Main-chain water-soluble polyphosphoesters: multi-functional polymers as degradable PEG-alternatives for biomedical applications

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Abstract

Polyphosphoesters (PPEs) are a class of (bio)degradable polymers with high chemical versatility and functionality. In particular, water-soluble PPEs with the phosphoester group in the polymer backbone are currently discussed as a potential alternative to poly(ethylene glycol) (PEG). Ring-opening polymerization of typically 5-membered cyclic phosphoesters gives straightforward access to various well-defined PPEs. Several PPE candidates have proven their biocompatibility *in vitro* in terms of cytocompatibility, antifouling properties, "stealth effect", degradability (hydrolytic and enzymatic), and some promising *in vivo* results in drug delivery vehicles. The possibility to control the properties with the appropriate tuning of the lateral chain makes PPEs especially appealing. This review summarizes recent developments of such PPEs for biomedical applications, e.g. in protein-polymer conjugates, hydrogels for tissue engineering, or nanocarriers for drug and gene delivery. We summarize the progress made over the years, highlighting the strengths and the shortcomings of PPEs for these applications to date. We critically evaluate the current state of the art, try to assess their potential and to predict future perspectives, shedding light on the pathway that needs to be followed to translate into clinics.

Keywords

Phosphorus; polyphosphoesters; poly(ethylene glycol); PEGylation; biodegradable, biocompatible.

Abbreviations:

$Al(O^iBu)_3$: aluminium triisobutanoate

$Al(O^iPr)_3$: aluminium triisopropanoate

AROP: Anionic Ring-Opening Polymerization

ATP: adenosine triphosphate

ATRP: Atom Transfer Radical Polymerization

BHT: 2,6-Di-tert-butyl-4-methylphenol

BSA: Bovine Serum Albumin

CMC: critic micelle concentration

CPT: camptothecin

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DFT: Density-Functional Theroy

DNA deoxyribonucleic acid

DOX: doroxubicin

ECDL: 1-[3'(dimethylamino)propyl]-3-ethylcarbodiimide methiodide

EDDA: 2,2'-(ethylenedioxy)bis(ethylamine)

ESI-MS: Electron Spray Ionization-Mass Spectrometry

FDA: Food and Drug Administration

GPC: Gel Permeation Chromatography

HES: hydroxy ethyl starch

HMPA: poly(*N*-(2-hydroxypropyl)methacrylamide)

HPPE: hyperbranched polyphosphoester

IgM: immunoglobulin M

My: myoglobin

NADH: nicotinamide adenine nucleotide

n-DSC: nano-Differential Scanning Calorimetry

n-DSF: nano-Differential Scanning Fluorimetry

NMR: Nuclear Magnetic Resonance

NPs: nanoparticles

P(EEP-*co*-EMEP): poly(ethyl ethylene-*co*-ethyl 2-methylehtylene phosphate)

PAEP: poly(allyl ethylene phosphate)

PBEP: poly(3-butenyl ethylene phosphate)

PBS: phosphate buffered saline

PBuEP: poly(butyl ethylene phosphonate)

PBYP: poly(3-butynyl ethylene phosphonate)

PCEP: poly{[(cholesteryl oxocarbonylamidoethyl) methyl bis(ethylene) ammonium iodide] ethyl phosphate}

PCL: poly(ε-caprolactone)

PDMAEMA: poly[2-(dimethylamino)ethyl methacrylate]

PDS: poly(disulfide)

PEBP: poly(2-ethylbutyl ethylene phosphonate)

PEEP: poly(ethyl ethylene phosphate)

PEG: poly(ethylene glycol)

PEI: poly(ethylene ammine)

PEMEP: poly(ethyl 1-methylethylene phosphate)

PEOMP: in-chain poly(ethyl (S)-methylethylene phopshoramidate)

PEP: poly(ethylene phosphate)

PEtEP: poly(ethyl ethylene phosphonate)

PG: poly(glycerol)

PGA: poly(glutamic acid)

PHMEP: poly(ethylene H-phoshponate)

P^{i}PrEP: poly(isopropyl ethylene phosphonate)

PLGA: poly(lactic-*co*-glycolic) acid

PLL: poly(L-lysine)

PLLA: poly(L-lactic acid)

PMeEP: poly(methyl ethylene phosphonate)

PMEP: poly(methyl ethylene phosphate)

PMMA: poly(methyl methacrylate)

PMOEPA: poly(2-metoxyethyl ethylene phosphoramidate)

Poly(CL-co-OPEA): poly(ε-caprolactone-*co*-[2-(2-oxo-1,3,2-dioxaphospholoyloxy) ethyl acrylate]

POxs: poly(2-oxazoline)s

PPAs: polyphosphoramidates

PPE3: poly(2-hydroxyethyl propylene phosphate)

PPE-EA: poly(2-aminoethyl propylene phosphate)

PPE-EA-Boc: poly(2-(*N*-tertbutoxycarbonylamino propylene phosphate)

PPE-HA: Poly(6-aminohexyl propylene phosphate)

PPEI: poly(ethylene phosphate) ionomer

PPE-MEA: poly(*N*-methyl-2-aminoethyl propylene phosphate)

PPEs: polyphosphoesters

PPLs: phospholipids

PS: polystyrene

PTX: paclitaxel

PVP: poly(vinylpyrrolidone)

RNA: ribonucleic acid

ROP: Ring-Opening Polymerization

SANS: Small Angle Neutron Scattering Spectroscopy

SC: succinimidyl carbonate

$SnOct_2$: tin octanoate

TBD: 1,5,7-triazabicyclo[4.4.0]dec-5-ene

TFPC: 7,8-dihydro-5,10,15,20-tetrakis(pentafluorophenyl)-21H,23H-porphine

Tris-Urea: 1,1',1''-(nitrilotris(ethane-2,1-diyl))tris(3-(3,5-bis(trifluoro-methyl)phenyl)urea)

TU: 1-1-[3,5-bis(trifluoromethyl)phenyl]- 3-cyclohexyl-2-thiourea

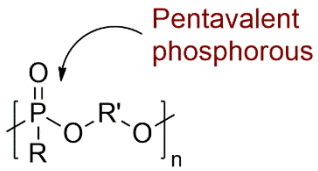
1. Introduction

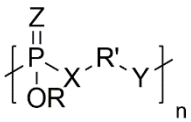
This review summarizes the application of PPEs in biomedical applications and compares the data to well-known PEG-based analogs.

Phosphorus-containing compounds play an important role in nature: DNA, RNA, ATP, NADH, PPLs are some examples of molecules involved in the metabolism bearing phosphorus in one or more phosphate units [1]. Less common, but still important in the living organisms, are compounds with a P-C bond, present in the so-called phosphono- lipids, glycans, and proteins, which play an important role in several metabolic pathways [2]. The large abundance of these compounds in nature has stimulated the interest of the scientific community over the years, fascinated by the possibility to synthesize PPEs that could mimic some properties of their natural analogs. The first studies on synthetic phosphorus-containing polymers were conducted in the 1950s [3], even though the high cost of the starting materials and the difficulties to control the synthesis have slowed down the research on this topic. Nonetheless, the interest in these polymers (in particular on the subclasses of polyphosphazenes and PPEs [4]) has grown over the years, due to their peculiar properties. To date, there are more than 13000 scientific publications on phosphorus-containing polymers (data from Web of Science, June 2020); the flame-retardant properties of some PPEs are exploited on an industrial scale [5].

The use of synthetic polymers in biomedical applications has been studied over more than 50 years. The most common water-soluble polymer currently used in bioapplications is probably PEG, an aliphatic polyether, prepared by ring-opening polymerization of ethylene oxide [6–8]. Very recently, some concerns on its long-term non-degradability and non-immunogenic properties (cf. *Section 2*) have triggered the search for potential alternatives. Among the potential substitutes, main-chain PPEs found their place as a promising biomimetic class of polymers, with broad potential use in the biomedical field, due to their controlled synthesis, additional chemical functionality, biodegradability, and biocompatibility.

Main-chain PPEs and PPAs are polymers based on phosphoric or phosphonic acid derivatives (esters and amides). A variety of chemical modifications around the central phosphorus gives access to polymers with different properties and chemical functionality. The most common classification varies the linking chemistry in the lateral chain, defining the subclasses of polyphosphates, poly(alkylene H-phosphonate)s, PPAs, and polyphosphonates, with respectively an -OR, an -H, an -NR_2 (or -NHR), and an -R group as the lateral group (*Scheme 1*). Besides the side chain, also the linkages in the main chain allow control over materials properties, especially backbone-hydrolysis, as in the case of in-chain polyphosphoramidates [9] or –diamidates [10] and in-chain polyphosphonates [11] (*Scheme 1*). More recently, also polythionophosphates (*Scheme 1*) have been reported, in which formally the P=O bond is replaced by the more hydrophobic P=S-moiety, inducing an additional handle on polymer properties, e.g. oxidative lability [12]. To date, these are the known members of the family, however, further structural modifications are possible.





R = alkyl,aryl	Polyphosphonates
R = OR''	Polyphosphates
R = H	Poly(alkylene H-phoshonate)s
R = NR''R'''(or NHR'')	Poly(phosphoramidate)s

X = N; Y,Z=O	In-chain poly(phosphoramidate)s
X,Y = N; Z=O	In-chain poly(phosphorodiamidate)s
X = CH_2; Y,Z =O	In-chain poly(phosphonate)s
X, Y = O; Z =S	Polythionophosphates

Scheme 1: Overview of the subclasses of PPEs reported to date.

Most PPEs are hydrophobic and prepared by classical polycondensation chemistry. However, pioneering works of Penczek and co-workers in the 1970s on the ring-opening polymerization of cyclic P-containing monomers paved the way for a broad family of hydrophilic (and hydrophobic) PPEs [13–15]. The chemical diversity is the major strength of the PPE-chemistry: the presence of the pentavalent phosphorus in the backbone allows the synthesis of polymers with a broad scope of functional groups in the side chain or

main chain, which allows controlling properties such as biocompatibility, hydrophilicity, degradability, crystallinity, thermal stability, etc.. The side chain could contain additional functional groups, that open the possibility to post-modification reactions, widely employed to prepare various kinds of co- and graft-polymers or to stimuli-responsive materials [16]. From a literature analysis [17], we could estimate more than 100 different PPE homopolymers synthesised to date by different strategies (e.g. polycondensation, polyaddition, ROP, metathesis), and the number of structures available rapidly increases if we consider all the post-modification reactions performed. The high variability of the structure represents one of the most competitive advantages of PPEs respect to the PEG and most of the other potential substitutes, however, it also makes the right choice difficult. The abundance of phosphorus-containing compounds in nature makes PPEs promising materials, as they are expected to show high compatibility with biological systems and low toxicity. Besides, with accurate miming of biological scaffolds, the polymers are expected to be biodegradable and producing non-toxic degradation products. The selection of the lateral chain substituent could also be useful for the tuning of the polymer degradability [18]. All these features render PPEs a promising platform for degradable and biocompatible materials for biomedicine. To date, they have not been reached clinical trials, because a systematic and comprehensive evaluation *in vitro* and *in vivo* still needs to be completed for some promising candidates. Figure 1 shows a timeline for the most important developments made concerning the features and applications of main-chain water-soluble PPEs.

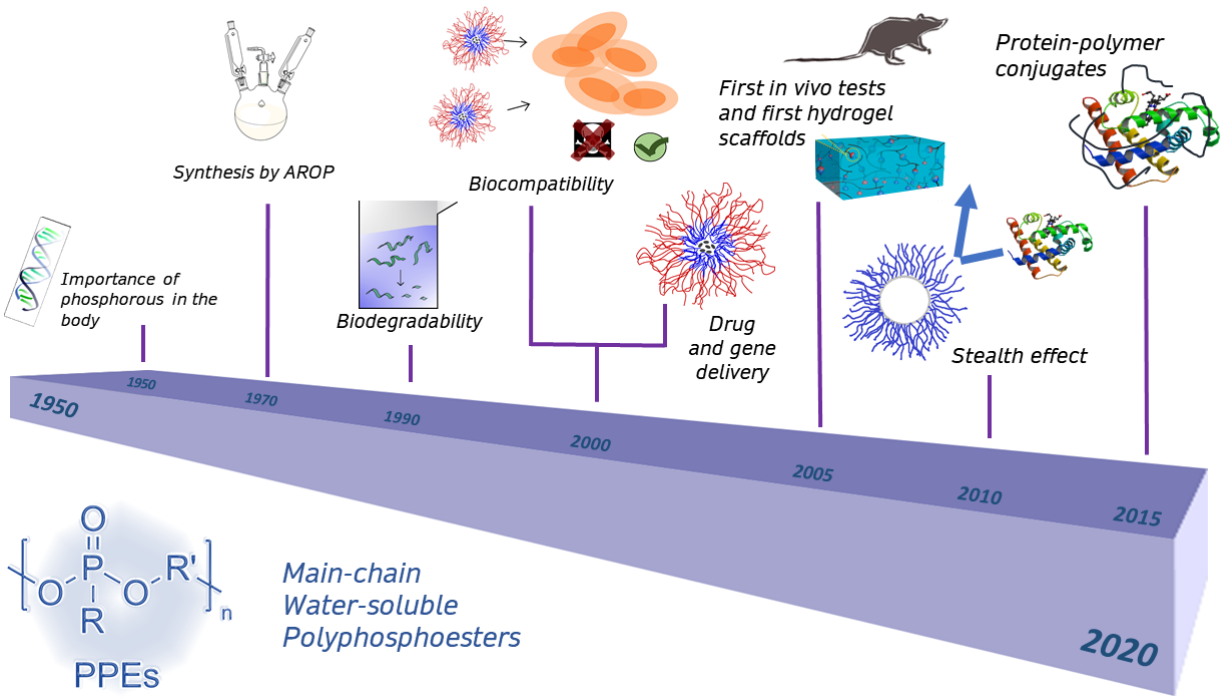


Figure 1: Timeline for the research and development of main-chain water-soluble PPEs.

In this review, we will focus on the competitive advantages given by the use of main-chain, water-soluble PPEs in biomedical applications, presenting the progress made through the years and the recent discoveries. Previous review articles about PPEs covered the synthesis, properties, and applications [4,19–23]. In 2017, our group published a comprehensive overview of PPEs history and synthesis [17]. The current article updates the former review but focuses on biomedical applications, adding a detailed evaluation of the polymer properties in comparison to PEG, and summarize selected examples of the field.

The review is divided into four sections: after a short motivation for PPEs as promising PEG alternatives, we will summarize the synthetic procedures and features of water-soluble PPEs, mainly prepared via ring-opening polymerization, including some very recent developments in the field. Later, we critically evaluate two fundamental properties for biomedical applications: biocompatibility and biodegradability and how these factors have been studied for PPEs *in vivo* and/or *in vitro*. In the last section, we report on selected examples relying on water-soluble PPEs for biomedical applications, in particular in protein-polymer conjugates ("PPEylation"), PPE-based hydrogels, and PPEs for drug and gene delivery.

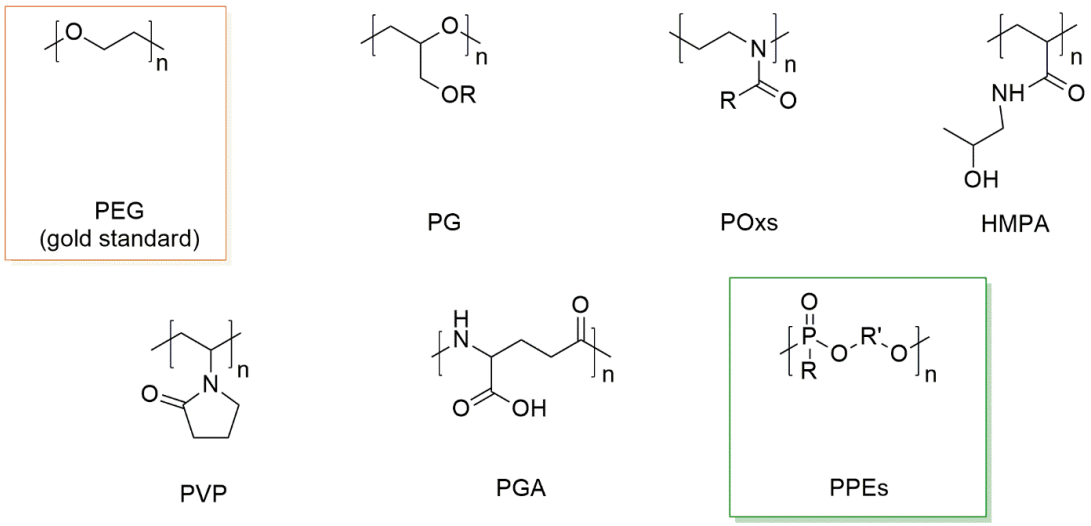
2. PPEs as an alternative for PEG

PEG is currently employed in many fields, e.g. in the food industry, cosmetics, textiles. PEG is also added as an additive in paints due to its antifouling properties [24]. Moreover, it is the most common water-soluble polymer in the conjugation field, ofter referred to as the "gold standard" [25–27]. Covalent attachment of PEG chains to proteins, peptides, nanocarriers, oligonucleotides, or other kinds of molecules is called "PEGylation" and it is widely used in the biomedical field. Today, 15 PEGylated pharmaceuticals have been approved by both the U.S. Food and Drug Administration Agency and the European Medicines Agency, and used for therapeutic purposes, while 36 PEGylated drugs, dendrimers, proteins, aptamers, or PEG-containing copolymers forming NPs are currently in clinical trials (Data from the U.S. Food and Drug Administration Agency and the European Medicines Agency websites) [26].

Despite their current use, PEGylated drugs have raised some concerns in the last 10 years, due to the observation of unexpected drawbacks. Long-term treatments with PEGylated drugs (required for the treatment of chronic diseases) could lead to polymer accumulation in the body, causing unwanted side effects [25]. Immunogenicity problems, hypersensitivity responses after the treatment and the formation of anti-PEG IgM antibodies have been reported, leading to an accelerated polymer clearance from the bloodstream [28–33]. Moreover, PEG has been shown to trigger complement activation pathway in the body, which can bring anaphylactic reaction in sensitive patients [34]. The chemical structure of PEG brings

additional disadvantages, such as low biodegradability in the human body, while it can be degraded in sewage-plants by certain microorganisms [35]. Besides, oxidative main-chain degradation can occur, leading to the formation of toxic compounds (e.g. 1,4-dioxane or formaldehyde) [25].

To overcome these issues, the search for PEG-substitutes has become an important research topic in the biomedical field. Both non-biodegradable polymers (such as PG, POxs, HMPA, PVP) and biodegradable alternatives (such as HES or poly(amino acid)s, e.g. PGA) have been proposed (*Scheme 2*). They have been discussed extensively in other reviews [25,36–39]. Besides these materials, PPEs are an emerging and promising alternative with peculiar features, some of them to be discussed herein. *Tables 1 and 2* compare the key properties of PPEs with PEG, HMPA, POxs, and PGA.



Scheme 2: Overview of water-soluble, synthetic polymers used in biomedical applications PEG alternatives (PG, POxs, HMPA, PVP, PGA, and PPEs; R and R' groups represent various (mostly) aliphatic residues).

	Versatility of chemical structure		Properties		Real applications*	
	Controlled synthesis	Postmodification reactions**	Biodegradability	Biocompatibility	Current clinical trials	Currently in-use
PPEs			 ***	 ***		
PEG					 (36 items)	 (15 items)
PHMPA					 ****	 ****
POxs					 (1 items)	
PGA					 (5 items)	

Table 1 and 2: Comparison between PEG and promising potential alternatives: water-soluble PPEs, HMPA, POxs, and PG.

Data from U.S. Food and Drug Administration Agency and European Medicines Agency websites; referred to drug-polymer conjugates of nanocarriers where the candidate is the whole polymer or a co-polymer moiety. In-chain post modification reactions, beyond the end-chain functionalization, possible for all the polymers.***Experiments made prevalently in vitro. **** No candidates are in a trial at the moment but some candidates have been subjected to discontinued clinical trials in the past years.*

	STRENGTHS	DRAWBACKS	OTHER CONSIDERATIONS
PPEs	**- Chemically versatile**: possible to add different groups in the lateral chain (tuning of properties, allowing post-modification reactions or NPs cross-linking, multiple linking with drugs); **- Stealth effect**, **biocompatible, biodegradable** *in vitro*; - Controlled synthesis.	- *In vivo* studies show promising results but are **still not enough to express a general evaluation**.	- This class of polymers is still young in the field; therefore, it is **still under evaluation**.
PEG	- **Enhanced pharmacokinetics** of PEGylated drugs in the body; - Biocompatible and stealth effect; **- Cheap and controlled synthesis**.	**- No chemically versatile**; - Issues related to assessed long term **non-biodegradability, hypersensitivity** reactions, and	- It is the **gold standard** (15 candidates currently in use, 36 in trials).

		antibody formation.	
POxs	- Behaviour comparable to PEG in terms of **pharmacokinetics** in the blood; - **Biocompatible**; - **Cheap and controlled synthesis**.	- In-chain post-polymerization reactions not allowed; - **Non-biodegradable**.	- **1 candidate** in a clinical trial.
PGA	- **Enhanced pharmacokinetics** of the candidates *in vivo*; - **Biodegradable**.	- **No chemically versatile**; - **Complement activation** in the body.	- **5 candidates** in clinical trials.
PHMPA	- **Enhanced pharmacokinetics** of the candidates *in vivo*; - Biocompatible.	- **Non-biodegradable**; - Some candidates have shown **marginal efficiency** in clinical trials.	- Some candidates are subjected to **discontinued clinical trials**.

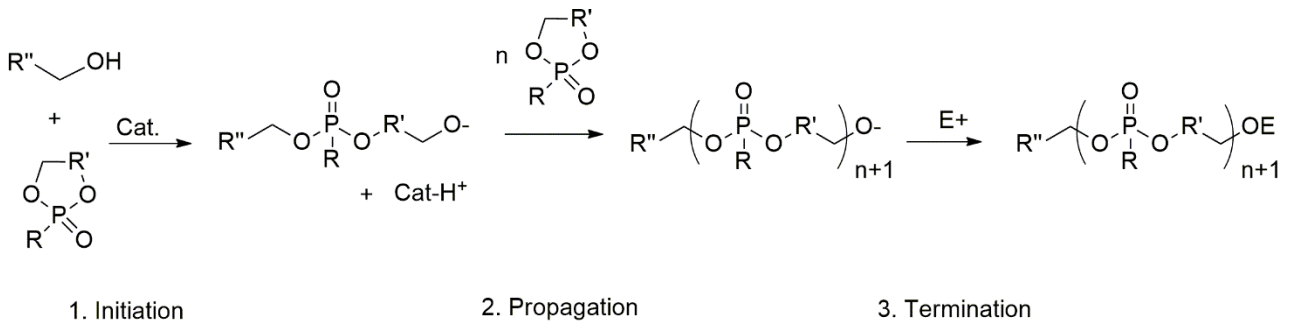
The differences in the chemistry, e.g. choice of linkages in the main- or side-chains lead to very different intrinsic properties, leading to different strengths and drawbacks for each material. In this context, we believe that PPEs find their place as very promising to substitute PEG and others, but also, as PPEs are one of the most recent materials in the field, a long way will be ahead; it was not even reported in the reviews that describe the possible alternative to PEG published before 2016 [25,40,41]. The chemical versatility in combination with degradability differentiates PPEs from the other candidates listed in Scheme 2, Tables 1 and 2, and opens new possibilities toward personalized medicine and drug-delivery.

