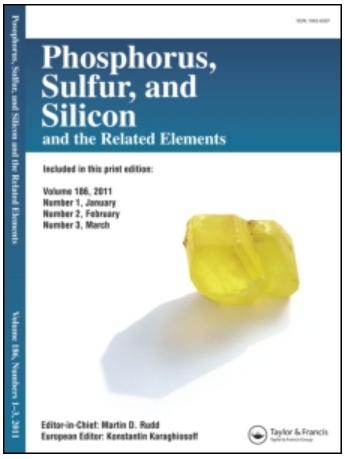

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Recent Advances in Polyphosphoester and Polyphosphoramidate-Based Biomaterials

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Recent Advances in Polyphosphoester and Polyphosphoramidate-Based Biomaterials

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Polyphosphoesters and polyphosphoramidates are important two classes of phosphorous-containing polymers, which have received more and more attention in the past decade for the application in biomedical field. The unique characteristic of biodegradation, biocompatibility and structure versatility of polyphosphoesters and polyphosphoramidates makes it possible to develop a variety of novel phosphate-based polymers for drug delivery, gene delivery and tissue engineering. In this review, we highlighted the recent advances in polyphosphoester and polyphosphoramidate-based biomaterials including synthesis and characterization, degradation, biocompatibility, and important application in drug delivery, gene delivery and nerve repairing.

Keywords Biodegradable; drug delivery; gene delivery; nerve guide conduit; polyphosphoester; polyphosphoramidate

INTRODUCTION

Synthetic biodegradable polymers, such as aliphatic polyesters, aliphatic polycarbonates, poly(ortho esters), polyanhydrides, polyphosphates, and so on, have been widely investigated and applied in biomedical field. Among the current biodegradable polymers, polyphosphates have attracted great attention because of their biocompatibility and pendant functionality. In the past decade, two important classes of phosphate-based polymers, including poly(phosphate ester)(polyphosphoester, PPE) and polyphosphoramidate (PPA), have

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been used in drug delivery, gene delivery, and tissue engineering. Earlier work has been well reviewed previously.[1–4] In this article, we introduced some recent advances in these fields.

SYNTHESIS OF NOVEL POLYPHOSPHOESTERS AND POLYPHOSPHORAMIDATES

The method for the synthesis of high-molecular-weight simple chains of poly(alkylene phosphate) was first developed by Penczek and Klosinski in the 1970s.[3] They used ring-opening polymerization of the five- or six-membered H-phosphonate with subsequent treatment to prepare polyphosphates. Ring-opening polymerization of five- or six-membered cyclic esters of phosphoric acid was also used. Another method for the synthesis of polyphosphates is polycondensation. A large number of phosphate-based homopolymers have been prepared using either methods of ring-opening polymerization or polycondensation mentioned above.[4]

The ring-opening polymerization of cyclic phosphate monomers often needs highly pure monomers, anhydrous conditions, and organometallic initiators. The metallic catalysts must be completely removed before they are used as biomaterials. To avoid the restriction of metallic catalysts, we developed an enzymatic ring-opening polymerization of cyclic phosphate (alkyl ethylene phosphate) to prepare a series of polyphosphoesters.[5,6] It was found that the length of alkyl groups in cyclic phosphates has no significant effect on polymerization degree of obtained polyphosphoesters, but greatly affects the yield of the polyphosphoesters. The yields of polyphosphoester increase with increasing the length of the alkyl groups in cyclic phosphates. Although the enzymatic homopolymerization of cyclic phosphates yield low molecular weight polyphosphoesters (Mn: 600–5000), relatively high molecular weight of poly(trimethylene carbonate-co-ethyl ethylene phosphate)(P(TMC-co-EEP)) (Mn: ~10000) was obtained by enzymatic ring-opening copolymerization of ethyl ethylene phosphate with trimethylene carbonate.[7] The polyphosphoesters with pendent chloroethyloxyl groups were also synthesized by enzymatic ring-opening polymerization.[8]

