the backbone and side chain structure (Fig. 10). The polymers with the L-Ser-L-Tyr backbone are considerably more hydrolytically labile than the L-Tyr-L-Tyr backbone. As the carbon chain length increases in the side chain, so does the stability of the polymers.

To test the adjuvanticity, polyphosphate (L-Tyr-L-Tyr backbone and

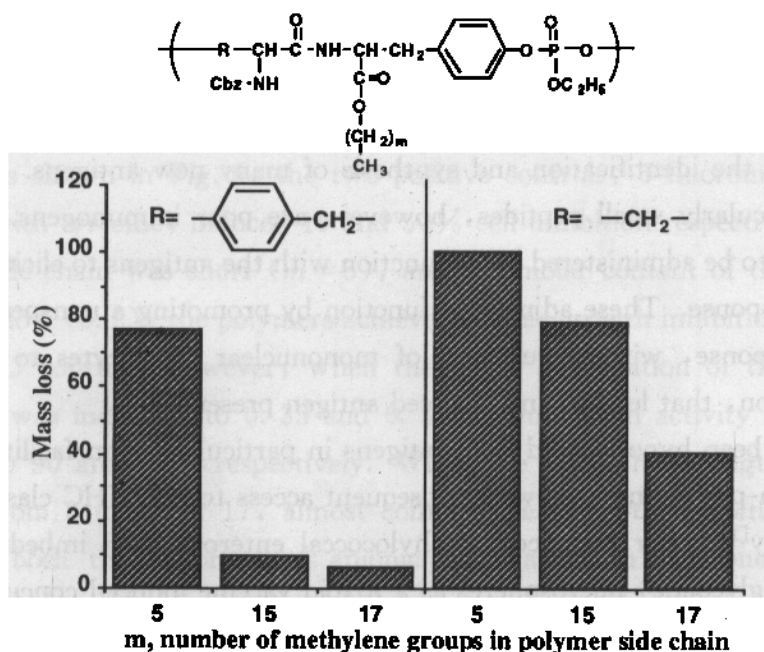


Fig. 10 In vitro mass loss of the poly (phosphoramidate)s at 10 days as a function of the backbone and side chain structures. The backbone contains either a L-Tyr-L-Tyr (left side of graph) or a L-Ser-L-Tyr (right side) dipeptide and an alkyl side chain of variable length

$m=5$) in the form of 10 μm particles was complexed with *Schistosoma japonicum* (SjAg), a human soluble antigen, by stirring for 24 hr in PBS at room temperature, and the suspension was injected into the subcutaneous space of mice. The antibody titers were then measured by ELISA as a function of time. As shown in Fig. 11, SjAg complexed with the poly (phosphate) elicited an antibody response as strong as that of SjAg in complete Freund's adjuvant (CFA). In contrast, SjAg in normal saline hardly elicited a measurable response except on week 5.

These results are promising since CFA is clinically unacceptable because of its severe tissue inflammatory reaction. It is also conceivable, with further optimization of the degradation rate of the polymer and the ratio between the polymer and the antigen, that the poly (phosphate) can surpass CFA as an immunopotentiating agent.

SUMMARY

This review provides a glimpse of the potential of the biodegradable phosphoryl-containing polymers in medical applications. Undoubtedly these polymers possess unique properties that are yet to be fully understood. Many areas warrant further investigation and much optimization remains to be done. The fascinating chemistry of phosphorus poses interesting hurdles but at the same time leaves ample room for polymer scientists to exercise their creativity in designing interesting biomaterials. As the mutual understanding between basic and clinical scientists on the need of medical devices and the capabilities of these new biomaterials expands, imaginative application of new biomaterials to other medical applications can be expected.

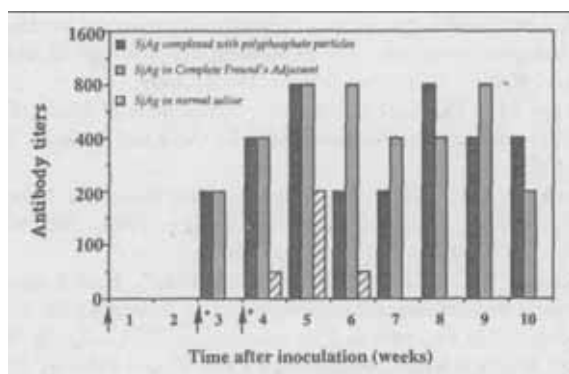


Fig. 11 Antibody titers against *Schistosoma japonicum* (SjAg) using polyphosphate as an adjuvant as comparison with Complete Freund's adjuvant (CFA). Antibody titers were determined by ELISA. 50 μ g of SjAg which was complexed with 2.5mg of poly (phosphate) particles (av. size 10 μ m) in 0.5 ml of PBS was injected (s.c.) into mice on day 0 ($\uparrow$), and boosted ($\uparrow^*$) with 20 μ g of SjAg complexed with 1.0 mg of poly (phosphate) particles in 0.2 ml of PBS on days 14 and 21, respectively. The same primary and booster doses of SjAg in CFA or normal saline were injected into mice as positive and negative control, respectively

Keywords:

Biodegradable polymers; Phosphoryl-containing backbone; Controlled drug delivery

Acknowledgment The authors would like to thank Hungnan Lo, Sudha Kadiyala, Michael Ponticiello, Basil Dahiyat, Zhong Zhao, Chang-lie Fan, and Jun Wang, who worked on the projects described in this review.

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