3. Synthesis of water-soluble PPEs

PPEs can be synthesized by polycondensation, transesterification, ring-opening polymerization, olefin metathesis, and some other strategies [17]. For water-soluble PPEs, mainly the AROP is used (*Scheme 3*) because of the high control over molar masses, dispersity, and chemical functionalization (either of the side chain or the chain termini).

Herein, we will define the polymers using the most used nomenclature: i) poly(alkyl alkylene phosphate)s for polyphosphates with the alkylene-group in the main chain (mostly ethylene-bridge) and the alkyl substituent present as an alkoxy group in the lateral chain; ii) poly(alkyl alkylene phosphonate)s for polyphosphonates defined with the same criteria (but in this case, the lateral alkyl-chain is directly linked to the phosphorus by a P-C-linkage); iii) poly(alkyl alkylene phosphoramidate)s to define PPAs with the same criteria (but in this case the lateral alkyl-chain is connected to phosphorus atom by a P-N-bond). In addition,

if polymers with the P-C or P-N bonds in the main-chain are mentioned, they will be called explicitly "in-chain polyphosphonates" or "in-chain PPAs" (in such cases, the substituent in the lateral chain is connected by a P-O bond). Other polymers are named individually, if necessary.



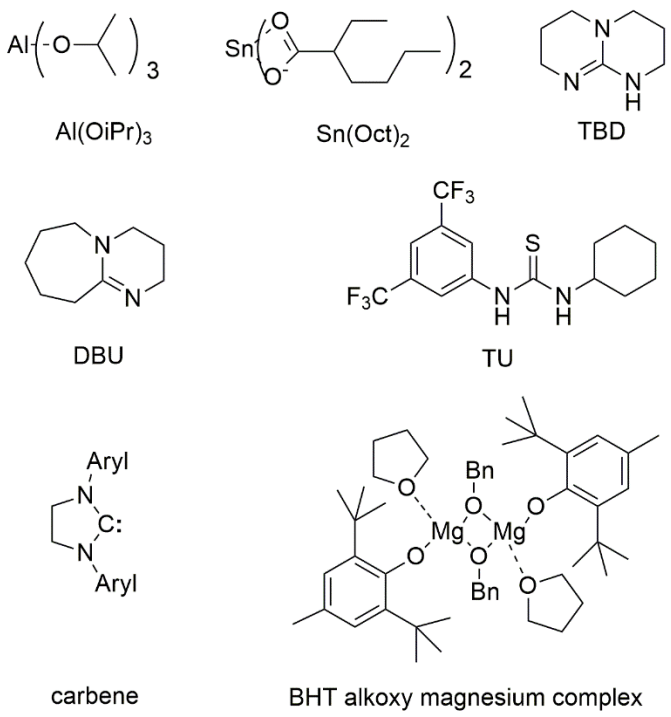
Scheme 3: AROP of cyclic phosphate monomers towards main-chain PPEs (R=alkyl, or O-alkyl; Cat.= catalyst, cf. Scheme 4; E^+= electrophilic termination reagent).

Ring-opening polymerization is a chain-growth technique, used for the synthesis of various classes of polymers, e.g. polyesters, polyamides, poly(ester amide)s, polyphosphoesters, which often allows the preparation of well-defined polymers with a low molar mass dispersity [42]. Besides cationic or metal-catalyzed ROP, the anionic ROP (AROP) is the most used technique to polymerize cyclic P-containing monomers (mainly five-membered cyclic phosphates). ^{31}P NMR spectroscopy is a powerful tool in monomer and polymer synthesis as the chemical shifts in ^{31}P NMR are highly sensitive to the chemical environment and allow fast assessment of ring-opened impurities during the monomer synthesis but also to follow polymerization kinetics (*Table 2*). One drawback of the AROP procedure is its high sensitivity to moisture and other nucleophiles, therefore the monomer needs to be carefully purified and dried to achieve control during the polymerization. The monomer purification itself is a delicate step, due to its high sensitivity to traces of water or other protic species that can easily open the ring, therefore high-vacuum distillation and subsequent dry storage are required. When stored properly, most cyclic phosphoester monomers are stable for at least several months. Most cyclic phosphate and phosphonate monomers that have been reported to date, react immediately with water, due to the high-ring strain of the five-membered ring. The only monomer that had been reported to withstand the hydrolysis with water for at least several hours is the phostone (see Table 3, line 2), recently reported by Bauer et al. [11].

The AROP of cyclic phosphoesters is initiated by a hydroxyl group of the initiator, which undergoes a nucleophilic attack at the phosphorus atom of the strained monomer. The use of different kinds of initiators has been reported in the literature, as aliphatic alcohols, benzylic alcohols (useful for the

determination of the polymer's absolute molar mass by NMR spectroscopy [43]), macroinitiators (for the formation of block copolymers [44–47]), or an anticancer drug (e.g. CPT [48] or PTX [49]). The last choice is an innovative pathway that led to the formation of nanoparticles for anticancer therapy, where the drug can be at the same time encapsulated and covalently linked.

Several catalysts have been employed for the ROP of cyclic phosphoester monomers. Initially, organometallic compounds (in particular $Al(O^iPr)_3$ [50] or $Sn(Oct)_2$ [44]) were used, in analogy with the polymerization of lactones to polyesters (*Scheme 4*). In 2010, Iwasaki reported the first organocatalyzed AROP using DBU or TBD (*Scheme 4*) for the synthesis of poly(isopropyl ethylene phosphate) [51]. In 2012, Clément et al. reported the combined use of the catalyst DBU and the co-catalyst TU to prepare PPEs with lower molar mass dispersity. They also hypothesized the mechanism of action for the three catalysts: DBU and TBD can activate the hydroxyl group of the initiator by hydrogen bonding and thus promoting the nucleophilic attack to the phosphorus centre of the monomer. TU, in contraat, is able to activate the P=O-bond in the monomer and increases its electrophilicity. A combined used of DBU and TU can promote the activation of both the nucleophile and the electrophile, enhancing the reaction rates and reducing the possibility of transerification side-reactions occurring during the polymerization similar to the organocatalytic ROP of other lactones [52]. The intermediates during AROP were recently studied in detail by Ninfant'ev and co-workers utilizing DFT calculations [53–55]. They were able to reveal that in the TBD-catalyzed polymerization of MEP, the transesterification reaction is energetically non-favourable and that the low-energy pathway for the catalyst action involves a "donor-acceptor" mechanism. Beyond the AROP (that nowadays is the polymerization strategy commonly used for the synthesis of PPEs for biomedical applications), new trends in ROP involve the use of catalysts such as *N*-heterocyclic carbenes [56] or heteroleptic BHT alkoxy magnesium complexes [57,58] (*Scheme 4*). *Table 3* summarizes the monomers that to date have been polymerized to form water-soluble main-chain PPEs.



Scheme 4: Catalysts used in AROP polymerization of main-chain water-soluble PPEs and the new generation of catalysts for PPE polymerization.

Table 3: Monomers that homopolymerize with ROP mechanism to form water-soluble main-chain PPEs.

Monomer	^{31}P NMR δ (ppm)[a]	Catalyst	Polymer	^{31}P NMR δ (ppm)[b]	Reference
(4S)-2-ethoxy-4-methyl-1,3,2-oxazaphospho-lidine 2-oxide	26.0-25.2	TBD	PEOMP	10	[9]
2-ethoxy-1,2-oxaphospholane 2-oxide (phostone)	49.3	TBD, DBU/TU, or DBU/Tris-urea	In-chain polyphosphonate	35	[11]
2-ethoxy-2-oxo-1,3,2-dioxaphospholane	16.8	$SnOct_2$, TBD, DBU	PEEP	-1	[43,44,59]
4-Methyl-2-oxo-2-hydro-1,3,2-dioxaphospholane	7.6	$Al(O^iBu)_3$	PHMEP (and PPE-EA, PPE-MEA, PPE-HA[c])	7.2 (and respectively 2, -4, -1)	[50,60,61]
2-methoxy-2-oxo-1,3,2-dioxaphospholane	17.6-16.8	$Al(O^iPr)_3$, BHT complex, TBD	PMEP	-0.4	[57,62]

2-methyl-2oxo-1,3,2-dioxaphospholane	48.8	DBU	PMeEP	32	[63]
2-ethyl-2oxo-1,3,2-dioxaphospholane	52.5	DBU	PEtEP	35	[64]
2-isopropyl-2oxo-1,3,2-dioxaphospholane	55.0	TBD	P^{i}PrEP	36.1	[64]
2-allyloxy-2oxo-1,3,2-dioxaphospholane	17.6	DBU/TU	PAEP	-1.4	[65]
N-Methoxyethyl phospholane amidate	25.8	TBD	PMOEPA (and PPEI[d])	10 (and 1.01)	[66]
2-Ethoxy-4-methyl-2-oxo-1,3,2-dioxaphospholane	15.7/15.8	TBD	PEMEP	-1.2/-3.5	[67]
2-(*N*-tert-butoxycarbonylamino) ethoxy-2-oxo-1,3,2-dioxaphospholane	Not reported	$SnOct_2$	PPE-EA-Boc (and PPE-EA[e])	Not reported	[68]

a: ^{31}P NMR of the monomer; b: ^{31}P NMR of the polymer; c: Obtained after chlorination and proper nucleophilic substitution; d: Obtained after hydrolysis of PMOEPA; e: Obtained after deprotection of the lateral chain.

Biomedical applications often require the development of a multi-functional polymeric structure to achieve their objectives [69]. For example, the formation of micelles or NPs in the aqueous environment requires polymer amphiphilicity, often achieved by the synthesis of block copolymers. PEEP is one of the most exploited in this area, being the hydrophilic part of copolymers with a wide range of hydrophobic polymers (e.g. PEG [70], PCL [44,45,71–73], PDS [46], PBYP [48]). Besides, post-modification reactions are also widely used to introduce new functionalities in the polymers. For example, the introduction of a triple bond and the subsequent click reaction is the key for the polymer covalent conjugation to drugs [48,74,75], to other polymers [76], or to change the nanoparticles surface charge [76–79]. More details about the PPEs structures developed for biomedical applications and their biological implications are reported in the dedicated section (*see below*).

4. Properties of water-soluble PPEs

4.1 Enzymatic and hydrolytic degradation

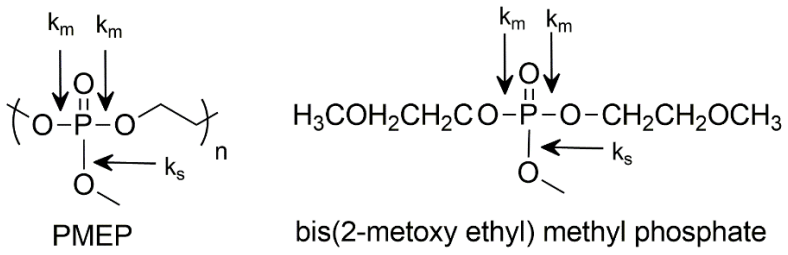
The degradation of polymers is an important property that strongly affects their applicability, in particular in the biomedical field [80]. Following the IUPAC definition, polymer degradation is a chemical change that leads to an alteration of its properties linked to a decrease of the molar mass. The process is called bio-degradation, when the breakdown of the substance is initiated by enzymes, *in vitro* or *in vivo* [81,82].

Degradability is desirable for every polymer that wants to be used as a protein-modifier, drug, or gene carrier, in the treatment of diseases. The gradual breaking of its structure is necessary to avoid complications related to the long-term presence of foreign material in the body, due to its accumulation. The tuning of the degradation rate lies in the thin line between the desired functionality of the polymer and its necessary clearance from the body, therefore it represents an important challenge in the design of new polymeric structures for biomedical applications [83].

PPEs present promising properties in this field, as they permit to control the degradation rate with an accurate choice of the substituent in the lateral chain.

Mechanisms of hydrolysis

Important investigations on kinetics and mechanism of PPEs degradation were performed by Penczek and Baran in 1995 [18]. They studied the hydrolysis of PMEP and bis(2-methoxy ethyl) methyl phosphate by NMR spectroscopy and titration, evaluating the degradation rate constants of the main chain (k_m) or the side chain (k_s) at different pH values (*Scheme 5*). The studies revealed that in acidic conditions, the hydrolysis of the lateral chain proceeded faster compared to the main chain ($k_s/k_m>1$), while under basic conditions the lateral or main chain was cleaved statistically. The authors explained these results by the occurrence of different degradation mechanisms: under acidic conditions, the α-carbon atom is attacked by a nucleophilic water molecule, therefore the attack at the side chain is favoured by less steric hindrance. Under basic conditions, OH^- attacks the phosphorus center and induces the formation of a trigonal bipyramidal geometry, in which the axial position (that can be occupied by either the lateral or the main-chain substituent) is preferentially broken (*Scheme 6*). Moreover, the similarities found between PMEP and bis(2-methoxy ethyl) methyl phosphate, led them to the assumption that the polymer degradation rate is comparable to small molecules.



Scheme 5: Definition of hydrolysis rate constants k_s and k_m for PMEP and bis(2-methoxy ethyl) methyl phosphate.

More recently, our group studied the hydrolytic degradation of PMEP and PEEP in detail [84]. We performed a comparative analysis by ^{31}P, ^{1}H, and ^{31}P DOSY NMR (*Figure 2a, 2b*), supported by additional GPC analyses and DFT calculations, from which they hypothesized a different predominant mechanism for the hydrolysis of PPEs in basic conditions, namely a backbiting degradation (reported in *Table 2* and *Scheme 6*). The formation of a five-membered cyclic intermediate from the terminal OH-group with the preferential cleavage of the main chain was observed for PEEP and PMEP. The mechanism was corroborated by the observation of a drastic reduction in the degradation kinetics when the OH-chain end was blocked by a stable urethane linkage (*Figure 2c*).

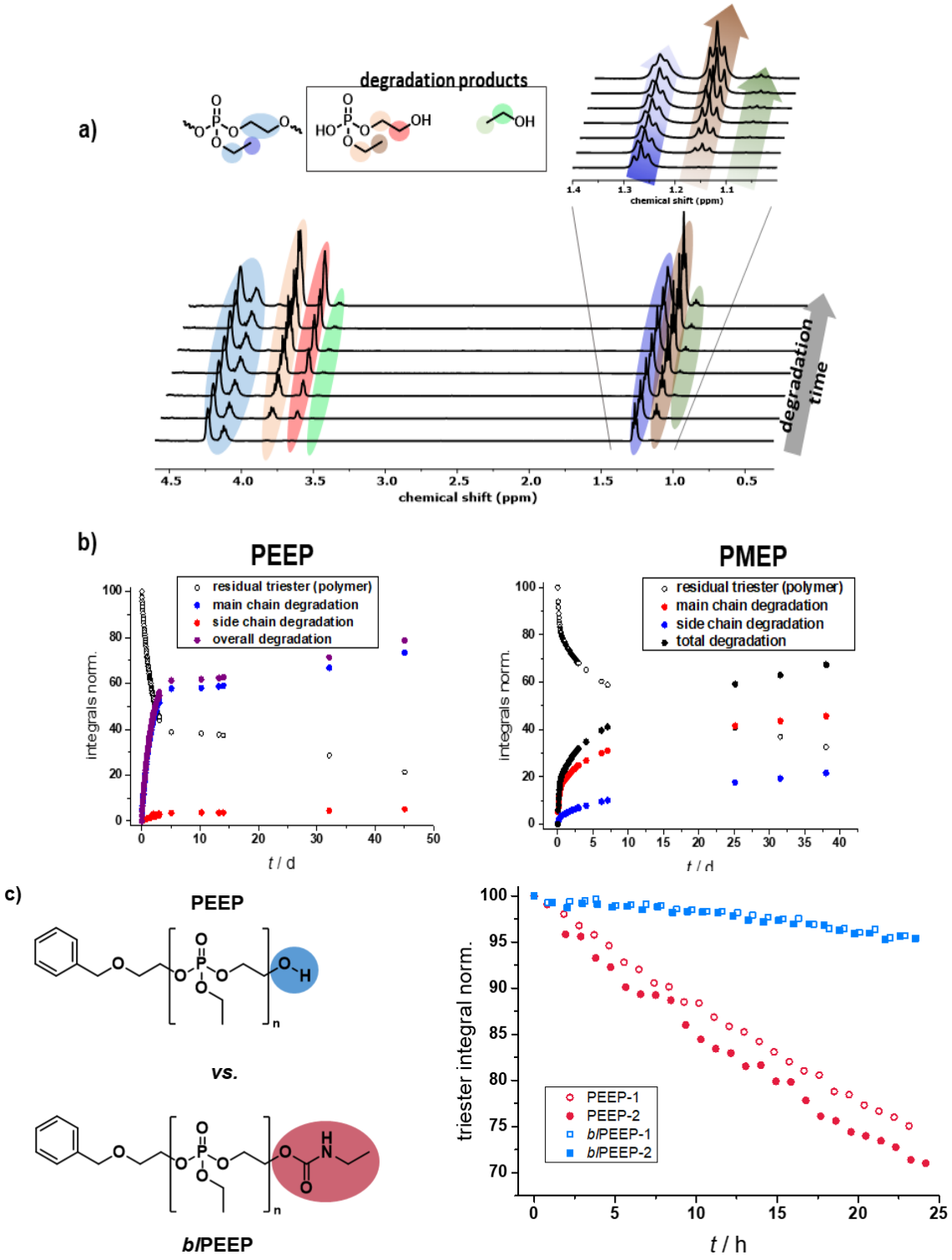
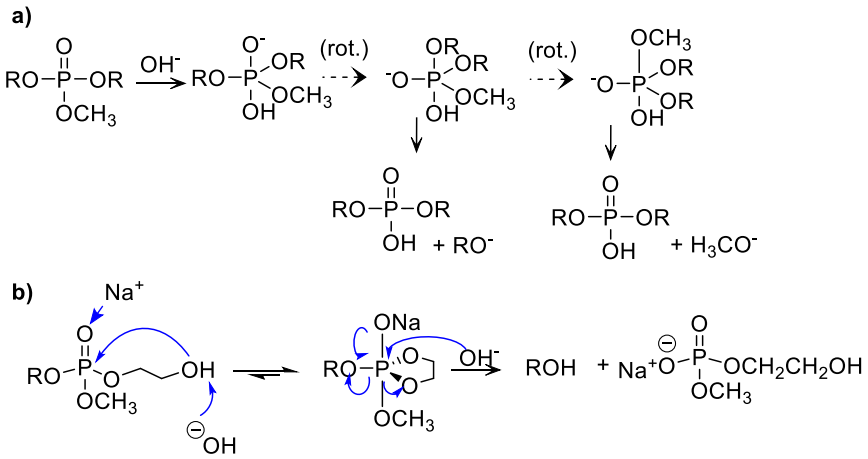


Figure 2: a) The overlay of several ^{1}H NMR spectra for PEEP, recorded at different degradation times. b) The degradation profiles of PEEP and PMEP at pH 11 derived from ^{1}H NMR spectra. c) Chemical structures of PEEP and b/PEEP, with respectively a terminal hydroxyl or a urethane functionality, and the respective degradation profiles derived from ^{31}P NMR spectra. Reproduced from: Mechanistic study on the hydrolytic degradation of polyphosphates, K.N. Bauer, L. Liu, M. Wagner, D. Andrienko, F.R. Wurm, Eur. Polym. J. 108 (2018) 286–294 [84]. Copyright © 2018, with permission from Elsevier.

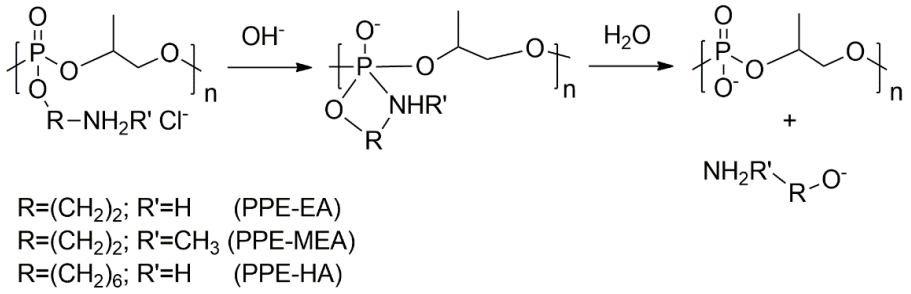


Scheme 6: Degradation mechanism of PMEP in basic conditions, suggested by a) Penczek and Baran [18] *and b) Bauer et al.* [84].

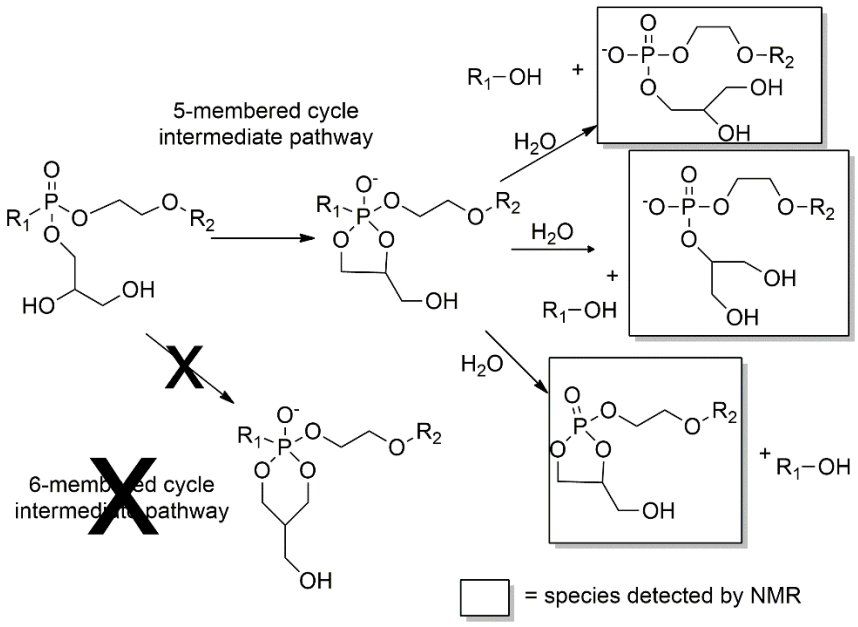
Wolf et al. highlighted the increased hydrolytic lability of the side-chain in polyphosphonates, with increasing hydrolysis rates going from the isopropyl, to the ethyl, to the methyl-substituted polymer [64] (*Table 4*). Interestingly, in-chain polyphosphonates prepared by ROP of phostones exhibited a much lower hydrolysis rate compared to both polyphosphonates and polyphosphates (with similar pendant groups) [11]. These differences in the experimental degradation rate of polyphosphonates and polyphosphates are probably caused by the electron density of the central phosphorus and the different tendency to form the 5-membered cyclic intermediate. Knowing that the major hydrolysis mechanism of PPEs synthesized by ROP relies on backbiting, allows further control of their degradation kinetics and the design of future applications.