Random copolymers, including poly(D,L-lactide-co-ethyl ethylene phosphate)(P(LA-co-EEP)),[9] poly(trimethylene carbonate-co-ethyl ethylene phosphate)((PTMC-co-EEP)), poly(2,2-dimethyl trimethylene carbonate-co-ethyl ethylene phosphate)(P(DTC-co-EEP)[10] and poly(p-dioxanone-co-ethyl ethylene phosphate)(P(DON-co-EEP)[11] (shown in Figure 1), were synthesized by ring-opening copolymerization of ethyl ethylene phosphate with D,L-lactide, trimethylene carbonate,

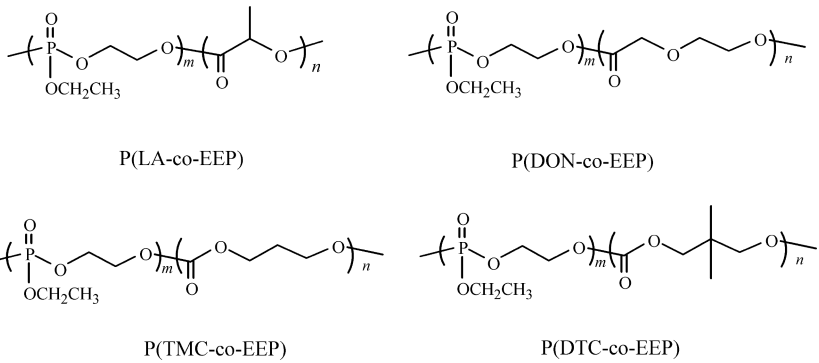


FIGURE 1 Chemical structures of random polyphosphoester copolymers.

2,2-dimethyl trimethylene carbonate and p-dioxanone, respectively. Polyphosphates, as components of copolymers, can increase their solubility in common organic solvents and lower the glass-transition temperature of copolymers. As a result, the processability of copolymers can be greatly improved.

Polyesters containing phosphoester units in the backbones, P(BHET-EOP/TC) and P(DAPG-EOP), were also synthesized by a two-step polycondensation.[4]

Wang and coworkers synthesized block poly(ε-caprolactone-co-ethyl ethylene phosphate), poly(ε-caprolactone-co-isopropyl ethylene phosphate) and poly(ε-caprolactone-co-methoxyethyl ethylene phosphate by initiating the polymerization of ε-caprolactone with $Al(O^iPr)_3$, followed by adding ethyl ethylene phosphate, isopropyl ethylene phosphate or methoxyethyl ethylene phosphate to the living PCL macroinitiation solution, respectively.[12,13] Similarly, amphiphilic brush-coil block copolymer consisting poly(ε-caprolactone) and PEGylated polyphosphoester was prepared by two-step sequential ring-opening copolymerization of

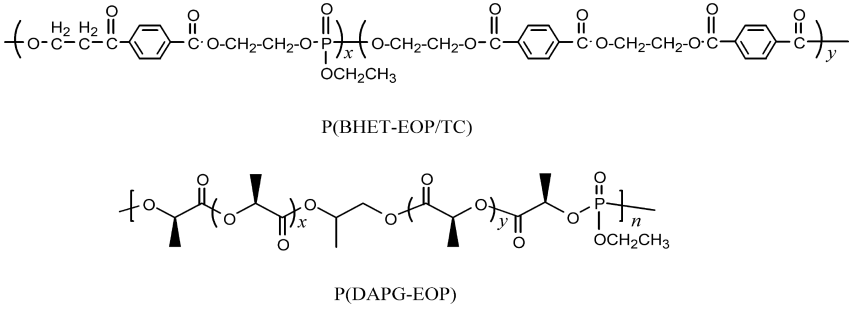
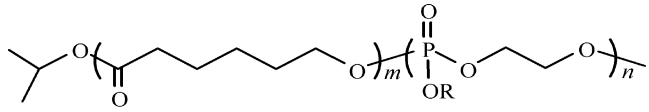


FIGURE 2 Chemical structures of P(BHET-EOP/TC) and P(DAPG-EOP).