Besides phosphoester-linkages, the more hydrolysis-labile P-N-bonds can be installed either in the lateral chain or in the polymer backbone. The group of Leong gave a significant contribution to this topic, reporting the degradation rate of different polymers in PBS at 37°C, determined through an evaluation of the polymer weight loss by GPC [50,61] (*Table 4*). They proposed a self-catalytic degradation mechanism, occurring via the nucleophilic attack of the substituent on the central phosphorus atom with the formation of a cyclic intermediate (*Scheme 7*). The degradation rate is therefore regulated by energetic factors, related to the number of atoms of the cyclic intermediate and the substituent polarity. The possibility of a nucleophilic attack of lateral chain substituent on the phosphorus atom was recently confirmed by Kosarev et al. with their study on the hydrolytic degradation in basic conditions of a 2,3-dihydroxy propyl functionalized polyphosphates [85]. They observed by NMR the degradation profile over time and, thanks to the molecular identification, they suggested the hypothesis of a degradation pathway that involves a 5-membered cycle intermediate, preferred to the 6-membered ring (*Scheme 8*). PPAs degradation implies a different mechanism, due to the acid-sensitive P-N-bond. The Wooley group has evaluated the degradation of PPAs with the P-N bond in the lateral chain [66] or the main chain [9], while Steinmann and co-workers

evaluated the degradation of main-chain polyphosphorodiamidates synthesized by acyclic diene metathesis polymerization [10] or thiol-ene reaction [86] (*Table 4*). PPAs with the P-N bonds forming the polymer backbone proved pH-dependent hydrolysis, namely an increase of polymer degradation with decreasing pH-values. It is important to note that for side-chain PPAs, the hydrolysis leads to the loosing of the side chain and the formation of a negatively charged polyphosphodiester, while backbone cleavage is achieved for main-chain PPAs. In the last case, the Wooley group evaluated more in detail the composition of the degradation products by ESI-MS, finding that the at 40% of conversion, the major degradation products were trimeric units (m/z= 512), subsequently object of further degradation, i.e. not following a backbiting mechanism.



Scheme 7: Self-catalytic degradation mechanism proposed by Leong and co-workers [50,61].



Scheme 8: Possible degradation mechanisms linked to the hydrolytic degradation of 2,3-dihydroxypropyl functionalized PPEs proposed by Kosarev et al. [85].

The degradation profile of various PPEs is reported in *Table 4*. Besides, the degradation profile of PPEs in block copolymers have been reported, in which a faster degradation of the PPE blocks compared to other polymers was found [79,87–89], confirming their potential for drug delivery or tissue engineering.

Table 4: Main-chain degradation by hydrolysis of main-chain water-soluble PPEs, calculated by ^{31}P NMR or GPC.

Polymer	Exp. Conditions	Method of analysis	Degradation %			Ref.
			After 24 h	After 7 days	After 30 days	
$PEOMP_{90}$	pH 7.4	^{31}P NMR[a]	100	100	/	[9]
	pH 5		98	80	/	
	pH 3		90	15	/	
$PPE\text{-}EA_{140}$	37°C PBS pH: 7	GPC[b]	90	33	/	[50]
$PPE\text{-}HA_{102}$	37°C PBS pH: 7	GPC[b]	91	88	40	[61]
$PPE\text{-}MEA_{44}$	37°C PBS pH: 7		91	90	80	
$PEtEP_{40}$	/	GPC[b]	Quantification not performed; degradation rate increase going from Me to Et substituent and increasing pH			[64]
$PMeEP_{21}$	/					
$PMEP_{97}$	pH 11	^{31}P NMR[a]	82	60	36	[84]
$PEEP_{93}$			80	38	28	
2,3-dihydroxypropyl-substituted poly(ethylene phosphate)$_{72}$	pH 8.5, 11	^{31}P NMR[a]	Quantification not performed; degradation rate increase at higher pH; considered faster than the others poly(alkyl ethylene phosphates)			[85]
$PEEP_{52}\text{-}PLLA_{29}\text{-}PEEP_{52}$	pH 10.9	GPC[b]	94	83	40[c]	[88]
	pH 7.4		98	96	71	
	pH 2.5		96	/	66[c]	
$PPE3_{35}$	37°C PBS pH: 7	GPC[b]	91	20	/	[90]

a: Calculated as the conversion of polymer signal in different species; b: Calculated as % of residue molecular weight respect to the initial one; c: After 20 days.

Degradation of PPE micelles and nanoparticles

To date, several studies on the hydrolytic degradation of PPEs as a constituent of micelles or nanoparticles have been reported. The degradation studies were usually conducted at physiological temperature (37°C) but different pH values, to simulate different environments (blood has pH 7.4; pH 5.0 mimics the conditions of endosomes/lysosomes or tumour tissues; pH 3.0 mimics the gastric fluids [1]).

In the last decade, the Wooley group has reported several elegant studies on the degradation of PPE-NPs, with interesting results. In 2019, they compared the hydrolysis of Au-NPs coated with citrate, PEG, and the polyphosphoester PBYP, monitoring the surface plasmon resonance (SPR) over 14 days (*Figure 3*) [77]. They reported that the Au-NPs coated with the zwitterionic polymer PBYP presented a broadening and a redshift of the SPR band over time, indicating an aggregation of the NPs induced by the degradation of the polymer coating. This result is important because it highlights the effective PPEs degradation when used for NP coating, while PEG is non-biodegradable under the same conditions. In addition, they performed a cross-linking reaction of the PBYP after the NP coating, to evaluate the eventual influence of this reaction on the properties of the NPs. The new NPs with cross-linked PBYP showed high stability over 14 days, suggesting the possibility to tune the polymer degradability with accurate control of the cross-linking degree, as already reported in other papers [91,92].

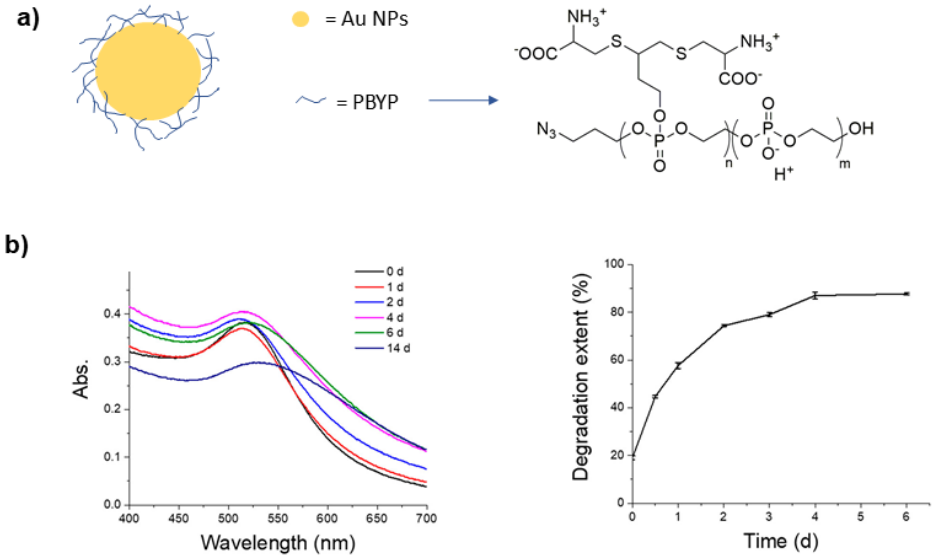


Figure 3: a) Chemical structure of AuNPs coated with the zwitterionic polymer PBYP; *b) On the left UV-VIS spectra of* Au-NPs coated with PBYP *recorded at 0, 1, 2, 4, 6, 14 days and on the right correspondent degradation profile over time. Adapted with permission from: Functional, Degradable Zwitterionic Polyphosphoesters as Biocompatible Coating Materials for Metal Nanostructures, R. Li, M. Elsabahy, Y. Song, H. Wang, L. Su, R.A. Letteri, S. Khan, G.S. Heo, G. Sun, Y. Liu, K.L. Wooley, Langmuir. 35 (2019) 1503–1512* [77] . *Copyright © 2018, American Chemical Society.*

The Wooley group has also investigated the effect of the chemical structure and charge of the substituent on the degradation rate of polymeric nanoparticles [91–94]. Elsabhay et al. published the biochemical evaluation of a set of PPE-based micelles and cross-linked nanoparticles with non-ionic, cationic, anionic, and zwitterionic surface charge, monitoring the size of the samples over time [91]. The size was retained for a longer time when the NPs were cross-linked, while the stability decreased going from of the anionic and non-ionic to zwitterionic, followed by the cationic NPs. The cationic NPs proved a size decreasing within several days, and a concomitant zeta potential reduction over time (from positive values to -45 mV) suggesting the formation of negatively charged-phosphates in the side-chain substituents during the degradation process. Overall, the degradation was higher when amino groups were present in the side chain and slightly faster at pH 7.4 than at pH 5.0, confirming the higher stability of PPEs in acidic conditions. The rapid loss of the positive charge of cationic NPs was confirmed by Shen and co-workers [92], who correlated the higher hydrolysis rate at pH 7.4 to a possible attack at the central phosphorus atom by the nucleophilic amino groups in the lateral chain, supporting the hypothesis (described in the previous paragraph) that it has an important role in the degradation mechanism.

Enzymatic degradation

Phosphodiester bonds are widely present in living systems; therefore, the enzymatic degradation of synthetic PPEs is important to be evaluated and could give more accurate hints on the behaviour of the polymeric material in a physiological environment. Some examples of enzymes that promote the degradation of PPEs have been reported in the literature, e.g. phosphodiesterase I [45,72,95], alkaline phosphatase [96,97], and phosphotriesterase [98]. Among the others, the alkaline phosphatase has great importance in the research of new target-specific drugs, because it is overexpressed in various cancer cells [99,100] and bacteria [101].

The accelerated degradation rate by enzymes could bring a higher release of the drug encapsulated in the micelles/nanoparticles, as shown by Wang et al., who obtained the release of 83.8% of DOX from their PPE-based nanocarriers after 140h using phosphodiesterase I, compared to the release of 30% obtained without the use of the enzyme [72]. The release of DOX encapsulated in hyperbranched PPEs, induced by alkaline phosphatase, was reported by Yao and co-workers [97]. The enzymatic degradation could be used to selectively degrade the other moiety linked to the PPEs in a block copolymer. For instance, the treatment of PPE-*b*-PLA block copolymers with proteinase K permitted the complete cleavage of the PLA block [93], and a similar result was obtained with the treatment of polycaprolactone-*b*-PPE block copolymers with *Pseudomonas* lipase [45,102].

To date, the hydrolytic and enzymatic degradation of PPEs was explored prevalently *in vitro,* and very few studies on *in vivo* evaluations have been reported. Chaubal et al. studied the degradation profile of a linear polylactide with phosphate units inserted in the chain, comparing the percentage of the polymer mass loss over time obtained *in vitro* (after dissolving the polymer in PBS at 37°C) and *in vivo* (after injection in mice) [87]. They found a significantly fast degradation *in vivo*, without any lag phase. Very recently, Liu et al. reported *in vivo* analyses on the antitumoral activity of NPs (called PPE-FP_2, *Figure 4*) composed of the probe TFPC conjugated to a homotelechelic PMEP [103]. Through real-time fluorescence imaging performed in mice, they found the accumulation of the NPs in the tumour site and the suppression of tumour growth after phototherapy. The spleen and kidneys of mice analysed after two months of treatment showed no damage caused by their use, in contrast to the severe damages caused after using the PEGylated analogues under the same conditions. The results were explained by a complete biodegradability of the PPE-FP_2 NPs, that exhibit with good performances their antitumoral action, without provoking damages to the spleen and kidneys caused by their accumulation.

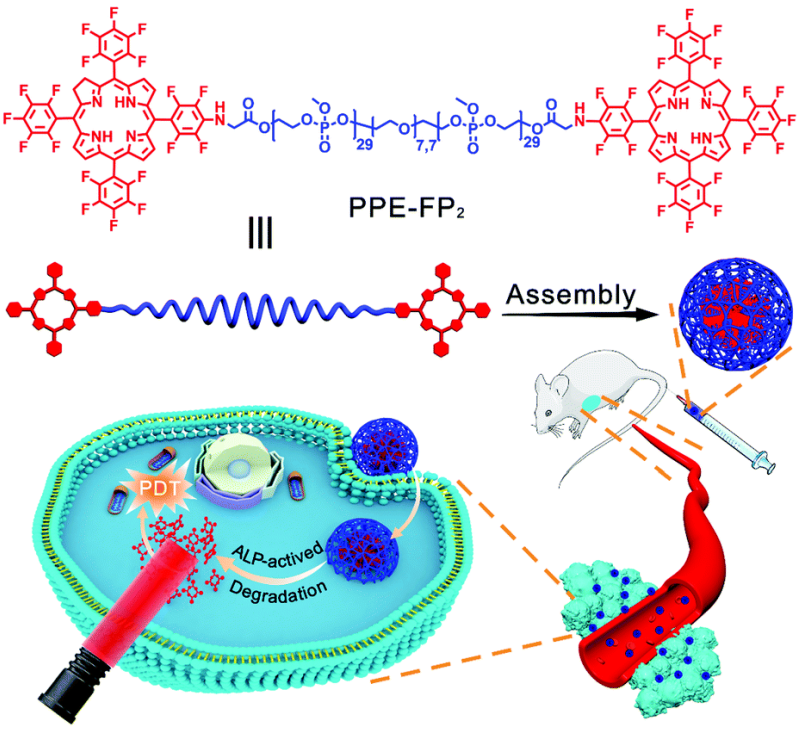


Figure 4: Schematic illustration of PPE-FP2 NPs and their biological action in photodynamic therapy in mice. Reproduced from: Hydrophilic polyphosphoester-conjugated fluorinated chlorin as an entirely biodegradable nano-photosensitizer for reliable and efficient photodynamic therapy, Z. Liu, M. Wu, Y. Xue, C. Chen, F.R. Wurm, M. Lan, W. Zhang, Chem. Commun. 56 (2020) 2415–2418. [103]*. Published by the Royal Society of Chemistry.*

Beyond these first results, other *in vivo* studies are still missing for main-chain water-soluble PPEs. The abundance of phosphorus-containing compounds in nature and the presence of enzymes for their digestion

make this class of polymers promising in terms of biodegradability, but the real behaviour needs to be tested, as in a living body unpredictable factors can influence the expected result. The alleged biodegradability is one of the key properties of PPEs because it could permit to overtake the problems linked to the non-biodegradability of PEG.

The good results in the biomedical field reported for PGA (another biodegradable candidate for the substitution of PEG) lead us to predict an increasing interest in the research on this sector. PPEs are expected to have comparable results to PGA in terms of biodegradability, with the further advantage to permit a fine-tuning of the properties, given by the proper choice of the substituent in the lateral chain or the chemistry around the central phosphorus, e.g. phosphonates vs. phosphates. Thus, we expect a rapid increase in the research interest on the *in vivo* biodegradability PPEs soon.

4.2 Biocompatibility

Cytocompatibility

Biocompatibility is defined by IUPAC as the ability of a material to be in contact with a biological system without producing an adverse effect [82]. It is a fundamental quality that needs to be assessed in any novel drug candidate, to avoid an undesired answer from the immune system of the patients. Even though the large presence of phosphate-containing compounds in the human body suggests the body acceptance of this kind of compounds, the variability in chemical structure and functionalisation brings the necessity of an evaluation case-by-case.

The determination of cytocompatibility (namely the capability to being non-toxic against cells) is a typical start to assess biocompatibility *in vitro,* and therefore it is one of the basic characterizations usually performed after the synthesis of novel polymers. For example, the polymer PEEP and the copolymer P(EEP-*co*-EMEP) have proven low cytotoxicity *in vitro* against HeLa cells up to a concentration of 600 µg/mL [43,67], while the polymers PMOEPA and PPEI, acid or sodium salt, resulted to be non-toxic up to a concentration of 1000 µg/mL [66]. Hyperbranched polyphosphates have been proven to be non-cytotoxic against COS-7 cells even at a concentration of 10 mg/mL [104], while polyphosphonates present a cytotoxic behaviour dependent on the length of the lateral substituent [63,64]. Besides, several examples of *in vitro* biocompatible PPEs-containing block copolymers are reported in the literature [46–48,88,89,105].

When PPE-based copolymers are used to form micelles or nanoparticles, another important parameter that influences the cytocompatibility is the surface charge. In particular, cationic charged nanoparticles are usually tolerated only in low concentrations (below 1-200 µg/mL), probably due to their interactions with negatively charged cell-membranes [79,91,106]. Leong and co-workers reported between 2001 and 2004

the synthesis and evaluation of a set of cationic PPEs used as gene carriers, all presenting lower cytotoxicity compared with other common polymers previously used as gene vector, e.g. PEI, or PLL [50,61,90,107]. All major results about the *in vitro* cytocompatibility of PPE-containing (co)polymers are collected in *Table 5*.

Table 5: Cytocompatibility of PPE-containing (co)polymers.

Polymer	M_n (kDa)	Cell lines	Incubation time (h)	Assay	Non-toxic conc. (mg/mL)[b]	Ref.
In-chain polyphosphonate	7.5, 25	RAW	48	ATP Cell Viability Assay	0.03[c]	[11]
PEEP	5	HeLa	48	Presto Blue fluorescence	0.6	[43]
PEEP-*b*-PDS-*b*-PEEP	9.8	L929, HeLa	48	MTT	200[d]	[46]
PAMAM-PBEP-PMP-FA	65	L929, HepG2	24	MTT	1[d]	[47]
Poly(BYP-*co*-EEP)	18	HeLa, HePG2, L929	48	MTT	0.2	[48]
PPE-EA	18	COS-7, HEK 293	24	MTT	0.1	[50]
PPE-MEA	13[a]	COS-7, HEK 293	24	MTT	0.1	[61]
PPE-HA	37[a]	COS-7, HEK 293	24	MTT	0.04[c]	[61]
PMeEP	5.6	HeLa	48	Presto Blue fluorescence	1	[63]
PEtEP	5.4	HeLa	24	CellTiter-Glo Luminescent Cell-viability	1	[64]
P^{i}PrEP	5.7	HeLa	24	CellTiter-Glo Luminescent Cell-viability	1	[64]
PBuEP	6.5	HeLa	24	CellTiter-Glo Luminescent Cell-viability	0.01	[64]
PMOEPA	from 3	HeLa,	24	CellTiter-Glo Luminescent	1	[66]

	to 9	RAW		Cell Viability		
PPEI acid or sodium salt	from 3 to 9	HeLa, RAW	24	CellTiter-Glo Luminescent Cell Viability	1	[66]
P(EEP-*co*-EMEP)	5.2, 5.4	HeLa	48	Presto Blue fluorescence	0.6	[67]
PEG-*b*-PEEP	28, 35	HEK 293	72	MTT	10	[89]
PEG-*b*-P(EEP-*co*-PEP)	35	HEK 293	72	MTT	10	[89]
PEEP-*b*-PLLA-*b*-PEEP	20	HEK 293	24	MTT	1	[88]
PPE3	6.3	COS-7, HEK 293	24	MTT	12.5	[90]
HPPE	4.2	COS-7	24	MTT	10	[104]
poly(CL-*co*-OPEA)	4.4	HepG2, HeLa	48	MTT	100	[105]
PCEP	4[a]	HeLa	24	WST-1 dye reduction	0.068[e]	[107]
HPHEEP-SP	9.2	HepG2, HUVEC	24	MTT	150	[108]

a: Weight average molecular weight, M_w; b: Maximum tested concentration at which the cell viability is 100% (within the experimental error); c: Maximum tested concentration at which the cell viability is more than 80% (within the experimental error); d: Maximum concentration of polymer tested at which the cell viability is more than 90% (within the experimental error); e: IC_{50}, namely conc. of 50% of cells death.

It is important to note that the results reported in Table 5 have been obtained on specific cell lines (in some cases cancer cell lines), therefore they are only a preliminary indication and can not substitute the more complete studies that must be performed with primary cells and additional *in vivo* studies. Moreover, given a real therapeutic application of the polymers, the toxicity of the degradation products needs to be carefully evaluated, because the biocompatibility of the polymer does not always imply the biocompatibility of its degradation products. For example, polylactide and poly(lactide-*co*-glycolide) have good biocompatibility, but their degradation process may lead to an inflammatory response, due to the decreasing of pH and the toxicity of the degradation products (lactic and glycolic acid) at high concentrations [109]. Moreover, some low molecular weight oligomers of ethylene glycol (in particular triethylene glycol and PEG with a molecular weight around 200 g/mol) are toxic at concentrations above

5mg/mL in *in vitro* experiments [110] and *in vivo* after the oral administration to rats [111,112] and monkeys [113].

To date, the cytocompatibility evaluation of PPEs degradation products is reported only in a few papers that show promising results. The degradation products of PEEP (mainly phosphates units with different substituents attached to the oxygens) have been evaluated not toxic to cells up to a concentration of 0.5 mg/mL in linear block copolymers PEG-*b*-PEEP [89], and of 10 mg/ mL in hyperbranched polyphosphates [104]. In addition, the Wooley group has reported that the degradation products obtained from the degradation of anionic, non-ionic, and zwitterionic PPE-based nanoparticles were non-toxic up to concentrations of 3 mg/mL, while for cationic nanoparticles the tolerated concentration was reduced to 0.6 mg/mL [91,92].

Overall, we have reported competitive results of PPEs respect to PEG in terms of cytocompatibility, considering that PEG with a molecular weight between 400 and 400 kDa is tolerate by HeLa cells at concentrations up to 10 mg/mL [110]. It is important to note that the concentrations, at which cytotoxicity for PPEs occurred, is well-above the concentrations required for the drug delivery [114]. In addition, the cytocompatibility of PPEs could further be varied by the substituent in the lateral chain, rendering PPEs promising PEG-alternatives. Additional analyses have been performed on real matrices: Wang and co-workers observed high blood compatibility of PEG-PEEP copolymers by observing the hemolysis of red blood cells. The polymers did not precipitate in blood plasma and no local inflammatory response in mouse muscles following intramuscular injections was detected [89]. The local tissue compatibility of poly(2-hydroxyethyl propylene phosphate) ("PPE3") was evaluated by Huang and co-workers [90] and compared to the well-known gene-carrier PEI. The two polymers were injected at different concentrations into the muscles of mice and subsequently biopsied after 3 and 7 days. The histologic images proved a lower level of necrosis for PPE3 compared to PEI, (*Figure 5*), suggesting the absence of an acute tissue response for PPE3.

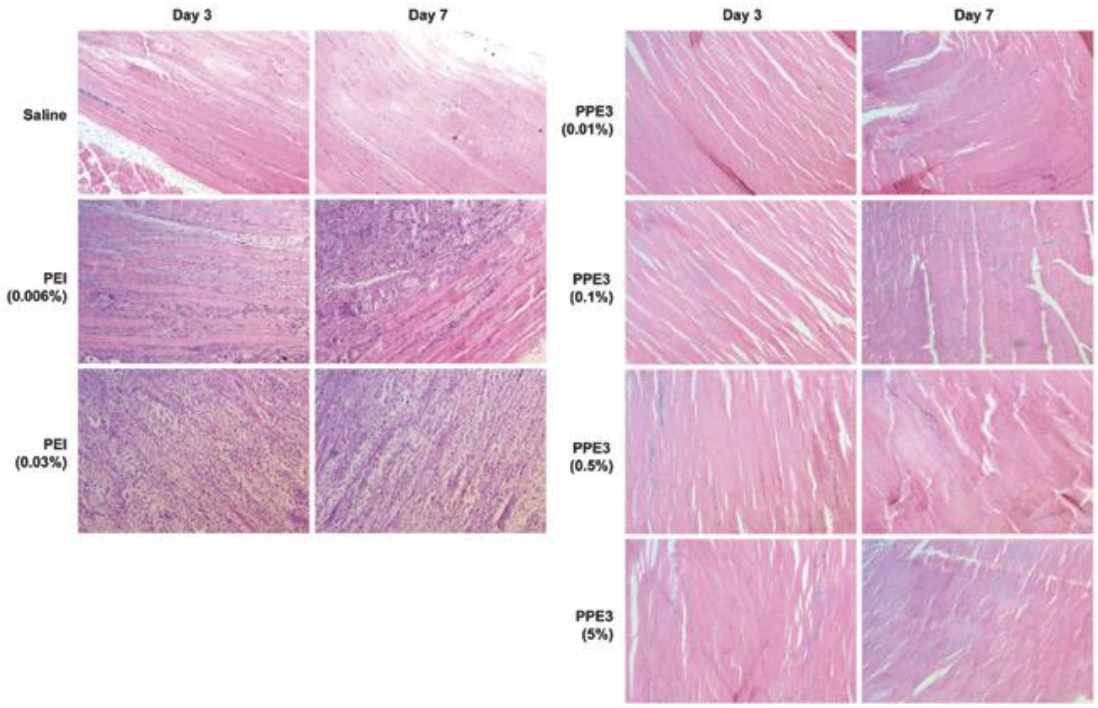


Figure 5: Histology images of mouse muscle samples injected with PEI and PPE3, harvested on days 3 and 7. Adapted with permission from: Water-soluble and non-ionic polyphosphoester: Synthesis, degradation, biocompatibility and enhancement of gene expression in mouse muscle, S.W. Huang, J. Wang, P.C. Zhang, H.Q. Mao, R.X. Zhuo, K.W. Leong, Biomacromolecules. 5 (2004) 306–311 [90]. *Copyright © 2004, American Chemical Society.*

As suggested by the international standard ISO published by the U.S. Food and Drug Administration Agency [115], the conditions necessary to define a material biocompatible and to exclude the occurring of an unacceptable adverse biological response, imply several tests, including a long- and short-term evaluation. The studies reported until now show promising features of PPEs, but the pathway to the real clinic use is still long. Similarly to the degradation behaviour, we expect soon a more systematic evaluation of PPEs biocompatibility (especially *in vivo).*

The "Stealth Effect" of PPEs

The use of PPEs in drug delivery requires their circulation in the bloodstream for a certain time to be recognized by immune cells or target cells. The polymer interactions with the plasma proteins are important to predict the eventual trigger of the immune system, the potential degradation by certain enzymes, and the pathway that leads to the cellular uptake by specific or unspecific recognition.

When a nanocarrier enters the bloodstream, it adsorbs proteins on its surfaces, leading to the formation of a protein shell (the so-called "protein corona") that alter the properties of the nanocarrier, such as size, charge, interactions with cells [116,117]. In many cases, the nanocarriers' chemical identity with properly installed targeting groups might be masked by the protein corona and the resulting "biological identity" behaves differently as intended. It has been reported by Dawson and co-workers that protein adsorption reduced the efficiency of specific cell targeting [118], therefore the possibility to control the protein corona to permit the use of targeted nanocarriers is one of the current challenges in drug delivery. Some proteins present in the corona could belong to the class of opsonins, namely antibodies, complement or circulating proteins that are responsible for the recognition of a foreign substance by the immune system and the subsequent clearance from the body. The evaluation of the protein corona composition is, therefore, a fundamental task to predict the biological fate of the nanocarrier.

PEG is currently used as a stealth coating for many drugs and nanocarriers as it decreases protein adsorption. However, certain protein types are "recruited" from the blood and still assembled on the nanocarriers' surface. This specific protein adsorption is believed to be responsible for the increased blood-half-life. This effect is generally called "stealth effect" and has been explained by several theories, linked to the polymer hydrophilicity, absence of charges, flexibility, and capacity of hydration (*Figure 6*) [119,120]. All these factors seem to influence the stealth behaviour of a polymer; they also allowed the design of various PEG-alternatives with additional features, such as degradability and chemical functionality. Recently, some concerns on the use of PEG after long-term treatments (e.g. the polymer accumulation and the development of anti-PEG antibodies and hypersensitivity reactions) have been reported, increasing the interest of research on novel polymers leading to a stealth effect [25,39]. Among the others, PPEs are a promising alternative. This section highlights their tuneable stealth properties and indicates several similarities, but also certain differences compared to PEG.

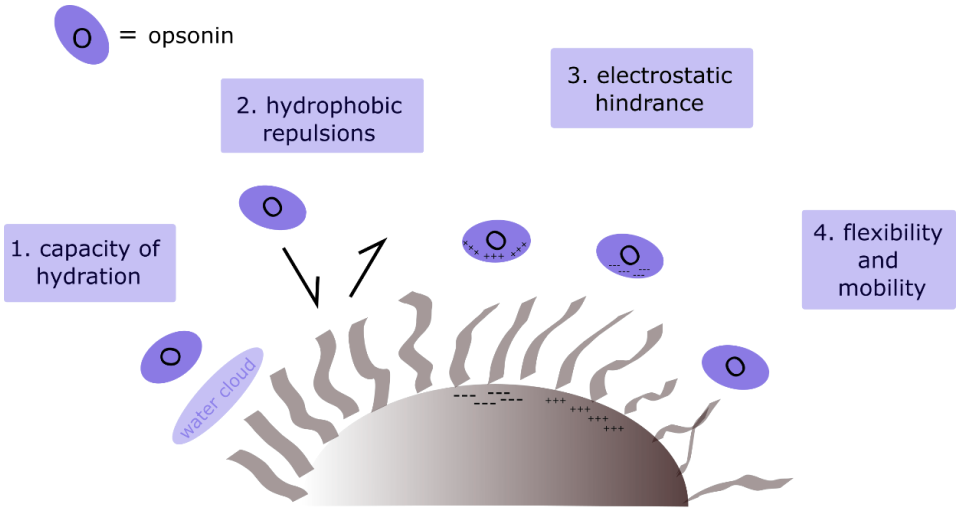


Figure 6: Schematic representation of the features that help to prevent the opsonization process as hypothesized for PEG.

The first studies about the protein adsorption on the surface of PPE-based nanoparticles had been reported in 2012 and 2013 [91,121]. Both the Wang and the Wooley groups reported that the protein adsorption was dependent on the surface charge, i.e. the zwitterionic NPs exhibited a very low protein adsorption, that increased going to neutral, then anionic and cationic NPs. Similar findings had been previously reported for other NPs, highlighting that the surface charge is a fundamental parameter in controlling the protein adsorption and the biological fate of the NPs [122–124].

The composition of the protein corona around polymer-coated NPs and the influence of the polymer structure were evaluated in detail in the following years: Schöttler et al. studied the protein adsorption and the cellular uptake of model-nanocarriers covalently modified with PEG or PEEP [125]. Both PEGylated and PPEylated nanocarriers exhibited low internalization into macrophages (cells with a key role in the clearance of foreign molecules from the bloodstream) when the nanocarriers were previously incubated with human blood plasma. In contrast, the same NPs exhibited high internalization in plasma-free conditions. This suggested that the stealth effect only occurred after selective recruitment of certain proteins from the blood, and this combination is responsible for the stealth effect against macrophages. The evaluation of the composition of the protein corona on both PEGylated and PPEylated nanocarriers highlighted the enrichment of clusterin (an apolipoprotein of 38kDa) while the non-modified samples exhibited a very different protein corona composition (*Figure 7*). Similar results were obtained by Müller et al., who evaluated the composition of the protein corona formed around NPs, however using a non-covalent coating of nanocarriers with PPE-surfactants [126].

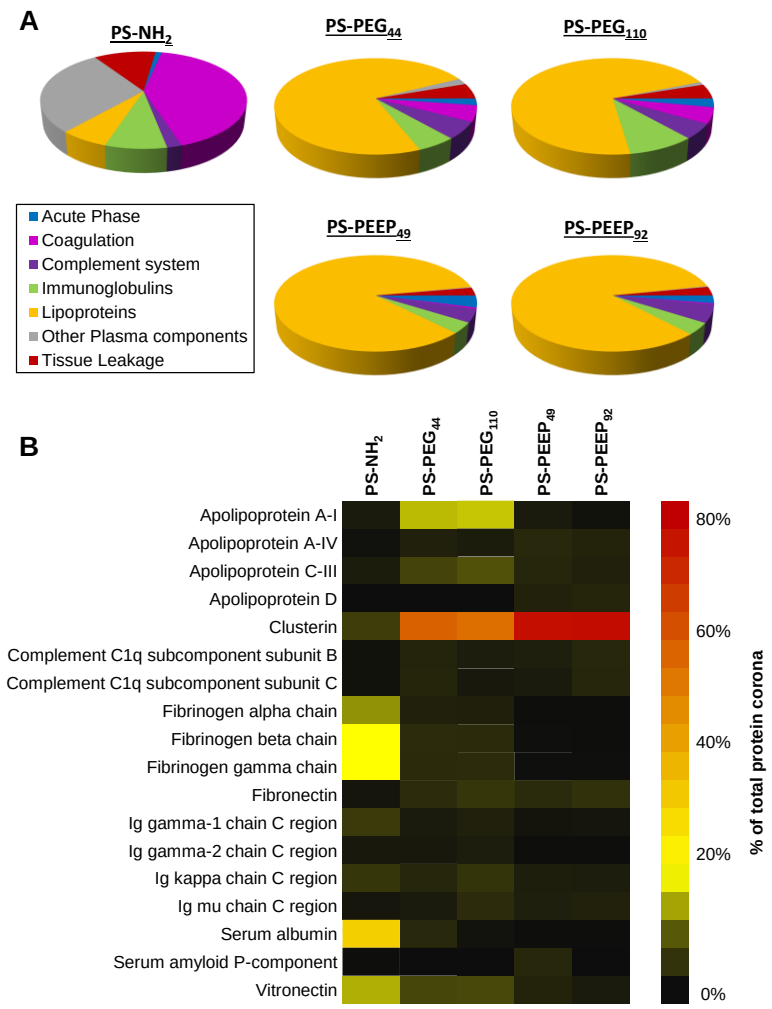
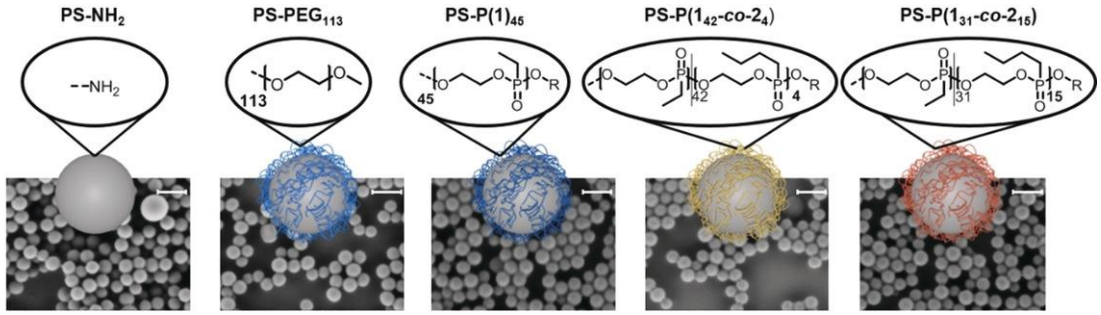


Figure 7: Proteomic analysis of protein corona on the surface of polystyrene nanocarriers naked (PS-NH_2), PEGylated (PS-PEG), PPEylated (PS-PEEP). Adapted from: Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers, S. Schöttler, G. Becker, S. Winzen, T. Steinbach, K. Mohr, K. Landfester, V. Mailänder, F.R. Wurm, Nat. Nanotechnol. 11 (2016) 372–377 [125]. *Copyright © 216, Springer Nature.*

In 2018, Simon et al. reported how the hydrophilicity of the polymer chain regulated the stealth properties of PPEs-coated nanocarriers [127]. They synthesized a set of PPEylated nanocarriers (analogue to the nanocarriers synthesized by Schöttler et al. [125]) using polyphosphonate-copolymers with a finely tuned hydrophilicity (*Figure 8*). A similar amount of "hard corona" proteins (the proteins more strongly adsorbed on the surface) was adsorbed on all the polymer-functionalised NPs, even though a significant difference in the protein pattern was detected by electrophoresis and proteomic mass spectrometry, depending on the polymer's hydrophilicity. The protein pattern changed systematically with increasing polymer-hydrophobicity in the way that the amount of clusterin decreased, while other proteins, such as albumin

increased and thus the cellular uptake (into macrophages) increased. Overall, the data confirmed the correlation (as already suggested by Schöttler et al. [125]) between the protein adsorption pattern and the polymer stealth properties, proposing, the possibility to control it with an accurate tuning of the polymer hydrophilicity. However, hydrophilicity is only one factor that might influence cellular uptake, other factors such as hydrogen bonding and charge must not be neglected.



modification	-	*m*-PEG$_{113}$	P(1)$_{45}$	P(1$_{42}$-co-2$_4$)	P(1$_{31}$-co-2$_{15}$)
chains / particle	-	5300	5500	6300	5400
D_h (H_2O) / nm	107 ± 9	124 ± 36	127 ± 28	105 ± 13	105 +61
D_h (NaCl 9 g L^{-1}) / nm	-	115 ± 27	111 ± 17	106 ± 24	155 ± 16
ζ-potential / mV	+47 ± 9	+ 7 ± 7	- 24 ± 5	- 16 ± 7	- 22 ± 4
ζ-potential in plasma / mV	-28 ± 9	-33 ± 7	-30 ± 19	-30 ± 5	-31 ± 7

Figure 8: Analytical data of polystyrene nanoparticles covalently linked with PEG, and polyphosphonates with a different degree of hydrophilicity. Scale bar: 200 nm. Adapted with permission from: Hydrophilicity Regulates the Stealth Properties of Polyphosphoester-Coated Nanocarriers, J. Simon, T. Wolf, K. Klein, K. Landfester, F.R. Wurm, V. Mailänder, Angew. Chemie - Int. Ed. 57 (2018) 5548–5553 [127]*. Copyright © 2018, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

One year later, Simon et al. were able to successfully combine the stealth effect with specific targeting to dendritic cells in the presence of blood plasma proteins [128]: they prepared PS and PMMA nanocarriers modified with PPEs carrying additional mannose target units. Thanks to their stealth properties, overall low adsorption of proteins was detected, and low internalization in monocytes was reported. However, a selective internalization by dendritic cells (that express receptor for mannose) was achieved (*Figure 9*), suggesting the combination of targeting and stealth properties as an useful strategy for the development of novel immunotherapies.

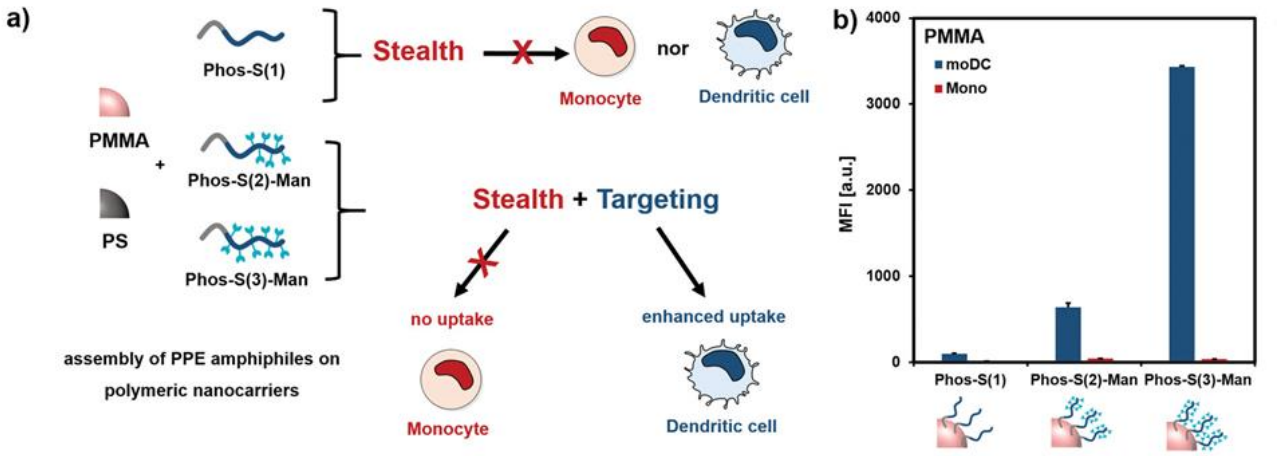


Figure 9: a) Schematic representation of PPE amphiphiles adsorbed on PS and PMMA NPs, that possess stealth and targeting properties; b) Cellular uptake toward dendritic cells (blue) or monocytes (red) quantified by flow cytometry after the exposure of human blood plasma to PMMA NPs for 2 hours. Values are expressed as mean ± SD from triplicates. Adapted from: Noncovalent Targeting of Nanocarriers to Immune Cells with Polyphosphoester-Based Surfactants in Human Blood Plasma, J. Simon, K.N. Bauer, J. Langhanki, T. Opatz, V. Mailänder, K. Landfester, F.R. Wurm, Adv. Sci. 6 (2019) [128]*. Copyright © 2019. The authors published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

Very recently, Bauer et al. published the synthesis of PS, PMMA, and HES nanocarriers functionalised by non-covalent adsorption of different non-ionic PPE-surfactants [129]. Three different polymers were used, composed by octadecanol as the hydrophobic tail and respectively poly(methyl ethylene phosphate), poly(methyl ethylene phosphonate) or in chain-poly(ethyl ethylene phosphonate) as hydrophilic parts, showing lower cytotoxicity than the common PEG-based surfactancts (e.g. Luthensol® AT 50) and a hydrolysis rate controlled by the chemical structure.

5. Biomedical applications

5.1 Protein-polymer conjugates

Protein-polymer conjugates are compounds with a covalent bond between one or more polymer chains and a protein. The first bioconjugations were reported in 1976 by Davis and Abuchowski, that published the covalent attachment of the polymer PEG to the proteins BSA and bovine liver catalase. The two conjugates showed a lower immunogenic response and a higher circulation time in animal models compared to native proteins [130,131].

The synthetic techniques used for the synthesis of protein-polymer conjugates have been improved through the years, and nowadays different approaches exist, widely discussed in other reviews [27,132–135]. The most common polymer used in bioconjugation is PEG. Today, there are 15 PEGylated proteins approved by U.S. Food and Drug Administration Agency and used for therapeutic purposes [26], while other proteins conjugated with PEG or other promising polymers are currently under investigation [40,41,136].

During the last five years, our group has reported the synthesis and characterization of different proteins conjugated with PPEs, namely the proteins BSA, uricase, and MPB conjugated with the polymer PMeEP [137,138]; BSA, bovine liver catalase, and myoglobin conjugated with the polymer PEEP [43,59] (*Figure 10*). All the conjugates were synthesized with a *grafting-to* method, through the non-site-specific reactions between the lysine groups available on the protein surface and a specific amount of polymer functionalised with a succinimidyl ester group. Before the reaction, the effective reactivity of the functionalised polymer was assessed evaluating that the rate of its hydrolysis and aminolysis reactions exhibit suitable values, comparable to those measured for other polymers typically used in bioconjugation reactions [43]. An important prerequisite for protein PPEylation is the stability of phosphoesters in presence of amines: in contrast to carboxylic acid esters, they do not undergo aminolysis quickly so that the polymer backbone stays intact [137].

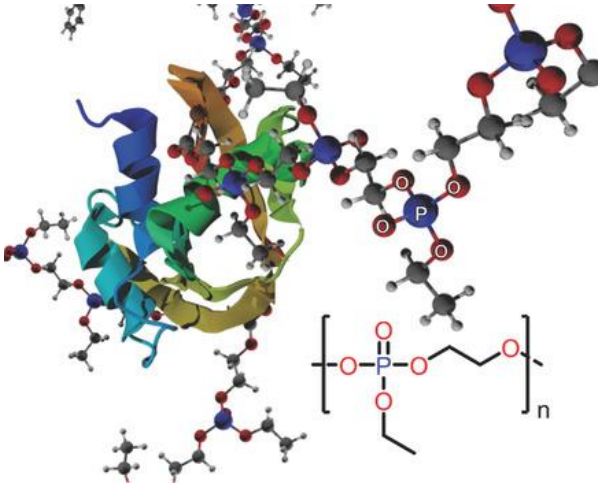


Fig.10: Graphical illustrations of the conjugate BSA-PEEP. Adapted with permission from: Reversible Bioconjugation: Biodegradable Poly(phosphate)-Protein Conjugates, T. Steinbach, G. Becker, A. Spiegel, T. Figueiredo, D. Russo, F.R. Wurm, Macromol. Biosci. (2017) [43]. Copyright © 2016. The authors published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. The polymer arrangement has illustrative purposes and it does not represent an actual configuration.

One of the first biochemical assays that need to be performed on a novel class of protein-polymer conjugates is the evaluation of the enzymatic activity. In fact, due to polymer conjugation, the enzymatic

activity in the conjugate is often altered: in most cases, the activity decreases due to partly denaturation and/or steric shielding of the active site by the attached polymer chains [139]. For example, the currently-in-use PEGylated interferon Pegasys® retained only 7% of the native antiviral activity of the protein [140]. Despite improved pharmacokinetics, high activity is beneficial, because it means a lower drug dosage for the patients and reduces costs. Compared to the conventional PEGylation, also the PPEylation resulted in decreased enzyme activities in a similar order of magnitude; the residual activities of the PPEylated conjugates are summarized in *Table 6*.

Table 6: Activity of PPEylated protein-polymer conjugates and their PEGylated analogues.

Conjugates	MW polymer (kDa)	Polymer chains attached	Activity %[a]	Ref.
Catalase-PEEP	3	1[b]	23	[43]
		2[b]	18	
Catalase-PEG	3	1[b]	22	
		2[b]	16	
My-PEEP	5	3[c]	86	[59]
		5[c]	79	
My PEG	5	3[c]	97	
		5 [c]	90	
Uricase-PMeEP	5	8[c]	53	[137]
Uricase-PEG			53	

a: Percentage of specific activity respect to pure proteins; b: Determined by experimental ratios; c: Calculated by GPC-UV/RI/MALLS.

Looking more closely at the data in *Table 6*, all conjugates show a reduction of the specific activity compared to the native protein. In particular, in the set of conjugates made with the protein My, a stronger decrease of the activity was observed, when the number of polymers or their degree of polymerization was increased, due to increased steric shielding of the active site or to the partial unfolding of the protein (caused probably by the formation of new interactions between the protein and the polymer chains). The influence of the bioconjugation procedure itself on the protein unfolding was excluded by an investigation made in our of our previous studies [59]. The remaining activities were comparable or higher than those found for similar conjugates made with different polymers.