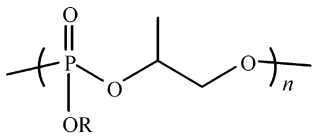


1 R=CH_3CH_2
2 R=$(CH_3)_2CH$
3 R=$CH_3OCH_2CH_2$
4 R=$CH_3O(CH_2CH_2O)_{14}CH_2CH_2$

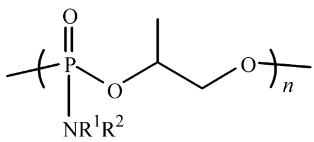
FIGURE 3 Chemical structures of PCL-polyphosphoester block copolymers.

ε-caprolactone and PEGylated cyclic phosphate monomer initiated by $Al(O^iPr)_3$.[14] The chemical structures of block copolymers are shown in Figure 3.

Most of the polyphosphoesters, homopolymers, and copolymers discussed above are hypdrophobic and water-insoluble. By conjugating hydrophilic amino groups or hydroxyl groups as side chains through P—O or P—N bonds, thus obtained polyphosphoesters or polyphosphoramidates become hydrophilic and water-soluble. Polyphosphoesters containing amino group side chains (PPE-EA, PPE-MEA and PPE-HEA)[15] and polyphosphoester containing hydroxyl group side chains (nonionic PPE in figure 4)[16] were synthesized starting from poly(1,2-propylene H-phosphonate) through chlorination, reaction with N-protected hydroxylalkylamine or O-protected diol, respectively, and deprotection. Polyphosphoramidates (PPA in Figure 4) were also prepared from



PPE-EA: R=$CH_2CH_2NH_3^+Cl^-$
PPE-MEA: R=$CH_2CH_2NH_2^+(Me)Cl^-$
PPE-HA: R=$(CH_2)_6NH_3^+Cl^-$
Nonionic PPE: R=CH_2CH_2OH



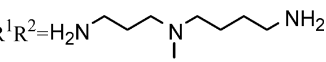
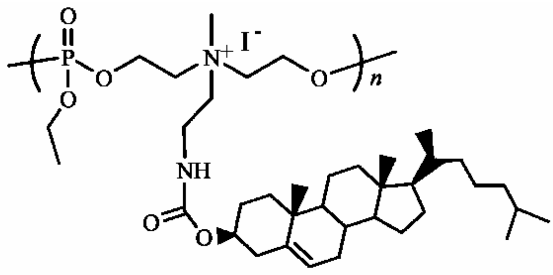
PPA-SP: NR^1R^2= 
PPA-EA: NR^1R^2=$NHCH_2CH_2NH_2$
PPA-BA: NR^1R^2=$NH(CH_2)_4NH_2$
PPA-MEA: NR^1R^2=$NHCH_2CH_2NH(Me)$
PPA-DMA: NR^1R^2=$NHCH_2CH_2N(Me)_2$
PPA-DEA: NR^1R^2=$NHCH_2CH_2N(Et)_2$
PPA-TMA:NR^1R^2=$NHCH_2CH_2N^+(Me)_3Cl^-$

FIGURE 4 Chemical structures of water-soluble polyphosphoesters and polyphosphoramidates.



PCEP

FIGURE 5 Chemical structure of amphiphilic cationic polyphosphoester (PCEP).

poly(1,2-propylene H-phosphonate) by direct reaction with N-protected diamine in the presence of CCl_4 and deprotection.[17]

Amphiphilic cationic polyphosphoester, poly[(cholesteryl oxocarbonylamidoethyl) methyl bis(ethylene) ammonium iodide] ethyl phosphate, as shown in Figure 5, was synthesized through polycondensation of ethyl dichlorophosphate with a diol carrying a positive charge and a cholesterol moiety.[18]