Beyond the characterization and the assessment of the basic biochemical properties, the conjugates can be studied from a biophysical point of view, to obtain more information on their structure, stability, and

response to external stimuli. To date, few studies on the conjugates' biophysical properties have been reported, even though the rationalization of all these features could enhance the fundamental knowledge on the topic and orientate the design of future drugs. Our group evaluated the thermal stability of the set of My-PEEP conjugates and their PEGylated analogues by n-DSF, n-DSC, and UV-VIS spectroscopy [59]. They measured the onset and the melting temperature of the protein unfolding, revealing that all the values present a higher reduction of both the temperatures (with respect to the pure protein) when increasing the number of polymer chains attached to the protein and their degree of polymerization. Further analysis of the thermograms was not feasible due to precipitation of the conjugates after the thermal unfolding. On the contrary, a more detailed thermal analysis on the PEGylated analogues was conducted, thanks to the action of PEG around the protein, that inhibits its aggregation after the unfolding, enhancing the reversibility of the process [141].

Circular dichroism and fluorescence spectroscopy were used to assess eventual changes in the protein's secondary and tertiary structure given by the bioconjugation reaction. The analyses made on the conjugates BSA-PEEP proved that the protein retained its secondary structure after the bioconjugation process, while the tertiary structure seems to slightly depend on the grafting degree [43] (*Figure 11*). Additional studies made by SANS revealed more precisely the partial loss of the protein tertiary structure at high grafting density and gave more information on the conjugates 3D structures. The authors reported a change from ellipsoid to globular shape when the number of polymer chains tethered to the protein increased, with a polymer conformation that compactly coat the protein in case of a low grafting, and goes to a star-like conformation when increasing the number of polymer chains attached [142].

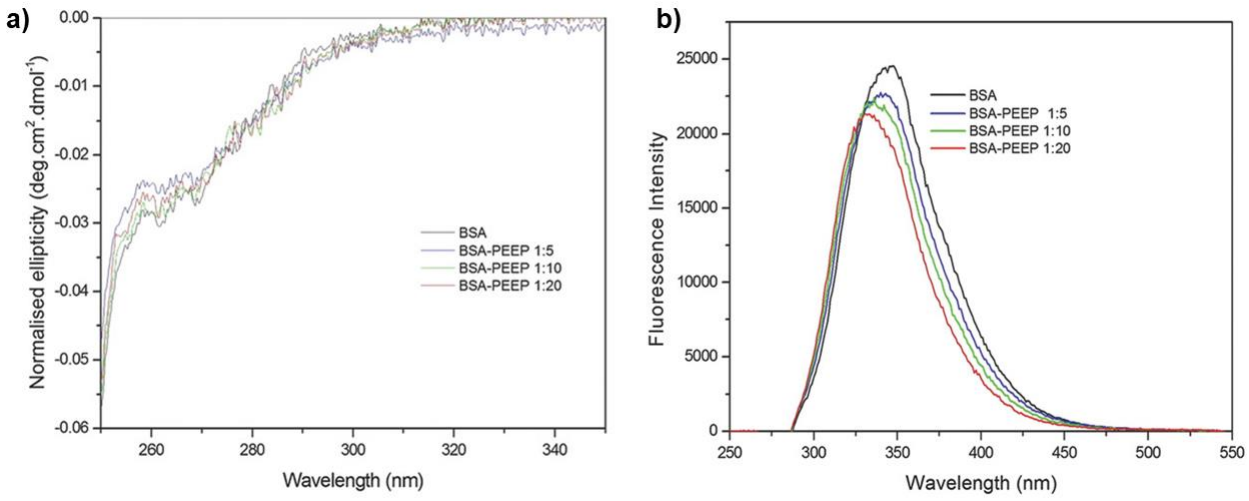


Figure 11: a): Far UV-CD spectra of native BSA and BSA-PEEP conjugates at room temperature, showing retention of the protein secondary structure after the bioconjugation process; b) Emission fluorescence spectra of native BSA and BSA-PEEP conjugates at room temperature, showing the dependence on the

protein tertiary structure from the polymer grafting. Adapted with permission from: Reversible Bioconjugation: Biodegradable Poly(phosphate)-Protein Conjugates, T. Steinbach, G. Becker, A. Spiegel, T. Figueiredo, D. Russo, F.R. Wurm, Macromol. Biosci. (2017) [43]. Copyright © 2016. The authors published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Russo et al. studied PPEylated conjugates using neutron scattering on the samples in the dry state and hydrated powders. They focused their attention on the dynamics of the samples in the nanosecond and picosecond timescale, using elastic, inelastic, and quasi-elastic neutron scattering on different PPEylated proteins (MBP-PMeEP [138], BSA-PEEP [143], and My-PEEP [144]). As dynamics is directly connected to the protein functionality, such fundamental analyses will allow tailoring the activity of future conjugates. The authors observed that the formation of interactions between the protein and the polymer enhanced the overall dynamics of the conjugates, which was larger than the sum of the two single contributes, i.e. the mobility of both the components was enhanced due to the presence of the other. In the BSA-PEEP conjugates, the polymer coating proved the same effect on protein dynamics than the hydration water and, also, it adsorbed the water molecules in hydrated powders, protecting the protein. The comparison between the three different studies revealed a non-trivial picture, in which the dynamics of the samples were influenced at the same time by several factors, e.g. the number of attached polymer chains, the size of the protein, the length of the polymer and its chemical structure.

5.2 PPEylated nanocarriers for drug delivery

The short lifetime and the low solubility of drugs in the bloodstream are two important challenges in drug delivery [69]. One of the successful strategies applied to overcome these problems is the encapsulation or the binding of hydrophobic drugs into nanocarriers, such as polymeric micelles. The polymeric micelles are usually made by amphiphilic block-copolymers, that can self-assemble in aqueous solutions, forming a structure with a hydrophobic core for the hydrophobic drugs and a hydrophilic shell that interacts with the environment [145]. PPEs are interesting candidates in drug delivery. Here we report a summary of the most important applications reported in the literature to date, highlighting the recent discoveries, intending to shed the light on future perspectives.

The choice of the substituent in the PPEs lateral chain permits a high control of the polymer hydrophilicity/hydrophobicity. In block copolymers, PPEs can thus act as either the hydrophobic part, e.g. with PEG as hydrophilic block [146,147], or the hydrophilic block, e.g. with PCL [44,72] or poly(lactide) [93,94] as the hydrophobic segments. Also, amphiphilic block copolymers, merely composed of PPEs with

different lateral substituents had been reported: the use of PPEs bearing reactive groups allows a further post-polymerization functionalization. Important contributions rely on thiol-ene or thiol-yne reactions or click chemistry (for example PBYP, or PAEP, cf. *Table 1*). The introduction of charged-groups [91,94,106], the conjugation with specific drugs, with other polymers or with dyes [74,76,148], was reported but also cross-linking after the formation of the micelles were studied [91,92].

Several papers studied the preparation of drug delivery nanocarriers utilizing PPEs relying on different chemistries in the literature. The drug, encapsulated or conjugated, could have an antitumoral (as in the case of PTX, DOX, or CPT [48,70,74,149]) or antimicrobial effect (silver [78,93,150–152]). Here, we describe some recent significant examples.

Chen et al. recently reported the double loading of silver cations and minocycline in PPE-based NPs for the antimicrobial treatment of *Pseudomonas Aeruginosa*, a bacterium detected in the lungs of around 50% of the patients with cystic fibrosis [78]. They use the copolymer PEBP-PBYP, functionalized with 3-mercaptopropanoic acid by a thiol−yne reaction, to form NPs in water, followed by cross-linking reactions. The sequential encapsulation of silver and minocycline provided a relatively high drug-loading (28% and 51%), significantly higher than the minocycline loading previously obtained with PEG-PLGA NPs [153]. Afterwards, the antimicrobial activity of the NPs was evaluated *in vitro*, proving that the combined administration of the two therapeutic agents reduced the dosage of each component needed to achieve the same antimicrobial effect, while the use of nanocarriers mitigated their side effects. TEM images of *Pseudomonas Aeruginosa* treated with silver acetate, minocycline, or both are reported in *Figure 11*.

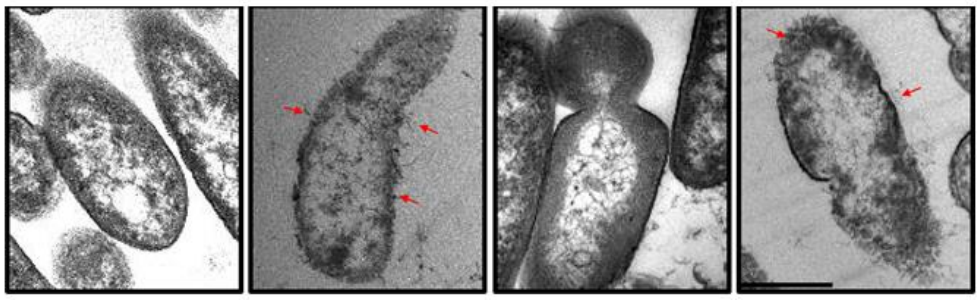


Figure 12: TEM images of Pseudomonas Aeruginosa treated with antimicrobial agents. 20k magnification, sale bar 0.5 µm. From left to right: no treatment; treatment with 4 µg/mL of silver acetate; treatment with 2 µg/mL of minocycline; treatment with 4 µg/mL of silver acetate + 2 µg/mL of minocycline. Adapted with permission from: Minocycline and Silver Dual-Loaded Polyphosphoester-Based Nanoparticles for Treatment of Resistant Pseudomonas aeruginosa, Q. Chen, K.N. Shah, F. Zhang, A.J. Salazar, P.N. Shah, R. Li, J.C. Sacchettini, K.L. Wooley, C.L. Cannon, Mol. Pharm. 16 (2019) 1606–1619 [78]. *Copyright © 2019, American Chemical Society.*

Wang et al. reported the first nanoparticles formed by the assembly of functional amphiphilic PPAs [154], physically loaded with the anticancer drug CPT. As the backbone of the polymer is acid-cleavable, the NPs degraded by decreasing pH value and released the cargo. The authors report an optimal drug loading of 10%, which was lower compared to the PEG-containing NPs previously proposed for CPT delivery (ca. 20%) [155], even though the high release efficiency compensated the lower loading, confirming the potential of PPEs as potential substitutes for PEG as drug carriers. One year later Dong et al. reported the synthesis of a novel pH/reduction dual-responsive polymeric prodrug, with simultaneous conjugation of the antitumoral drugs CPT and DOX [48]. A CPT derivative, with a disulfide bridge and a hydroxyl functionality (CPT-ss-OH), was used as initiator for the PPE-based co-polymer, while DOX was efficiently incorporated in the PPE lateral chain through a hydrazone bond (*Figure 12*). The copolymer self-assembled in water into spherical NPs with a diameter of ca. 90 nm. As the drugs CPT and DOX are linked to the NPs by either a disulfide or a hydrazone linkage, the release of the drugs inside of tumour cells was expected (the pH of the tumour cells is 6.5-7.2 instead of 7.4, and the glutathione concentration is 2-10 mM instead of 2-10 µM [156]). The drug release was studied in various buffers in the presence or absence of glutathione and the authors demonstrated that the drug was released in acidic or reductive conditions. In addition, the effective cellular uptake and intracellular drug release were monitored by real-time imaging of HeLa cells after different incubation times with the NPs. As shown in *Figure 12*, DOX fluorescence was observed in the cytoplasm of HeLa cells, and exhibited higher intensity when increasing the incubation time, indicating the successful internalization of the NPs and an efficient time-dependent drug release in the cells.

Other papers reported the preparation of responsive nanocarriers, e.g. by redox-labile disulfide linkages [46,71] or diselenide bonds [75] in the polymeric chain, or by attaching a drug via an acid-labile hydrazone bond [157]. Another current challenge in drug delivery is the targeted drug release. For PPE-based micelles, Zhang et al. used covalently attached transferrin to direct their action to brain cells [73]. Other papers reported the conjugation of folic acid, able to bind to certain tumour-associated antigens [95,158].

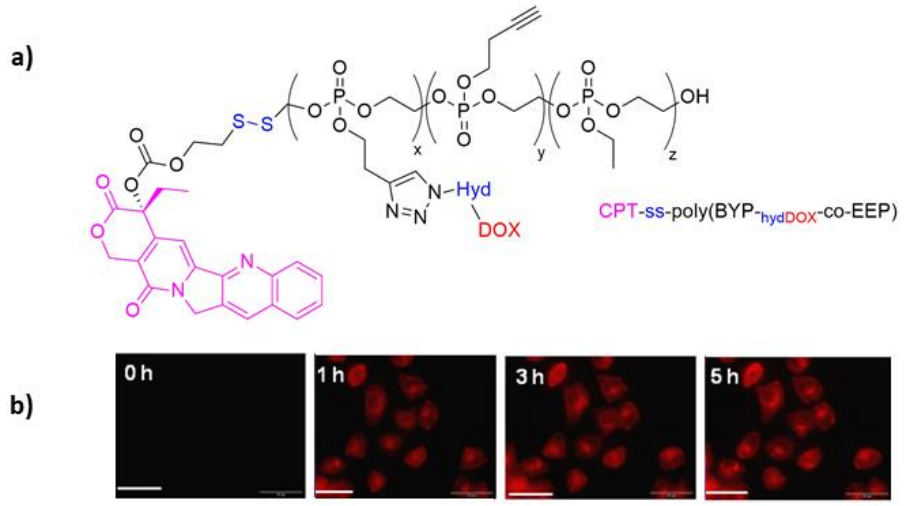


Figure 13: a) Chemical structure of pH/reduction dual-responsive polymeric prodrug synthesized by Dong et al. b) Fluorescence images of HeLa cells incubated with the prodrug, for different incubation times, stained by DOX The (scale bars: 50 µm). Adapted with permission from: Multifunctional Polymeric Prodrug with Simultaneous Conjugating Camptothecin and Doxorubicin for pH/Reduction Dual-Responsive Drug Delivery, S. Dong, Y. Sun, J. Liu, L. Li, J. He, M. Zhang, P. Ni, ACS Appl. Mater. Interfaces. 11 (2019) 8740–8748 [48]. *Copyright © 2019, American Chemical Society.*

Besides linear PPEs, hyperbranched architectures have shown promising results as carrier polymers. In 2012, Chen et al. proposed a hyperbranched polyphosphate, functionalised with a hydrophobic photochromic spiropyran dye that undergoes a reversible isomerization under UV irradiation, forming the hydrophilic merocyanine [108] (*Figure 1*3). The changes in the properties of the dye permitted the formation of UV-responsive micelles. Some years later, Yao et al. described the synthesis of amphiphilic hyperbranched polyphosphoesters with 6-carbons long alkyl chains and PEG chains linked by phosphate bonds as the branching points [97] (*Figure 13*). The polymers were assembled by a nanoprecipitation method, encapsulating the anticancer drugs DOX and the photothermal agent IR-780. The system exhibited good serum stability, preferential accumulation in tumour cells, and a relevant drug release, with effective tumour suppression in mice. In addition, they found that the drug release was accelerated by the presence of alkaline phosphatase, which is an interesting result because the enzyme is overexpressed in some tumour cells [159]. Last year another interesting paper was published by Zhang and co-workers, proposing the formation of supramolecular micelles starting from a multi-arm block copolymer, namely a poly(amido amine) core with arms formed by block copolymers PBEP-PMEP conjugated with the folic acid [47] (*Figure 13*). The branched polymeric structure was inspired to poly(amidoamine) dendrimers, that present higher stability in a fluid environment compared to micelles based on linear polymers. The presence in the polymeric structure of the other two PPE moieties permitted the formation of a cavity, allowing a high drug

loading and a controlled release. Moreover, the multi-arm block copolymer has biodegradable arms (formed by the PPE moieties) and it is easier to be synthesized if compared with highly symmetric dendrimers.

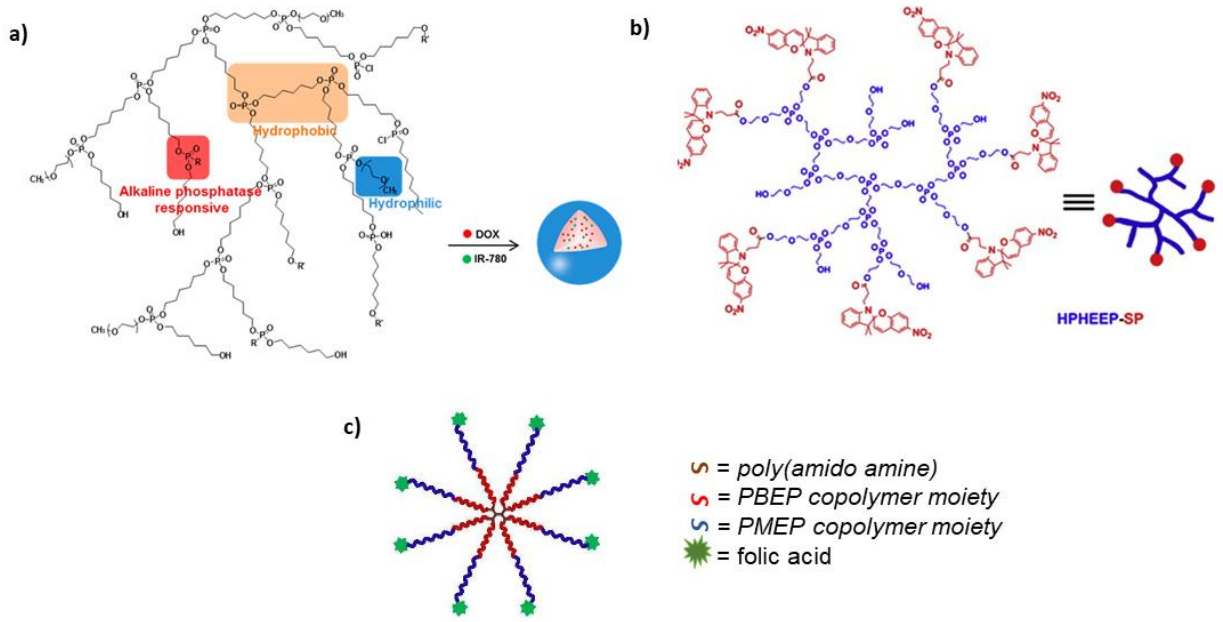


Figure 14: PPE-based branched copolymers used for biomedical applications. a) Hyperbranched phosphate functionalized with spiropyran molecules. Adapted from Reversibly light-responsive micelles constructed via a simple modification of hyperbranched polymers with chromophores, C.J. Chen, Q. Jin, G.Y. Liu, D.D. Li, J.L. Wang, J. Ji, Polymer (Guildf). 53 (2012), 3695–3703 [108]. Copyright © 2012, with permission from Elsevier; b) Amphiphilic hyperbranched PPEs with phosphate bond as the branching point. Adapted with permission from: Enzyme Degradable Hyperbranched Polyphosphoester Micellar Nanomedicines for NIR imaging-guided Chemo-Photothermal Therapy of Drug-Resistant Cancers, M. Yao, Y. Ma, H. Liu, M.I. Khan, S. Shen, S. Li, Y. Zhao, Y. Liu, G. Zhang, X. Li, F. Zhong, W. Jiang, Y. Wang Biomacromolecules. 19 (2018) 1130–1141 [97]. Copyright © 2018, American Chemical Society; c) Multi-arm block co-polymer, namely a poly(amido amine) core with arms formed by block copolymers conjugated with the folic acid. Adapted with permission from: Supramolecular micellar drug delivery system based on multi-arm block copolymer for highly effective encapsulation and sustained-release chemotherapy, L. Zhang, D. Shi, C. Shi, T. Kaneko, M. Chen, J. Mater. Chem. B. 7 (2019) 5677–5687 [47]. Copyright © 2019, with permission from Royal Society of Chemistry.

The use of PPEs in drug delivery vehicles opens many possibilities for the field. As shown by these selected examples, several strategies from other polymer classes were adopted and extended to PPEs due to the additional degradability or chemical functionality. We expect a further improvement that could bring some of the nanocarriers to be evaluated in clinical trials.

5.3 PPEylated nanocarriers for gene delivery

Gene therapy has been progressively developed in the last years [160]. The use of synthetic vectors is a promising strategy, even though the non-biocompatibility and non-biodegradability of some of them are problematic. PPEs have promising features to be used as effective biodegradable and polyfunctional gene delivery vectors and their applicability in this field was tested since the beginning of the current century.

Synthetic polymeric vectors are mostly polycations, able to interact with negatively charged nucleic acids, deliver and release them into the cytosol of the specific target cell. The first examples of PPE-based gene vectors were reported by the Leong group between 2001 and 2003 [50,61]. They synthesized a set of cationic poly(aminoalkyl propylene phosphate)s with different substituents, with a positively charged amino group in the lateral chain, and they tested their gene transfer efficiency by electrophoresis and by the evaluation of the luciferase expression *in vitro* and *in vivo* in mouse muscles. They found that the nanocarriers could encapsulate the plasmid DNA and released it inside of the specific target, with a transfection efficiency dependent on the degradation profile of the micelles, and therefore linked to the polymer stability. One year later, the same research group proposed a new PPE-based drug carrier with inverted polarity features, namely a polymer bearing a cationic group in the backbone and a lipophilic substituent in the lateral chain [107].

In 2010, Zhang and co-workers published the synthesis and evaluation of a diblock copolymer PEEP-*b*-PDMAEMA, proposed as a potential biodegradable substitute of the PEGylated analog for gene delivery [161]. Copolymers with different compositions were synthesized by a combination of ROP and ATRP, which were able to self-assemble in aqueous solutions with particle sizes and morphologies dependent on the pH of the medium. Further studies by electrophoresis demonstrated the effective capacity of the micelles to bind DNA. In particular, micelles composed by $PEEP_{32}$-*b*-$PDMAEMA_{67}$ with DNA added in an N/P ratio of 3 (ratio between the amino groups on the polymer and the phosphate groups on the DNA complex) were able to form spherical, discrete polyplexes with a mean diameter of 95 nm and a surface charge close to neutrality, able therefore to hide the negative charge of the nucleic acid, required to enhance the cellular uptake.

In the same period, the group of Wang studied triblock copolymers made by PEG, PCL, and PPEEA with different segment lengths for gene delivery (*Figure 15*) [45,162,163]. The effective formation of micelles by self-assembly in an aqueous environment was confirmed by DLS, TEM, zeta potential, and fluorescence measurements, showing a spherical morphology and diameters between 60 and 120 nm; zeta potential: 45-48 mV; CMC: 2.7 x 10^{-3} mg/mL. The micelles were loaded with siRNA without losing the uniformity, forming the so-called micelleplex, that proved high internalization of siRNA and its subsequent release in two

different cancer lines (HEK293 and BT474). Afterward, the simultaneous loading of the anticancer drug PTX and the genetic material siRNA, that can be delivered at the same time to the cancerous cells, was reported to have a synergic effect [163]. The multiple loading into a PPE-based gene nanocarrier was reported also some years later by Elzeny and co-workers [76].

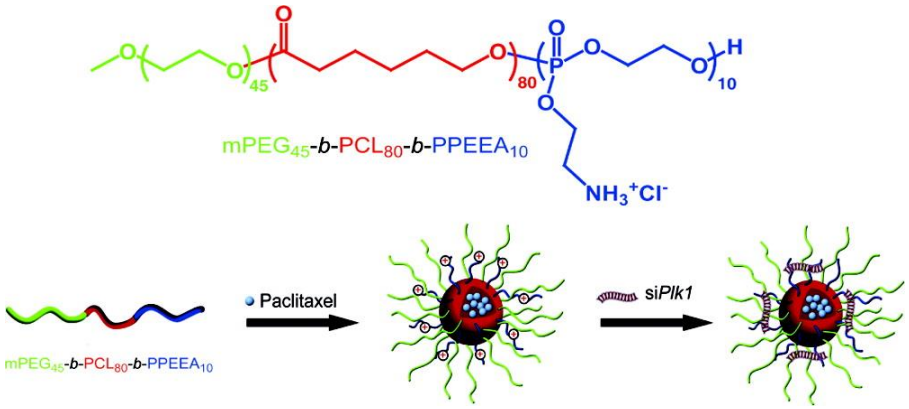


Figure 15: Chemical structure of the tri-block co-polymer mPEG-PCL-PPEEA, representative illustration of micelleplex formation, and the subsequent loading of paclitaxel and siRNA. Adapted with permission from: Simultaneous delivery of siRNA and paclitaxel via a "two-in-one" micelleplex promotes synergistic tumor suppression, T.M. Sun, J.Z. Du, Y.D. Yao, C.Q. Mao, S. Dou, S.Y. Huang, P.Z. Zhang, K.W. Leong, E.W. Song, J. Wang, ACS Nano. 5 (2011) 1483–1494 [163]*. Copyright © 2011, American Chemical Society.*

In the following years, more complex structures have been exploited. In particular, we cite the tumor acidity-responsive PEGylated polymers (synthesized with a click reaction between the PEG-PAEP di-block copolymer and cysteamine) [164], and the recent and innovative fully degradable phosphonium-functionalized amphiphilic di-block copolymer [79], both self-assembling into well-defined micellar systems for efficient siRNA intracellular delivery.

The development of gene delivery systems is a young topic, therefore the research is still ongoing and full of possibilities to be explored. The good results obtained with PPE-based nanocarriers suggest a growth of their applicability in the field, expected in the next years.

5.4 PPE-based hydrogels

Main-chain PPEs are promising for tissue engineering, in particular for bone regeneration. The Leong group reported already in 2006 the coupling of acrylated PEG with the biodegradable PPE-HA (a polyphosphoester previously used as a gene carrier, *see Section 5.2*), with subsequent photocrosslinking to obtain hydrogel

scaffolds [165]. The hydrogels exhibited good cytocompatibility against several cell lines. A lower swelling behavior, lower degradation rate but a higher mechanical strength was determined for hydrogels with increasing the content of acrylate groups and the cross-linking ratio. After this initial report, several PPEs were used to prepare hydrogels in combination with other natural scaffolds (e.g. catechols [166] and hyaluronic acid [167]) or with other synthetic polymers (e.g. PEG [168,169]). The versatile structure of PPEs played a key role in the design of the hydrogels because the proper choice of the lateral substituent permitted the introduction of the desired hydrophilicity or cross-linking group, e.g. by using click reactions (*see Section 3*). For example, Wang et al. published the synthesis of an innovative hydrogel with high adhesive properties, resulting from interactions between complementary nucleobases in the gel structure [169] (*Figure 16*).

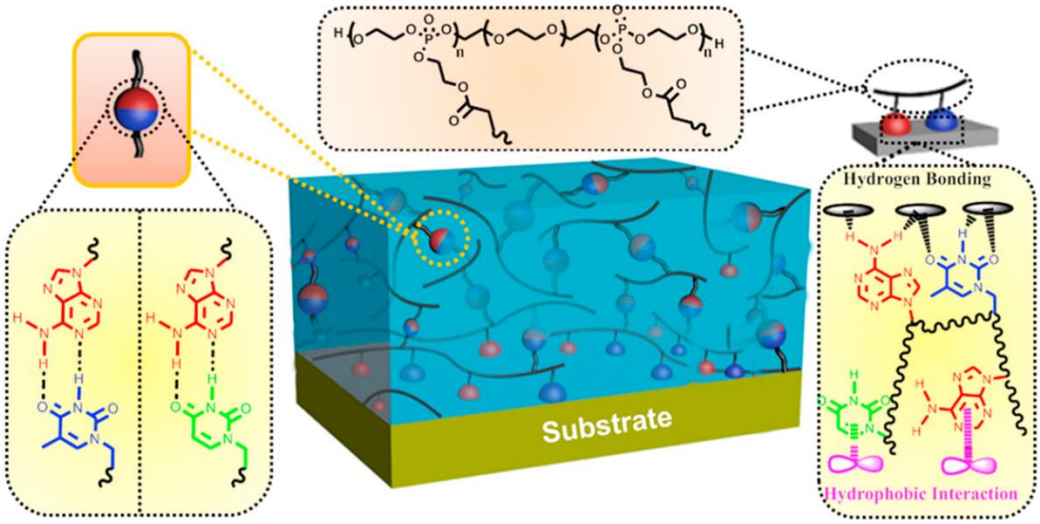
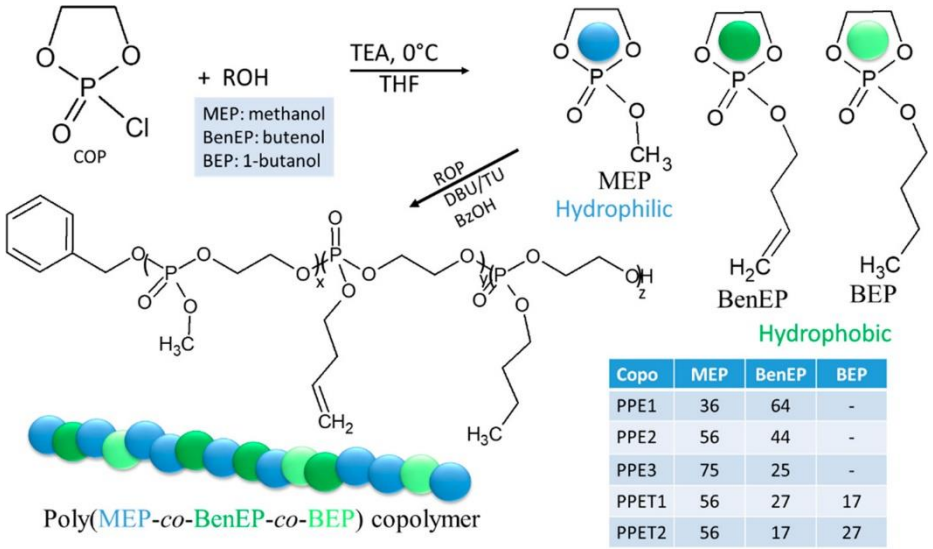


Figure 16: Schematic illustration of the hydrogel network proposed by Wang et al., with a focus on the interactions between the nucleobases. Adapted with permission from: DNA-Inspired Adhesive Hydrogels Based on the Biodegradable Polyphosphoesters Tackified by a Nucleobase, W. Wang, S. Liu, B. Chen, X. Yan, S. Li, X. Ma, X. Yu, Biomacromolecules 20 (2019), 3672–3683 [169]. *Copyright © 2019, American Chemical Society.*

Other PPE hydrogels enabled controlled biodegradation of the scaffold: for example, Liu et al. reported the formation of PEG hydrogels with phosphoesters as cross-linking points, with tunable degradation rates in water by varying the molecular weight of the PEG chain [170]. Padmavathy et al. recently published the synthesis of a novel “smart hybrid nanogel” composed of copper oxide and PPEs, in which the action of the enzymes phosphatase or phospholipase could trigger the cleavage of the PPE segments, resulting in the controlled release of Cu ions [171].

Tee et al. recently prepared PPE-based hydrogels by UV-crosslinking of methacrylated PMEP and proved the low cell adhesion to the hydrogels and their hydrolytic degradation at neutral pH [172]. In 2020, Jerome and co-workers reported the synthesis of a set of polyphosphate copolymers bearing different pendent groups (methyl, butyl, or 1-butynyl), which were crosslinked into hydrogels through UV irradiation [173] (*Figure 17*). They observed a dependence of the mechanical properties, the swelling behavior, and the degradation rates from the microstructure of the polymer and the degree of cross-linking. The possibility to tune the macroscopic properties of the hydrogels by varying the ratio of the comonomers, together with the material compatibility, makes their approach a promising scaffold for tissue engineering. With similar anti-fouling properties as PEG but additional adjustable biodegradability, we expect an extended use of PPE-based hydrogels in biomedical research. Besides, as most PPEs release phosphate, they might be especially interesting for application in bone or cartilage.



Copo	MEP	BenEP	BEP
PPE1	36	64	-
PPE2	56	44	-
PPE3	75	25	-
PPET1	56	27	17
PPET2	56	17	27

Figure 17: PPE hydrogels: Composition and synthesis of polyphosphate copolymers, which can be cross-linked by UV irradiation. Adapted with permission from: Design of Degradable Polyphosphoester Networks with Tailor-Made Stiffness and Hydrophilicity as Scaffolds for Tissue Engineering, R. Riva, U. Shah, J. Thomassin, Z. Yilmaz, A. Lecat, A. Colige, C. Jerome, Biomacromolecules 21 (2020) 349–355 [173]. *Copyright © 2020, American Chemical Society.*

6. Summary and Outlook

Main-chain water-soluble PPEs have been proposed as a competitive alternative to PEG in various biomedical applications. We have reviewed and summarized important advantages peculiar of PPEs with potential for the biomedical field. After a summary of the synthesis of water-soluble PPEs, we performed a critical evaluation of the state of art regarding their biocompatibility and -degradability, two major issues required for the applications in medicine. We have chosen representative examples from recent literature,

which highlight the potential of PPEs (mainly as a substitute for the non-biodegradable PEG) in protein-polymer conjugates, hydrogels, and drug or gene delivery.

We found an increasing interest in PPEs for biomedical applications with a rising number of publications, after the basic synthetic procedures had been established, such as expanded monomer scope, metal-free synthesis, cyto- and blood compatibility, stealth effect, etc.). Several candidates of the broad spectrum of PPEs have been identified as for clinical trials that will probably be reported soon. To date, several PPEs exhibit promising results based on *in vitro* studies, while only a few *in vivo* studies have been reported, which will probably be explored in the next future.

The critical evaluation of the developments of water-soluble PPEs allowed us to show up certain areas of their potential for the biomedical field; however also shed light on some critical issues, such as limited *in vivo* data to date. The future of main-chain water-soluble PPEs, e.g. as biodegradable, versatile substitutes for PEG shedding need interdisciplinary research between chemists and clinicians to initiate clinical trials.

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Bibliography

[1] M.M. Cox, D.L. Nelson, Lehninger Principles of Biochemistry, 4th ed., New York, 2005.

[2] W.W. Metcalf, W.A. van der Donk, Biosynthesis of Phosphonic and Phosphinic Acid Natural Products, Annu. Rev. Biochem. 78 (2009) 65–94. doi:10.1146/annurev.biochem.78.091707.100215.

[3] V.W. Buls, W. Creek, R.C. Morris, Polyesters of phosphoryl-substitute alcohols and polybasic phosphorus acids, 1953. doi:10.1145/178951.178972.

[4] P. Strasser, I. Teasdale, Main-Chain Phosphorus-Containing Polymers for Therapeutic Applications, Molecules. 25 (2020) 1716–1763. doi:10.3390/molecules25071716.

[5] M.M. Velencoso, A. Battig, J.C. Markwart, B. Schartel, F.R. Wurm, Molecular Firefighting—How Modern Phosphorus Chemistry Can Help Solve the Challenge of Flame Retardancy, Angew. Chemie - Int. Ed. 57 (2018) 10450–10467. doi:10.1002/anie.201711735.

[6] P.L. Turecek, M.J. Bossard, F. Schoetens, I.A. Ivens, PEGylation of Biopharmaceuticals: A Review of

Chemistry and Nonclinical Safety Information of Approved Drugs, J. Pharm. Sci. 105 (2016) 460–475. doi:10.1016/j.xphs.2015.11.015.

[7] J. Herzberger, K. Niederer, H. Pohlit, J. Seiwert, M. Worm, F.R. Wurm, H. Frey, Polymerization of ethylene oxide, propylene oxide, and other alkylene oxides: Synthesis, novel polymer architectures, and bioconjugation, Chem. Rev. 116 (2016) 2170–2243. doi:10.1021/acs.chemrev.5b00441.

[8] R. Klein, F.R. Wurm, Aliphatic polyethers: Classical polymers for the 21st century, Macromol. Rapid Commun. 36 (2015) 1147–1165. doi:10.1002/marc.201500013.

[9] H. Wang, L. Su, R. Li, S. Zhang, J. Fan, F. Zhang, T.P. Nguyen, K.L. Wooley, Polyphosphoramidates that undergo acid-Triggered backbone degradation, ACS Macro Lett. 6 (2017) 219–223. doi:10.1021/acsmacrolett.6b00966.

[10] M. Steinmann, M. Wagner, F.R. Wurm, Poly(phosphorodiamidate)s by Olefin Metathesis Polymerization with Precise Degradation, Chem. A Eur. J. 22 (2016) 17329–17338. doi:10.1002/chem.201603990.

[11] K.N. Bauer, L. Liu, D. Andrienko, M. Wagner, E. K. Macdonald, M. P. Shaver, F. R. Wurm, Polymerizing Phostones: A Fast Way to In-Chain Poly(phosphonate)s with Adjustable Hydrophilicity, Macromolecules. 51 (2018) 1272–1279. doi:10.1021/acs.macromol.7b02473.

[12] G. M. Salamończyk, M. Kuźnikowski, A. Skowrońska, A divergent synthesis of thiophosphate-based dendrimers, Tetrahedron Lett. 41 (2000) 1643–1645. doi:10.1016/S0040-4039(00)00005-8.

[13] S. Penczek, J. Libiszowski, Polymerization of 2-methoxy-2-oxo-1,3,2-dioxaphospholane, Die Makromol. Chemie. 189 (1988) 1765–1785. doi:10.1002/macp.1988.021890803.

[14] J. Libiszowski, K. Kaluzynski, S. Penczek, Polymerization of Cyclic Esters of Phosphoric Acid - 6. Poly(Alkyl Ethylene Phosphates). Polymerization of 2-Alkoxy-2-Oxo-1,3,2-Dioxaphospholans and Structure of Polymers., J Polym Sci Polym Chem Ed. 16 (1978) 1275–1283. doi:10.1002/pol.1978.170160610.

[15] S. Penczek, Polymerization of cyclic esters of phosphoric acid, in: Polym. Heterocycles (Ring Opening), 1977: pp. 363–371.

[16] I. Teasdale, Stimuli-Responsive Phosphorus-Based Polymers, Eur. J. Inorg. Chem. (2019) 1445–1456. doi:10.1002/ejic.201801077.

[17] K.N. Bauer, H.T. Tee, M.M. Velencoso, F.R. Wurm, Main-chain poly(phosphoester)s: History, syntheses, degradation, bio-and flame-retardant applications, Prog. Polym. Sci. 73 (2017) 61–122. doi:10.1016/j.progpolymsci.2017.05.004.

[18] J. Baran, S. Penczek, Hydrolysis of Polyesters of Phosphoric Acid. 1. Kinetics and the pH Profile, Macromolecules. 28 (1995) 5167–5176. doi:10.1021/ma00119a002.

[19] S. Maiti, S. Banerjee, S.K. Palit, Phosphorus-containing polymers, Prog. Polym. Sci. 18 (1993) 227–261.

[20] Z. Zhao, J. Wang, H.Q. Mao, K.W. Leong, Polyphosphoesters in drug and gene delivery, Adv. Drug Deliv. Rev. 55 (2003) 483–499. doi:10.1016/S0169-409X(03)00040-1.

[21] S. Monge, B. Canniccioni, A. Graillot, J.J. Robin, Phosphorus-containing polymers: A great opportunity for the biomedical field, Biomacromolecules. 12 (2011) 1973–1982. doi:10.1021/bm2004803.

[22] T. Steinbach, F.R. Wurm, Poly(phosphoester)s: A New Platform for Degradable Polymers, Angew. Chemie - Int. Ed. 54 (2015) 6098–6108. doi:10.1002/anie.201500147.

[23] Z.E. Yilmaz, C. Jérôme, Polyphosphoesters: New Trends in Synthesis and Drug Delivery Applications, Macromol. Biosci. 16 (2016) 1745–1761. doi:10.1002/mabi.201600269.

[24] J.H. Milton, Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications, 1st ed., Springer US, 1992.

[25] K. Knop, R. Hoogenboom, D. Fischer, U.S. Schubert, Poly(ethylene glycol) in drug delivery: Pros and cons as well as potential alternatives, Angew. Chemie - Int. Ed. 49 (2010) 6288–6308. doi:10.1002/anie.200902672.

[26] I. Ekladious, Y.L. Colson, M.W. Grinstaff, Polymer–drug conjugate therapeutics: advances, insights and prospects, Nat. Rev. Drug Discov. 18 (2019) 273–294. doi:10.1038/s41573-018-0005-0.

[27] L.H. Belén, C. De Oliveira Rangel-Yagui, J.F. Beltrán Lissabet, B. Effer, M. Lee-Estevez, A. Pessoa, R.L. Castillo, J.G. Farías, From synthesis to characterization of site-selective pegylated proteins, Front. Pharmacol. 10 (2019) 1–16. doi:10.3389/fphar.2019.01450.

[28] T. Ishida, M. Ichihara, X.Y. Wang, K. Yamamoto, J. Kimura, E. Majima, H. Kiwada, Injection of PEGylated liposomes in rats elicits PEG-specific IgM, which is responsible for rapid elimination of a second dose of PEGylated liposomes, J. Control. Release. 112 (2006) 15–25. doi:10.1016/j.jconrel.2006.01.005.

[29] S. Shah, T. Prematta, N.F. Adkinson, F.T. Ishmael, Hypersensitivity to polyethylene glycols, J. Clin. Pharmacol. 53 (2013) 352–355. doi:10.1177/0091270012447122.

[30] T. Ishida, H. Kiwada, Anti－polyethyleneglycol Antibody Response to PEGylated Substances, Biol.

Pharm. Bull. 36(6) (2013) 889–891. doi:10.1248/bpb.b13-00107.

[31] S.M. Poppenborg, J. Wittmann, W. Walther, G. Brandenburg, R. Krähmer, J. Baumgart, F. Leenders, Impact of anti-PEG IgM antibodies on the pharmacokinetics of pegylated asparaginase preparations in mice, Eur. J. Pharm. Sci. 91 (2016) 122–130. doi:10.1016/j.ejps.2016.06.007.

[32] K. Shiraishi, K. Kawano, Y. Maitani, T. Aoshi, K.J. Ishii, Y. Sanada, S. Mochizuki, K. Sakurai, M. Yokoyama, Exploring the relationship between anti-PEG IgM behaviors and PEGylated nanoparticles and its significance for accelerated blood clearance, J. Control. Release. 234 (2016) 59–67. doi:10.1016/j.jconrel.2016.05.010.

[33] L. Hong, Z. Wang, X. Wei, J. Shi, C. Li, Antibodies against polyethylene glycol in human blood: A literature review, J. Pharmacol. Toxicol. Methods. 102 (2020) 106678. doi:10.1016/j.vascn.2020.106678.

[34] I. Hamad, A.C. Hunter, J. Szebeni, S.M. Moghimi, Poly(ethylene glycol)s generate complement activation products in human serum through increased alternative pathway turnover and a MASP-2-dependent process, Mol. Immunol. 46 (2008) 225–232. doi:10.1016/j.molimm.2008.08.276.

[35] T. Mackul'ak, J. Prousek, L. Švorc, J. Ryba, J. Škubák, M. Drtil, Treatment of industrial wastewater with high content of polyethylene glycols by Fenton-like reaction system (Fe0/H2O2/H2SO4), Desalin. Water Treat. 51 (2013) 4489–4496. doi:10.1080/19443994.2013.769704.

[36] T.X. Viegas, M.D. Bentley, J.M. Harris, Z. Fang, K. Yoon, B. Dizman, R. Weimer, A. Mero, G. Pasut, F.M. Veronese, Polyoxazoline: Chemistry, properties, and applications in drug delivery, Bioconjug. Chem. 22 (2011) 976–986. doi:10.1021/bc200049d.

[37] A. Richard, A. Margaritis, Poly(glutamic acid) for biomedical applications, Crit. Rev. Biotechnol. 21 (2001) 219–232. doi:10.1080/07388550108984171.

[38] R. Duncan, M.J. Vicent, Do HPMA copolymer conjugates have a future as clinically useful nanomedicines? A critical overview of current status and future opportunities, Adv. Drug Deliv. Rev. 62 (2010) 272–282. doi:10.1016/j.addr.2009.12.005.

[39] T.T.H. Thi, E.H. Pilkington, D.H. Nguyen, J.S. Lee, K.D. Park, N.P. Truong, The importance of Poly(ethylene glycol) alternatives for overcoming PEG immunogenicity in drug delivery and bioconjugation, Polymers (Basel). 12 (2020) 298. doi:10.3390/polym12020298.

[40] Y. Qi, A. Chilkoti, Protein-polymer conjugation-moving beyond PEGylation, Curr. Opin. Chem. Biol. 28 (2015) 181–193. doi:10.1016/j.cbpa.2015.08.009.

[41] E.M. Pelegri-O'day, E.W. Lin, H.D. Maynard, Therapeutic protein-polymer conjugates: Advancing

beyond pegylation, J. Am. Chem. Soc. 136 (2014) 14323–14332. doi:10.1021/ja504390x.

[42] G. Becker, F.R. Wurm, Functional biodegradable polymers: Via ring-opening polymerization of monomers without protective groups, Chem. Soc. Rev. 47 (2018) 7739–7782. doi:10.1039/c8cs00531a.

[43] T. Steinbach, G. Becker, A. Spiegel, T. Figueiredo, D. Russo, F.R. Wurm, Reversible Bioconjugation: Biodegradable Poly(phosphate)-Protein Conjugates, Macromol. Biosci. 17 (2017) 1600377. doi:10.1002/mabi.201600377.

[44] Y.C. Wang, X.Q. Liu, T.M. Sun, M.H. Xiong, J. Wang, Functionalized micelles from block copolymer of polyphosphoester and poly(e{open}-caprolactone) for receptor-mediated drug delivery, J. Control. Release. 128 (2008) 32–40. doi:10.1016/j.jconrel.2008.01.021.

[45] Y.C. Wang, L.Y. Tang, T.M. Sun, C.H. Li, M.H. Xiong, J. Wang, Self-assembled micelles of biodegradable triblock copolymers based on poly(ethyl ethylene phosphate) and poly(ε-caprolactone) as drug carriers, Biomacromolecules. 9 (2008) 388–395. doi:10.1021/bm700732g.