DEGRADATION OF POLYPHOSPHOESTERS AND POLYPHOSPHORAMIDATES

Generally, the degradation rate of polyphosphoesters is faster than aliphatic polyesters, polycarbonates and poly(p-dioxanones) because of relatively high hydrophilicity and low crystallizability of polyphosphoesters. As a result, the introduction of polyphosphoester units in the backbone of biodegradable polymers, such as polyesters, polycarbonates and poly(p-dioxanones), accelerated the degradation of copolymers. For example, the weight loss of poly(TMC-co-EEP) (2:8) copolymer incubated in PBS buffer (pH = 7.4) at 37°C is 100% after 28 days, however, the weight loss of PTMC homopolymer is only 11.5%.[10] The degradation rates of copolymers increase with increasing the percentages of polyphosphoester units in copolymers. Similar results were also observed in the in vitro degradation of poly(D,L-lactide-co-ethyl ethylene phosphate)[19] and poly(p-dioxanone-co-ethyl ethylene phosphate).[11] The in vivo degradation of poly(D,L-lactide-co-ethyl ethylene phosphate) was also investigated and found that the in vivo weight loss is significantly faster than in vitro.

The degradation rate of water-soluble polyphosphoesters is much faster than hydrophobic polyphosphoester homopolymers and copolymers. The weight average molecular weight (Mw) of polyphosphoester containing hydroxyl group side chains (nonionic PPE in Figure 4) decreased by 9% and 33% after 1 day and 7 days incubation in PBS buffer (pH = 7.4) at 37°C, respectively. [16] Although the degradation rate of polyphosphoester containing amino group side chains (PPE-EA) is in the same order of magnitude as that of nonionic PPE, the introduction of amino group side chains into polyphosphoester induces faster degradation as compared to polyphosphoester containing hydroxyl group side chains.[15]The degradation of polyphosphoramidates is much slower than water-soluble PPE. For example, the weight average molecular weight (Mw) of PPA-SP only decreases 7% after 30-day incubation in PBS (pH = 7.4). [1]

BIOCOMPATIBILITY OF POLYPHOSPHOESTERS AND POLYPHOSPHORAMIDATES

Biocompatibility is important for biomedical polymers design. Generally, hydrophobic polyphosphoester homopolymers and copolymers show low in vitro cytotoxicity. For example, P(LA-co-EEP) is less toxic in HeLa cells. Its in vivo biocompatibility was tested in mouse brain and the results indicated that there are no significant difference in inflammatory reaction in the brain section injected with saline and P(LA-co-EEP) microspheres.[19]

Water-soluble polyphosphoester also shows very low cytotoxicity. Polyphosphoester containing hydroxyl group side chains (nonionic PPE) has minimal toxicity to both COS 7 cells and HEK 293 cells at a polymer concentration as high as 12.5 $mgmL^{-1}$. Nonionic PPE also shows low level of acute tissue responses in mouse muscle.[16] In contrast to polyphosphoester containing hydroxyl group side chains, polyphosphoesters containing amino group side chains show relatively high in vitro cytotoxicity and tissue responses in mouse muscle, however, the in vitro cytotoxicity and tissue responses of cationic PPE and polyphosphoramidates are much lower than polyethylenimine and poly(L-lysine)—two polycations widely used in nonviral gene delivery.[1,15]

BIOMEDICAL APPLICATION OF POLYPHOSPHOESTERS AND POLYPHOSPHORAMIDATES

Drug Delivery

Drugs can be encapsulated in polymer systems, such as polymer microparticles and nanoparticles, and released through surface or bulk

erosion of the particle, diffusion of the drug through the polymer matrices, or swelling followed by diffusion. Poly(LA-co-EEP)s, an important kind of polyphosphoester copolymers, have been used in drug delivery in preclinical and clinical studies. PACLIMER microspheres, containing 10% (W/W) paclitaxel in poly(LA-co-EEP), have been evaluated in both a relevant animal model (OVCAR-3) and a phase I human trial for advanced ovarian cancer, respectively. The results indicated that the tumor growth inhibition using PACLIMER microspheres is greater than that using Taxol solution and vehicle controls. [1]