[46] P. Ju, J. Hu, F. Li, Y. Cao, L. Li, D. Shi, Y. Hao, M. Zhang, J. He, P. Ni, A biodegradable polyphosphoester-functionalized poly(disulfide) nanocarrier for reduction-triggered intracellular drug delivery, J. Mater. Chem. B. 6 (2018) 7263–7273. doi:10.1039/c8tb01566j.

[47] L. Zhang, D. Shi, C. Shi, T. Kaneko, M. Chen, Supramolecular micellar drug delivery system based on multi-arm block copolymer for highly effective encapsulation and sustained-release chemotherapy, J. Mater. Chem. B. 7 (2019) 5677–5687. doi:10.1039/c9tb01221d.

[48] S. Dong, Y. Sun, J. Liu, L. Li, J. He, M. Zhang, P. Ni, Multifunctional Polymeric Prodrug with Simultaneous Conjugating Camptothecin and Doxorubicin for pH/Reduction Dual-Responsive Drug Delivery, ACS Appl. Mater. Interfaces. 11 (2019) 8740–8748. doi:10.1021/acsami.8b16363.

[49] Y. Sun, X. Du, J. He, J. Hu, M. Zhang, P. Ni, Dual-Responsive Core-Crosslinked Polyphosphoester-Based Nanoparticles for pH/Redox-Triggered Anticancer Drug Delivery, J. Mater. Chem. B. 5 (2017) 3771–3782. doi:10.1039/C7TB00440K.

[50] J. Wang, H.Q. Mao, K.W. Leong, A novel biodegradable gene carrier based on polyphosphoester [19], J. Am. Chem. Soc. 123 (2001) 9480–9481. doi:10.1021/ja016062m.

[51] Y. Iwasaki, E. Yamaguchi, Synthesis of well-defined thermoresponsive polyphosphoester macroinitiators using organocatalysts, 2010. doi:10.1021/ma100242s.

[52] B. Grignard, L. Koole, C. Je, Metal-Free Strategies for the Synthesis of Functional and Well-Defined Polyphosphoesters, Macromolecules. 45 (2012) 4476–4486.

[53] I.E. Nifant'ev, A. V. Shlyakhtin, A.N. Tavtorkin, M.A. Kosarev, D.E. Gavrilov, P.D. Komarov, S.O. Ilyin, S.G. Karchevsky, P. V. Ivchenko, Mechanistic study of transesterification in TBD-catalyzed ring-opening polymerization of methyl ethylene phosphate, Eur. Polym. J. 118 (2019) 393–403. doi:10.1016/j.eurpolymj.2019.06.015.

[54] I.E. Nifant'ev, P. Ivchenko, DFT Modeling of Organocatalytic Ring-Opening Polymerization of Cyclic Esters: A Crucial Role of Proton Exchange and Hydrogen Bonding, Polymers (Basel). 11 (2019) 2078. doi:10.3390/polym11122078.

[55] I. Nifant'ev, P. Ivchenko, Coordination ring-opening polymerization of cyclic esters: A critical overview of DFT modeling and visualization of the reaction mechanisms, Molecules. 24 (2019). doi:10.3390/molecules24224117.

[56] T.S. Stukenbroeker, D. Solis-Ibarra, R.M. Waymouth, Synthesis and topological trapping of cyclic poly(alkylene phosphates), Macromolecules. 47 (2014) 8224–8230. doi:10.1021/ma501764c.

[57] I.E. Nifant'Ev, A. V. Shlyakhtin, V. V. Bagrov, P.D. Komarov, M.A. Kosarev, A.N. Tavtorkin, M.E. Minyaev, V.A. Roznyatovsky, P. V. Ivchenko, Controlled ring-opening polymerisation of cyclic phosphates, phosphonates and phosphoramidates catalysed by heteroleptic BHT-alkoxy magnesium complexes, Polym. Chem. 8 (2017) 6806–6816. doi:10.1039/c7py01472d.

[58] I. Nifant'ev, A. Shlyakhtin, M. Kosarev, S. Karchevsky, P. Ivchenko, Mechanistic insights of BHT-Mg-catalyzed ethylene phosphate's coordination ring-opening polymerization: DFT modeling and experimental data, Polymers (Basel). 10 (2018). doi:10.3390/polym10101105.

[59] C. Pelosi, C. Duce, D. Russo, M.R.R. Tiné, F.R.R. Wurm, PPEylation of proteins: Synthesis, Activity, and Stability of Myoglobin-Polyphosphoester Conjugates, Eur. Polym. J. 108 (2018) 357–363. doi:10.1016/j.eurpolymj.2018.09.019.

[60] K. Oussadi, V. Montembault, M. Belbachir, L. Fontaine, Ring-Opening Bulk Polymerization of Five- and Six-Membered Cyclic Phosphonates Using Maghnite, a Nontoxic Proton Exchanged Montmorillonite Clay, J. Appl. Polym. Sci. 122 (2011) 891–897. doi:10.1002/app.

[61] J. Wang, S.W. Huang, P.C. Zhang, H.Q. Mao, K.W. Leong, Effect of side-chain structures on gene transfer efficiency of biodegradable cationic polyphosphoesters, Int. J. Pharm. 265 (2003) 75–84. doi:10.1016/j.ijpharm.2003.07.006.

[62] S. Penczek, T. Biela, P. Klosinski, G. Lapienis, Polymerization of phosphorus containing cyclic monomers: synthesis of polymers related to biopolymers, Makromol. Chem., Macromol. Symp. 153 (1986) 123–153.

[63] T. Steinbach, S. Ritz, F.R. Wurm, Water-soluble poly(phosphonate)s via living ring-opening polymerization, ACS Macro Lett. 3 (2014) 244–248. doi:10.1021/mz500016h.

[64] T. Wolf, T. Steinbach, F.R. Wurm, A Library of Well-Defined and Water-Soluble Poly(alkyl phosphonate)s with Adjustable Hydrolysis, Macromolecules. 48 (2015) 3853–3863. doi:10.1021/acs.macromol.5b00897.

[65] B. Clément, D.G. Molin, C. Jérôme, P. Lecomte, Synthesis of polyphosphodiesters by ring-opening polymerization of cyclic phosphates bearing allyl phosphoester protecting groups, J. Polym. Sci. Part A Polym. Chem. 53 (2015) 2642–2648. doi:10.1002/pola.27732.

[66] S. Zhang, H. Wang, Y. Shen, F. Zhang, K. Seetho, J. Zou, J. a Taylor, A.P. Dove, K.L. Wooley, A Simple and Efficient Synthesis of an Acid-Labile Polyphosphoramidate by Organobase-Catalyzed Ring-Opening Polymerization and Transformation to Polyphosphoester Ionomers by Acid Treatment, Macromolecules. 46 (2013) 5141–5149. doi:https://doi.org/10.1021/ma400675m.

[67] T. Steinbach, R. Schröder, S. Ritz, F.R. Wurm, Microstructure analysis of biocompatible phosphoester copolymers, Polym. Chem. 4 (2013) 4469–4479. doi:10.1039/c3py00563a.

[68] T. Sun, J. Du, L. Yan, H. Mao, J. Wang, Biomaterials Self-assembled biodegradable micellar nanoparticles of amphiphilic and cationic block copolymer for siRNA delivery, Biomaterials. 29 (2008) 4348–4355.

[69] R. Haag, F. Kratz, Polymer therapeutics: Concepts and applications, Angew. Chemie - Int. Ed. 45 (2006) 1198–1215. doi:10.1002/anie.200502113.

[70] C.Y. Sun, S. Dou, J.Z. Du, X.Z. Yang, Y.P. Li, J. Wang, Doxorubicin conjugate of poly(ethylene glycol)-block-polyphosphoester for cancer therapy, Adv. Healthc. Mater. 3 (2014) 261–272. doi:10.1002/adhm.201300091.

[71] L.Y. Tang, Y.C. Wang, Y. Li, J.Z. Du, J. Wang, Shell-detachable micelles based on disulfide-linked block copolymer as potential carrier for intracellular drug delivery, Bioconjug. Chem. 20 (2009) 1095–1099. doi:10.1021/bc900144m.

[72] F. Wang, Y.C. Wang, L.F. Yan, J. Wang, Biodegradable vesicular nanocarriers based on poly(ε-caprolactone)-block-poly(ethyl ethylene phosphate) for drug delivery, Polymer (Guildf). 50 (2009) 5048–5054. doi:10.1016/j.polymer.2009.09.007.

[73] P. Zhang, L. Hu, Q. Yin, Z. Zhang, L. Feng, Y. Li, Transferrin-conjugated polyphosphoester hybrid micelle loading paclitaxel for brain-targeting delivery: Synthesis, preparation and in vivo evaluation, J. Control. Release. 159 (2012) 429–434. doi:10.1016/j.jconrel.2012.01.031.

[74] F. Zhang, S. Khan, R. Li, J.A. Smolen, S. Zhang, G. Zhu, L. Su, A.A. Jahnke, M. Elsabahy, X. Chen, K.L. Wooley, Design and development of multifunctional polyphosphoester-based nanoparticles for ultrahigh paclitaxel dual loading, Nanoscale. 9 (2017) 15773–15777. doi:10.1039/c7nr05935c.

[75] G. Ma, J. Liu, J. He, M. Zhang, P. Ni, Dual-Responsive Polyphosphoester-Doxorubicin Prodrug Containing a Diselenide Bond: Synthesis, Characterization, and Drug Delivery, ACS Biomater. Sci. Eng. 4 (2018) 2443–2452. doi:10.1021/acsbiomaterials.8b00429.

[76] H. Elzeny, F. Zhang, E.N. Ali, H.A. Fathi, S. Zhang, R. Li, M.A. El-Mokhtar, M.A. Hamad, K.L. Wooley, M. Elsabahy, Polyphosphoester nanoparticles as biodegradable platform for delivery of multiple drugs and siRNA, Drug Des. Devel. Ther. 11 (2017) 483–496. doi:10.2147/DDDT.S128503.

[77] R. Li, M. Elsabahy, Y. Song, H. Wang, L. Su, R.A. Letteri, S. Khan, G.S. Heo, G. Sun, Y. Liu, K.L. Wooley, Functional, Degradable Zwitterionic Polyphosphoesters as Biocompatible Coating Materials for Metal Nanostructures, Langmuir. 35 (2019) 1503–1512. doi:10.1021/acs.langmuir.8b02033.

[78] Q. Chen, K.N. Shah, F. Zhang, A.J. Salazar, P.N. Shah, R. Li, J.C. Sacchettini, K.L. Wooley, C.L. Cannon, Minocycline and Silver Dual-Loaded Polyphosphoester-Based Nanoparticles for Treatment of Resistant Pseudomonas aeruginosa, Mol. Pharm. 16 (2019) 1606–1619. doi:10.1021/acs.molpharmaceut.8b01288.

[79] Y.P. Borguet, S. Khan, A. Noel, S.P. Gunsten, S.L. Brody, M. Elsabahy, K.L. Wooley, Development of Fully Degradable Phosphonium-Functionalized Amphiphilic Diblock Copolymers for Nucleic Acids Delivery, Biomacromolecules. 19 (2018) 1212–1222. doi:10.1021/acs.biomac.8b00069.

[80] H. Azevedo, R. Reis, Understanding the Enzymatic Degradation of Biodegradable Polymers and Strategies to Control Their Degradation Rate, Biodegrad. Syst. Tissue Eng. Regen. Med. (2004) 177–202. doi:10.1201/9780203491232.ch12.

[81] Iupac, Glossary for chemist for terms used in biotechnology (IUPAC Recommendations 1992), Compend. Chem. Terminol. (The Golden Book). 64 (1992) 143–168. doi:10.1351/goldbook.

[82] M. Vert, Y. Doi, K.-H. Hellwich, M. Hess, P. Hodge, P. Kubisa, M. Rinaudo, F. Schué, Terminology for Biorelated Polymers and Applications (IUPAC Recommendations 2012), Pure Appl. Chem. 84 (2012) 377–410.

[83] C. Bastioli, Handbook of biodegradable polymers, 2nd ed., Smithers, 2005.

[84] K.N. Bauer, L. Liu, M. Wagner, D. Andrienko, F.R. Wurm, Mechanistic study on the hydrolytic degradation of polyphosphates, Eur. Polym. J. 108 (2018) 286–294. doi:10.1016/j.eurpolymj.2018.08.058.

[85] M.A. Kosarev, D.E. Gavrilov, I.E. Nifant'ev, A. V. Shlyakhtin, A.N. Tavtorkin, V.P. Dyadchenko, V.A. Roznyatovsky, P. V. Ivchenko, Ultrafast hydrolytic degradation of 2,3-dihydroxypropyl functionalized poly(ethylene phosphates), Mendeleev Commun. 29 (2019) 509–511. doi:10.1016/j.mencom.2019.09.010.

[86] M. Steinmann, F.R. Wurm, Water-soluble and degradable polyphosphorodiamidates via thiol-ene polyaddition, Polym. Degrad. Stab. 179 (2020) 109224. doi:10.1016/j.polymdegradstab.2020.109224.

[87] M. V. Chaubal, G. Su, E. Spicer, W. Dang, K.E. Branham, J.P. English, Z. Zhao, In vitro and in vivo degradation studies of a novel linear copolymer of lactide and ethylphosphate, J. Biomater. Sci. Polym. Ed. 14 (2003) 45–61. doi:10.1163/15685620360511137.

[88] X.Z. Yang, Y.C. Wang, L.Y. Tang, H. Xia, J. Wang, Synthesis and Characterization of Amphiphilic Block Copolymer of Polyphosphoester and Poly(L-lactic aacid), J. Polym. Sci. Part a Polym. Chem. 46 (2008) 6425–6434. doi:10.1002/pola.22951.

[89] Y.C. Wang, L.Y. Tang, Y. Li, J. Wang, Thermoresponsive block copolymers of poly(ethylene glycol) and polyphosphoester: Thermo-induced self-assembly, biocompatibility, and hydrolytic degradation, Biomacromolecules. 10 (2009) 66–73. doi:10.1021/bm800808q.

[90] S.W. Huang, J. Wang, P.C. Zhang, H.Q. Mao, R.X. Zhuo, K.W. Leong, Water-soluble and nonionic polyphosphoester: Synthesis, degradation, biocompatibility and enhancement of gene expression in mouse muscle, Biomacromolecules. 5 (2004) 306–311. doi:10.1021/bm034241l.

[91] M. Elsabahy, S. Zhang, F. Zhang, Z.J. Deng, Y.H. Lim, H. Wang, P. Parsamian, P.T. Hammond, K.L. Wooley, Surface charges and shell crosslinks each play significant roles in mediating degradation, biofouling, cytotoxicity and immunotoxicity for polyphosphoester-based nanoparticles, Sci. Rep. 3 (2013). doi:10.1038/srep03313.

[92] Y. Shen, S. Zhang, F. Zhang, A. Loftis, A. Pavía-Sanders, J. Zou, J. Fan, J.S.A. Taylor, K.L. Wooley, Polyphosphoester-based cationic nanoparticles serendipitously release integral biologically-active components to serve as novel degradable inducible nitric oxide synthase inhibitors, Adv. Mater. 25 (2013) 5609–5614. doi:10.1002/adma.201302842.

[93] Y.H. Lim, K.M. Tiemann, G.S. Heo, P.O. Wagers, Y.H. Rezenom, S. Zhang, F. Zhang, W.J. Youngs, D.A. Hunstad, K.L. Wooley, Preparation and in vitro antimicrobial activity of silver-bearing degradable polymeric nanoparticles of polyphosphoester-block-poly(l-lactide), ACS Nano. 9 (2015) 1995–2008. doi:10.1021/nn507046h.

[94] Y.H. Lim, G.S. Heo, S. Cho, K.L. Wooley, Construction of a reactive diblock copolymer, polyphosphoester- block -poly(l -lactide), as a versatile framework for functional materials that are capable of full degradation and nanoscopic assembly formation, ACS Macro Lett. 2 (2013) 785–789. doi:10.1021/mz400229m.

[95] G. Zhang, M. Zhang, J. He, P. Ni, Synthesis and characterization of a new multifunctional polymeric prodrug paclitaxel-polyphosphoester-folic acid for targeted drug delivery, Polym. Chem. 4 (2013) 4515–4525. doi:10.1039/c3py00419h.

[96] K. Tanaka, N. Kitamura, Y. Chujo, Biodegradable main-chain phosphate-caged fluorescein polymers for the evaluation of enzymatic activity, Macromolecules. 43 (2010) 6180–6184. doi:10.1021/ma1009066.

[97] M. Yao, Y. Ma, H. Liu, M.I. Khan, S. Shen, S. Li, Y. Zhao, Y. Liu, G. Zhang, X. Li, F. Zhong, W. Jiang, Y. Wang, Enzyme Degradable Hyperbranched Polyphosphoester Micellar Nanomedicines for NIR Imaging-Guided Chemo-Photothermal Therapy of Drug-Resistant Cancers, Biomacromolecules. 19 (2018) 1130–1141. doi:10.1021/acs.biomac.7b01793.

[98] D.F. Xiang, A.N. Bigley, Z. Ren, H. Xue, K.G. Hull, D. Romo, F.M. Raushel, Interrogation of the Substrate Profile and Catalytic Properties of the Phosphotriesterase from Sphingobium sp. Strain TCM1: An Enzyme Capable of Hydrolyzing Organophosphate Flame Retardants and Plasticizers, Biochemistry. 54 (2015) 7539–7549. doi:10.1021/acs.biochem.5b01144.

[99] J. Zhou, X. Du, C. Berciu, H. He, J. Shi, D. Nicastro, B. Xu, Enzyme-Instructed Self-Assembly for Spatiotemporal Profiling of the Activities of Alkaline Phosphatases on Live Cells, Chemistry (Easton). 1 (2016) 246–263. doi:10.1016/j.chempr.2016.07.003.

[100] S.R. Rao, A.E. Snaith, D. Marino, X. Cheng, S.T. Lwin, I.R. Orriss, F.C. Hamdy, C.M. Edwards, Tumour-derived alkaline phosphatase regulates tumour growth, epithelial plasticity and disease-free survival in metastatic prostate cancer, Br. J. Cancer. 116 (2017) 227–236. doi:10.1038/bjc.2016.402.

[101] K.S. Nilgiriwala, A. Alahari, A.S. Rao, S.K. Apte, Cloning and overexpression of alkaline phosphatase PhoK from Sphingomonas sp. strain BSAR-1 for bioprecipitation of uranium from alkaline solutions, Appl. Environ. Microbiol. 74 (2008) 5516–5523. doi:10.1128/AEM.00107-08.

[102] J.Z. Du, D.P. Chen, Y.C. Wang, C.S. Xiao, Y.J. Lu, J. Wang, G.Z. Zhang, Synthesis and micellization of amphiphilic brush - Coil block copolymer based on poly(∈-caprolactone) and PEGylated polyphosphoester, Biomacromolecules. 7 (2006) 1898–1903. doi:10.1021/bm051003c.

[103] Z. Liu, M. Wu, Y. Xue, C. Chen, F.R. Wurm, M. Lan, W. Zhang, Hydrophilic polyphosphoester-

conjugated fluorinated chlorin as an entirely biodegradable nano-photosensitizer for reliable and efficient photodynamic therapy, Chem. Commun. 56 (2020) 2415–2418. doi:10.1039/d0cc00142b.

[104] J. Liu, W. Huang, Y. Pang, X. Zhu, Y. Zhou, D. Yan, Hyperbranched polyphosphates for drug delivery application: Design, synthesis, and in vitro evaluation, Biomacromolecules. 11 (2010) 1564–1570. doi:10.1021/bm100188h.

[105] Y. Tao, J. He, M. Zhang, Y. Hao, J. Liu, P. Ni, Galactosylated biodegradable poly(ε-caprolactone-co-phosphoester) random copolymer nanoparticles for potent hepatoma-targeting delivery of doxorubicin, Polym. Chem. 5 (2014) 3443–3452. doi:10.1039/c4py00024b.

[106] S. Zhang, J. Zou, F. Zhang, M. Elsabahy, S. Felder, J. Zhu, D.J. Pochan, K.L. Wooley, Rapid and versatile construction of diverse and functional nanostructures derived from a polyphosphoester-based biomimetic block copolymer system, J. Am. Chem. Soc. 134 (2012) 18467–18474. doi:10.1038/jid.2014.371.

[107] J. Wen, H.Q. Mao, W. Li, K.Y. Lin, K.W. Leong, Biodegradable polyphosphoester micelles for gene delivery, J. Pharm. Sci. 93 (2004) 2142–2157. doi:10.1002/jps.20121.

[108] C.J. Chen, Q. Jin, G.Y. Liu, D.D. Li, J.L. Wang, J. Ji, Reversibly light-responsive micelles constructed via a simple modification of hyperbranched polymers with chromophores, Polymer (Guildf). 53 (2012) 3695–3703. doi:10.1016/j.polymer.2012.06.024.

[109] A.A. Ignatius, L.E. Claes, In vitro biocompatibility of bioresorbable polymers: Poly(L, DL-lactide) and poly(L-lactide-co-glycolide), Biomaterials. 17 (1996) 831–839. doi:10.1016/0142-9612(96)81421-9.

[110] G. Liu, Y. Li, L. Yang, Y. Wei, X. Wang, Z. Wang, L. Tao, Cytotoxicity study of polyethylene glycol derivatives, RSC Adv. 7 (2017) 18252–18259. doi:10.1039/c7ra00861a.

[111] H.F. Smyth, C.P. Carpenter, C.S. Weil, The toxicology of the polyethylene glycols., J. Am. Pharm. Assoc. 39 (1950) 349–354. doi:10.1002/jps.3030390615.

[112] H.F. Smyth, C.P. Carpenter, C.S. Weil, The chronic oral toxicology of the polyethylene glycols., J. Am. Pharm. Assoc. 44 (1955) 27–30. doi:10.1002/jps.3030440111.

[113] D.E. Prentice, S.K. Majeed, Oral toxicity of polyethylene glycol (PEG 200) in monkeys and rats, Toxicol. Lett. 2 (1978) 119–122. doi:10.1016/0378-4274(78)90084-X.

[114] H. Jeong, J. Hwang, H. Lee, P.T. Hammond, J. Choi, J. Hong, In vitro blood cell viability profiling of polymers used in molecular assembly, Sci. Rep. 7 (2017) 1–13. doi:10.1038/s41598-017-10169-5.

[115] J. Goode, Use of International Standard ISO 10993-1, “Biological evaluation of medical devices - Part

1: Evaluation and testing within a risk management process," Dep. Heal. Hum. Serv. Food Drug Adm. (2016).

[116] P. Del Pino, B. Pelaz, Q. Zhang, P. Maffre, G.U. Nienhaus, W.J. Parak, Protein corona formation around nanoparticles - From the past to the future, Mater. Horizons. 1 (2014) 301–313. doi:10.1039/c3mh00106g.

[117] S. Salmaso, P. Caliceti, Stealth Properties to Improve Therapeutic Efficacy of Drug Nanocarriers, J. Drug Deliv. 2013 (2013) 1–19. doi:10.1155/2013/374252.