BSA as a macromolecular model drug was encapsulated in poly(LA-co-EEP) microspheres. The in vitro release of BSA from poly(LA-co-EEP) microspheres included an initial burst (9%) in the first day, 4% release in the following 40 days, and 91% release of BSA in a nearly linear manner in the further 80 days. This is greatly different from release of BSA from PLA microspheres. The SDS-PAGE data indicated that the structure of BSA was not significantly affected in the process of preparation, storage and release.[19]

The sustained release of nerve growth factor (NGF), a water-soluble neurotrophic protein, from polyphosphoester P(DAPG-EOP) microspheres was also investigated. Bioactive NGF could be released for at least 10 weeks from the P(DAPG-EOP) microspheres.[20]

Gene Delivery

Polycations as nonviral vectors provide a simple, safe and cost-effective method for gene delivery and have attracted more and more attention in the past decade. A large number of cationic polymers, such as polyethylenimine (PEI), polyamidoamine dendrimer, poly(L-lysine) etc, were used to deliver foreign DNA into cells and enhance protein expression in cells.[21] Cationic polyphosphoesters and polyphosphoramidates have been successfully developed as gene delivery vectors.[15,17] They can bind plasmid DNA to form polyelectrolyte complexes nanoparticles and in vitro transfect many cells efficiently. The transfection efficiencies depend on the polymer structure and the ratio of polycation to DNA (N/P). Under optimized conditions, the transfection efficiency of PPA-SP in HEK 293 cell line is comparable to that of PEI, one of the most efficient cationic polymer carriers.

The in vivo transfection of CNS neurons was carried out by peripheral intramuscular injection of complexes of PPA and therapeutic gene bcl-2. The bcl-2 gene expression of PPA-SP/DNA is comparable to that of PEI/DNA, however, higher in vivo expression of PPA-DMA/DNA was observed as compared with PEI/DNA. The in vivo results are inconsistent with the in vitro results. This phenomenon is common in polymeric

gene delivery system, which implies that it is necessary and important to investigate the difference of mechanism between in vitro and in vivo transfection.[22]

The unique hydrolytically-degradable characteristic of cationic PPE makes it possible to develop a novel DNA controlled release system. The electrophoresis analysis of cationic PPE/DNA complexes incubated in PBS7.4 at 37°C demonstrated that the release of DNA from the complexes was a function of N/P ratio of complexes and incubation time. Higher N/P ratio leads to slower release of DNA from cationic PPE/DNA complexes. The release of DNA is triggered by the hydrolytic degradation of cationic PPE and losing the ability to bind DNA.[15] This method of controlled DNA release has been successfully applied in intramuscular gene delivery.[23,24]Sustained local release of DNA from cationic PPE/DNA complexes from the injection site protected DNA from degradation, enhanced and prolonged the gene expression in the muscle cells.

Nerve Guide Conduit

Biodegradable hydrophobic polyphosphoesters have also been applied in treatment for injury-induced nerve defect. Nerve guide conduits, prepared from biodegradable polyphosphoester, have been developed to bridge the nerve gaps by inserting the severed nerve stumps into the two ends of the conduits. Because of the good processability, relatively fast degradation and no crystallization, P(BHET-EOP/TC) was selected to prepare nerve guide conduits. Thus obtained nerve guide conduits show non-toxicity in vitro and low inflammatory response after in vivo implantation and promote the axonal regeneration.[25,26]

Sustained release of nerve growth factor (NGF) from NGF-containing polyphosphoester (P(DAPG-EOP))microspheres, loaded in silicon or P(BHET-EOP/TC) conduits, provides a long-term promoting regeneration of injured peripheral nerves. The advantages of the combination of microspheres NGF delivery system and nerve guide conduits include sustained local action of a trophic factor and reduction of the total amount of trophic factor proteins needed to generate a satisfactory biological effect.[27]

CONCLUSIONS

In summary, applications of polyphosphoesters and polyphosphoramidates in drug delivery, gene delivery, and tissue engineering have attracted more and more attention in the past decade because of biodegradation and structure versatility. The future work in the field of phosphate-based biomaterials will be focused on the structure-function

relationships and new applications in biomedical field. It is believed that more and more phosphate-based biomaterials will be clinically investigated and applied in the future.

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