[118] M.P. Monopoli, C. Åberg, A. Salvati, K.A. Dawson, Biomolecular coronas provide the biological identity of nanosized materials, Nat. Nanotechnol. 7 (2012) 779–786. doi:10.1038/nnano.2012.207.

[119] S.-D. Li, L. Huang, Stealth Nanoparticles: High Density but Sheddable PEG is a Key for Tumor Targeting, J. Control. Release. 145 (2010) 178–181. doi:10.1016/j.jconrel.2010.03.016.

[120] M.D. Howard, M. Jay, T.D. Dziubla, X. Lu, PEGylation of nanocarrier drug delivery systems: State of the art, J. Biomed. Nanotechnol. 4 (2008) 133–148. doi:10.1166/jbn.2008.021.

[121] Y.Y. Yuan, C.Q. Mao, X.J. Du, J.Z. Du, F. Wang, J. Wang, Surface charge switchable nanoparticles based on zwitterionic polymer for enhanced drug delivery to tumor, Adv. Mater. 24 (2012) 5476–5480. doi:10.1002/adma.201202296.

[122] F. Alexis, E. Pridgen, L.K. Molnar, O.C. Farokhzad, Factors affecting the clearance and biodistribution of polymeric nanoparticles, Mol. Pharm. 5 (2008) 505–515. doi:10.1021/mp800051m.

[123] R.R. Arvizo, O.R. Miranda, D.F. Moyano, C.A. Walden, K. Giri, R. Bhattacharya, J.D. Robertson, V.M. Rotello, J.M. Reid, P. Mukherjee, Modulating pharmacokinetics, tumor uptake and biodistribution by engineered nanoparticles, PLoS One. 6 (2011) 3–8. doi:10.1371/journal.pone.0024374.

[124] Z.J. Deng, M. Liang, I. Toth, M. Monteiro, R.F. Minchin, Plasma protein binding of positively and negatively charged polymer-coated gold nanoparticles elicits different biological responses, Nanotoxicology. 7 (2013) 314–322. doi:10.3109/17435390.2012.655342.

[125] S. Schöttler, G. Becker, S. Winzen, T. Steinbach, K. Mohr, K. Landfester, V. Mailänder, F.R. Wurm, Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers, Nat. Nanotechnol. 11 (2016) 372–377. doi:10.1038/nnano.2015.330.

[126] J. Müller, K.N. Bauer, D. Prozeller, J. Simon, V. Mailänder, F.R. Wurm, S. Winzen, K. Landfester, Coating nanoparticles with tunable surfactants facilitates control over the protein corona, Biomaterials. 115 (2017) 1–8. doi:10.1016/j.biomaterials.2016.11.015.

[127] J. Simon, T. Wolf, K. Klein, K. Landfester, F.R. Wurm, V. Mailänder, Hydrophilicity Regulates the Stealth Properties of Polyphosphoester-Coated Nanocarriers, Angew. Chemie - Int. Ed. 57 (2018) 5548–5553. doi:10.1002/anie.201800272.

[128] J. Simon, K.N. Bauer, J. Langhanki, T. Opatz, V. Mailänder, K. Landfester, F.R. Wurm, Noncovalent Targeting of Nanocarriers to Immune Cells with Polyphosphoester-Based Surfactants in Human Blood Plasma, Adv. Sci. 6 (2019). doi:10.1002/advs.201901199.

[129] K.N. Bauer, J. Simon, V. Mailander, K. Landfester, F.R. Wurm, Polyphosphoester Surfactants as General Stealth Coatings for Polymeric Nanocarriers, Acta Biomater. (2020). doi:10.1016/j.actbio.2020.09.016.

[130] A. Abuchowski, J.R. Mccoy, N.C. Palczuk, Effect of Covalent Attachment of Polyethylene Glycol on Immunogenicity and Circulating Life of Bovine Liver Catalase, J. Biol. Chem. 252 (1976) 3582–3586.

[131] A. Abuchowski, T. van Es, N.C. Palczuk, Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol., J. Biol. Chem. 252 (1977) 3578–81.

[132] N. V. Katre, The conjugation of proteins with polyethylene glycol and other polymers. Altering properties of proteins to enhance their therapeutic potential, Adv. Drug Deliv. Rev. 10 (1993) 91–114. doi:10.1016/0169-409X(93)90005-O.

[133] M.J. Roberts, M.D. Bentley, J.M. Harris, Chemistry for peptide and protein PEGylation, Adv. Drug Deliv. Rev. 54 (2002) 459–476. doi:10.1016/j.addr.2012.09.025.

[134] M.A. Gauthier, H.-A. Klok, Peptide/protein–polymer conjugates: synthetic strategies and design concepts, Chem. Commun. (2008) 2591. doi:10.1039/b719689j This.

[135] D. Pfister, M. Morbidelli, Process for protein PEGylation, J. Control. Release. 180 (2014) 134–149. doi:10.1016/j.jconrel.2014.02.002.

[136] Y. Hou, H. Lu, Protein PEPylation: A New Paradigm of Protein-Polymer Conjugation, Bioconjug. Chem. 30 (2019) 1604–1616. doi:10.1021/acs.bioconjchem.9b00236.

[137] T. Steinbach, F.R. Wurm, Degradable Polyphosphoester-Protein Conjugates: “PPEylation” of Proteins, Biomacromolecules. 17 (2016) 3338–3346. doi:10.1021/acs.biomac.6b01107.

[138] D. Russo, M. Plazanet, J. Teixeira, M. Moulin, M. Härtlein, F.R. Wurm, T. Steinbach, Investigation into the Relaxation Dynamics of Polymer-Protein Conjugates Reveals Surprising Role of Polymer Solvation on Inherent Protein Flexibility, Biomacromolecules. 17 (2016) 141–147. doi:10.1021/acs.biomac.5b01269.

[139] T.A. Wright, R.C. Page, D. Konkolewicz, Polymer conjugation of proteins as a synthetic post-translational modification to impact their stability and activity, Polym. Chem. 10 (2019) 434–454. doi:10.1039/c8py01399c.

[140] P. Bailon, A. Palleroni, C.A. Schaffer, C.L. Spence, W.-J. Fung, J.E. Porter, G.K. Ehrlich, W. Pan, Z.-X. Xu, M.W. Modi, A. Farid, W. Berthold, Rational Design of a Potent, Long-Lasting Form of Interferon: A 40 kDa Branched Polyethylene Glycol-Conjugated Interferon a-2a for the Treatment of Hepatitis C, Bioconjug. Chem. 12 (2001) 195–202.

[141] C. Pelosi, F. Saitta, F.R. Wurm, D. Fessas, M.R. Tinè, C. Duce, Thermodynamic stability of myoglobin-poly(ethylene glycol) bioconjugates: A calorimetric study, Thermochim. Acta. 671 (2019) 26–31. doi:10.1016/j.tca.2018.11.001.

[142] D. Russo, A. de Angelis, F.R. Wurm, C.J. Garvey, M.S. Appavou, S. Prevost, Effect of polymer chain density on protein-polymer conjugate conformation, Biomacromolecules. 20 (2019) 1944–1955.

[143] D. Russo, A. De Angelis, A. Paciaroni, B. Frick, N. De Sousa, F.R. Wurm, J. Teixeira, Protein−Polymer Dynamics as Affected by Polymer Coating and Interactions, Langmuir. 35 (2019) 2674–2679. doi:10.1021/acs.langmuir.8b03636.

[144] D. Russo, C. Pelosi, F.R. Wurm, B. Frick, J. Ollivier, J. Teixeira, Insight into Protein–Polymer Conjugate Relaxation Dynamics: The Importance of Polymer Grafting, Macromol. Biosci. 1900410 (2020) 16–18. doi:10.1002/mabi.201900410.

[145] S. Mitragoti, P.A. Burke, R. Langer, Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies, Nat. Rev. Drug Discov. 13 (2014) 655–672. doi:10.1016/j.physbeh.2017.03.040.

[146] S. Vanslambrouck, B. Clèment, R. Riva, L.H. Koole, D.G.M. Molin, G. Broze, P. Lecomte, C. Jerome, Synthesis and tensioactive properties of PEO-b- polyphosphate copolymers, RCS Adv. 5 (2015) 27330–27337.

[147] Z.E. Yilmaz, S. Vanslambrouck, S. Cajot, J. Thiry, A. Debuigne, P. Lecomte, C. Jérôme, R. Riva, Core cross-linked micelles of polyphosphoester containing amphiphilic block copolymers as drug nanocarriers, RSC Adv. 6 (2016) 42081–42088. doi:10.1039/c6ra07422g.

[148] T.P. Gustafson, Y.H. Lim, J.A. Flores, G.S. Heo, F. Zhang, S. Zhang, S. Samarajeewa, J.E. Raymond, K.L. Wooley, Holistic assessment of covalently labeled core-shell polymeric nanoparticles with fluorescent contrast agents for theranostic applications, Langmuir. 30 (2014) 631–641. doi:10.1021/la403943w.

[149] S. Zhang, J. Zou, M. Elsabahy, A. Karwa, A. Li, D.A. Moore, R.B. Dorshow, K.L. Wooley, Poly(ethylene oxide)-block-polyphosphester-based paclitaxel conjugates as a platform for ultra-high paclitaxel-loaded multifunctional nanoparticles, Chem. Sci. 4 (2013) 2122–2126. doi:10.1039/c3sc50252j.

[150] F. Zhang, J.A. Smolen, S. Zhang, R. Li, P.N. Shah, S. Cho, H. Wang, J.E. Raymond, C.L. Cannon, K.L. Wooley, Degradable polyphosphoester-based silver-loaded nanoparticles as therapeutics for bacterial lung infections, Nanoscale. 7 (2015) 2265–2270. doi:10.1039/c4nr07103d.

[151] T.A. Aweda, S. Zhang, C. Mupanomunda, J. Burkemper, G.S. Heo, N. Bandara, M. Lin, C.S. Cutler, C.L. Cannon, W.J. Youngs, K.L. Wooley, S.E. Lapi, Investigating the pharmacokinetics and biological distribution of silver-loaded polyphosphoester-based nanoparticles using 111Ag as a radiotracer, J. Label. Compd. Radiopharm. 58 (2015) 234–241. doi:10.1002/jlcr.3289.

[152] R. Li, H. Wang, Y. Song, Y.N. Lin, M. Dong, Y. Shen, S. Khan, S. Zhang, J. Fan, F. Zhang, L. Su, K.L. Wooley, In situ production of Ag/polymer asymmetric nanoparticles via a powerful light-driven technique, J. Am. Chem. Soc. 141 (2020) 19542–19545. doi:10.1021/jacs.9b10205.

[153] T.S.J. Kashi, S. Eskandarion, M. Esfandyari-Manesh, S.M.A. Marashi, N. Samadi, S.M. Fatemi, F. Atyabi, S. Eshraghi, R. Dinarvand, Improved drug loading and antibacterial activity of minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method, Int. J. Nanomedicine. 7 (2012) 221–234.

[154] H. Wang, M. Dong, S. Khan, L. Su, R. Li, Y. Song, Y.N. Lin, N. Kang, C.H. Komatsu, M. Elsabahy, K.L. Wooley, Acid-Triggered Polymer Backbone Degradation and Disassembly to Achieve Release of Camptothecin from Functional Polyphosphoramidate Nanoparticles, ACS Macro Lett. 7 (2018) 783–788. doi:10.1021/acsmacrolett.8b00377.

[155] Y. Wen, Y. Wang, X. Liu, W. Zhang, X. Xiong, Z. Han, X. Liang, Camptothecin-based nanodrug delivery systems, Cancer Biol. Med. 14 (2017) 363–370. doi:10.20892/j.issn.2095-3941.2017.0099.

[156] M.P. Gamcsik, M.S. Kasibhatla, S.D. Teeter, O.M. Colvin, Glutathione levels in human tumors, Biomarkers. 17 (2012) 671–691. doi:10.3109/1354750X.2012.715672.

[157] J.Z. Du, X.J. Du, C.Q. Mao, J. Wang, Tailor-Made dual pH-sensitive polymer-doxorubicin nanoparticles for efficient anticancer drug delivery, J. Am. Chem. Soc. 133 (2011) 17560–17563. doi:10.1021/ja207150n.

[158] Y. Cao, J. He, J. Liu, M. Zhang, P. Ni, Folate-Conjugated Polyphosphoester with Reversible Cross-Linkage and Reduction Sensitivity for Drug Delivery, ACS Appl. Mater. Interfaces. 10 (2018) 7811–7820. doi:10.1021/acsami.7b18887.

[159] L.L. Stolbach, M.J. Krant, W.H. Fishman, Ectopic production of an alkaline phosphatase isoenzyme in patients with cancer, N. Engl. J. Med. 281 (1969) 757-. doi:10.1056/NEJM196910022811403.

[160] S.Y. Wu, G. Lopez-Berestein, G.A. Calin, A.K. Sood, Targeting the undruggable: Advances and obstacles in current RNAi therapy, Sci. Transl. Med. 6 (2014) 1–15. doi:10.1016/0040-5809(84)90022-4.

[161] X. Liu, P. Ni, J. He, M. Zhang, Synthesis and micellization of pH/temperature-responsive double-hydrophilic diblock copolymers polyphosphoester- block -poly[2-(dimethylamino)ethyl methacrylate] prepared via ROP and ATRP, Macromolecules. 43 (2010) 4771–4781. doi:10.1021/ma902658n.

[162] C.Q. Mao, J.Z. Du, T.M. Sun, Y.D. Yao, P.Z. Zhang, E.W. Song, J. Wang, A biodegradable amphiphilic and cationic triblock copolymer for the delivery of siRNA targeting the acid ceramidase gene for cancer therapy, Biomaterials. 32 (2011) 3124–3133. doi:10.1016/j.biomaterials.2011.01.006.

[163] T.M. Sun, J.Z. Du, Y.D. Yao, C.Q. Mao, S. Dou, S.Y. Huang, P.Z. Zhang, K.W. Leong, E.W. Song, J. Wang, Simultaneous delivery of siRNA and paclitaxel via a “two-in-one” micelleplex promotes synergistic tumor suppression, ACS Nano. 5 (2011) 1483–1494. doi:10.1021/nn103349h.

[164] X.Z. Yang, J.Z. Du, S. Dou, C.Q. Mao, H.Y. Long, J. Wang, Sheddable ternary nanoparticles for tumor acidity-targeted siRNA delivery, ACS Nano. 6 (2012) 771–781. doi:10.1021/nn204240b.

[165] Q. Li, J. Wang, S. Shahani, D.D.N. Sun, B. Sharma, J.H. Elisseeff, K.W. Leong, Biodegradable and photocrosslinkable polyphosphoester hydrogel, Biomaterials. 27 (2006) 1027–1034. doi:10.1016/j.biomaterials.2005.07.019.

[166] G. Becker, L.M. Ackermann, E. Schechtel, M. Klapper, W. Tremel, F.R. Wurm, Joining Two Natural Motifs: Catechol-Containing Poly(phosphoester)s, Biomacromolecules. 18 (2017) 767–777. doi:10.1021/acs.biomac.6b01613.

[167] Y. Hao, J. He, X. Ma, L. Feng, M. Zhu, Y. Zhai, Y. Liu, P. Ni, G. Cheng, A fully degradable and photocrosslinked polysaccharide-polyphosphate hydrogel for tissue engineering, Carbohydr. Polym. 225 (2019) 115257. doi:10.1016/j.carbpol.2019.115257.

[168] J.Z. Du, T.M. Sun, S.Q. Weng, X.S. Chen, J. Wang, Synthesis and characterization of photo-cross-linked hydrogels based on biodegradable polyphosphoesters and poly(ethylene glycol) copolymers, Biomacromolecules. 8 (2007) 3375–3381. doi:10.1021/bm700474b.

[169] W. Wang, S. Liu, B. Chen, X. Yan, S. Li, X. Ma, X. Yu, DNA-Inspired Adhesive Hydrogels Based on the Biodegradable Polyphosphoesters Tackified by a Nucleobase, Biomacromolecules. 20 (2019) 3672–

3683. doi:10.1021/acs.biomac.9b00642.

[170] Z. Liu, L. Wang, C. Bao, X. Li, L. Cao, K. Dai, L. Zhu, Cross-linked PEG via degradable phosphate ester bond: Synthesis, water-swelling, and application as drug carrier, Biomacromolecules. 12 (2011) 2389–2395. doi:10.1021/bm2004737.

[171] N. Padmavathy, P.K. Samantaray, L. Das Ghosh, G. Madras, S. Bose, Selective cleavage of the polyphosphoester in crosslinked copper based nanogels: Enhanced antibacterial performance through controlled release of copper, Nanoscale. 9 (2017) 12664–12676. doi:10.1039/c7nr02446k.

[172] H. Tee, R. Zipp, K. Koynov, W. Tremel, F.R. Wurm, Poly(methyl ethylene phosphate) Hydrogels: Degradable and Cell-Repellent Alternatives to PEG-Hydrogels, Eur. Polym. J. (2020). doi:Accepted.

[173] R. Riva, U. Shah, J.M. Thomassin, Z. Yilmaz, A. Lecat, A. Colige, C. Jérôme, Design of Degradable Polyphosphoester Networks with Tailor-Made Stiffness and Hydrophilicity as Scaffolds for Tissue Engineering, Biomacromolecules. 21 (2020) 349–355. doi:10.1021/acs.biomac.9b01276.

SCHEME CAPTIONS

Scheme 1: Overview of the PPEs subclasses reported to date.

Scheme 2: Overview of water-soluble, synthetic polymers used in biomedical applications PEG alternatives (PG, POxs, HMPA, PVP, PGA, and PPEs).

Scheme 3: AROP of cyclic phosphate monomers towards main-chain PPEs (R=alkyl, or O-alkyl; Cat.= catalyst, cf. Scheme 4; E^+= electrophilic termination reagent).

Scheme 4: Catalysts used in AROP polymerization of main-chain water-soluble PPEs and the new generation of catalysts for PPE polymerization.

Scheme 5: Definition of hydrolysis rate constants k_s and k_m for PMEP and bis(2-methoxy ethyl) methyl phosphate.

Scheme 6: Degradation mechanism of PMEP in basic conditions, suggested by a) Penczek and Baran [18] *and b) Bauer et al.* [84].

Scheme 7: Self-catalytic degradation mechanism proposed by Leong and co-workers [50,61].

Scheme 8: Possible degradation mechanisms linked to the hydrolytic degradation of 2,3-Dihydroxypropyl functionalized polyphosphates proposed by Kosarev et al. [85].

FIGURES CAPTIONS

Figure 1: Overview of the most important discoveries made through the years concerning the features and applications of main-chain water-soluble PPEs.

Figure 2: a) The overlay of several ^{1}H NMR spectra for PEEP, recorded at different degradation times. b) The degradation profiles of PEEP and PMEP at pH 11 derived from ^{1}H NMR spectra. c) Chemical structures of PEEP and b/PEEP, with respectively a terminal hydroxyl or a urethane functionality, and the respective degradation profiles derived from ^{31}P NMR spectra. Reproduced from: Mechanistic study on the hydrolytic degradation of polyphosphates, K.N. Bauer, L. Liu, M. Wagner, D. Andrienko, F.R. Wurm, Eur. Polym. J. 108 (2018) 286–294 [84]. Copyright © 2018, with permission from Elsevier.

Figure 3: a) Chemical structure of AuNPs coated with the zwitterionic polymer PBYP; *b) On the left UV-VIS spectra of* Au-NPs coated with PBYP *recorded at 0, 1, 2, 4, 6, 14 days and on the right correspondent degradation profile over time. Adapted with permission from: Functional, Degradable Zwitterionic Polyphosphoesters as Biocompatible Coating Materials for Metal Nanostructures, R. Li, M. Elsabahy, Y. Song, H. Wang, L. Su, R.A. Letteri, S. Khan, G.S. Heo, G. Sun, Y. Liu, K.L. Wooley, Langmuir. 35 (2019) 1503–1512* [77] . *Copyright © 2018, American Chemical Society.*

Figure 4: Schematic illustration of PPE-FP2 NPs and their biological action in photodynamic therapy in mice. Reproduced from: Hydrophilic polyphosphoester-conjugated fluorinated chlorin as an entirely biodegradable nano-photosensitizer for reliable and efficient photodynamic therapy, Z. Liu, M. Wu, Y. Xue, C. Chen, F.R. Wurm, M. Lan, W. Zhang, Chem. Commun. 56 (2020) 2415–2418. [103]. *Published by the Royal Society of Chemistry.*

Figure 5: Histology images of mouse muscle samples injected with PEI and PPE3, harvested on days 3 and 7. Adapted with permission from: Water-soluble and non-ionic polyphosphoester: Synthesis, degradation, biocompatibility and enhancement of gene expression in mouse muscle, S.W. Huang, J. Wang, P.C. Zhang, H.Q. Mao, R.X. Zhuo, K.W. Leong, Biomacromolecules. 5 (2004) 306–311 [90]. *Copyright © 2004, American Chemical Society.*

Figure 6: Schematic representation of the features that help to prevent the opsonization process as hypothesized for PEG.

Figure 7: Proteomic analysis of protein corona on the surface of polystyrene nanocarriers naked (PS-NH_2), PEGylated (PS-PEG), PPEylated (PS-PEEP). Adapted from: Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers, S. Schöttler, G. Becker, S. Winzen, T.

Steinbach, K. Mohr, K. Landfester, V. Mailänder, F.R. Wurm, Nat. Nanotechnol. 11 (2016) 372–377 [125]. *Copyright © 216, Springer Nature.*

Figure 8: Analytical data of polystyrene nanoparticles covalently linked with PEG, and polyphosphonates with a different degree of hydrophilicity. Scale bar: 200 nm. Adapted with permission from: Hydrophilicity Regulates the Stealth Properties of Polyphosphoester-Coated Nanocarriers, J. Simon, T. Wolf, K. Klein, K. Landfester, F.R. Wurm, V. Mailänder, Angew. Chemie - Int. Ed. 57 (2018) 5548–5553 [127]. *Copyright © 2018, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

Figure 9: a) Schematic representation of PPE amphiphiles adsorbed on PS and PMMA NPs, that possess stealth and targeting properties; b) Cellular uptake toward dendritic cells (blue) or monocytes (red) quantified by flow cytometry after the exposure of human blood plasma to PMMA NPs for 2 hours. Values are expressed as mean ± SD from triplicates. Adapted from: Noncovalent Targeting of Nanocarriers to Immune Cells with Polyphosphoester-Based Surfactants in Human Blood Plasma, J. Simon, K.N. Bauer, J. Langhanki, T. Opatz, V. Mailänder, K. Landfester, F.R. Wurm, Adv. Sci. 6 (2019) [128]. *Copyright © 2019. The authors published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.*

Fig.10: Graphical illustrations of the conjugate BSA-PEEP. Adapted with permission from: Reversible Bioconjugation: Biodegradable Poly(phosphate)-Protein Conjugates, T. Steinbach, G. Becker, A. Spiegel, T. Figueiredo, D. Russo, F.R. Wurm, Macromol. Biosci. (2017) [43]. Copyright © 2016. The authors published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. The polymer arrangement has illustrative purposes and it doesn't represent an actual configuration.

Figure 11: a): Far UV-CD spectra of native BSA and BSA-PEEP conjugates at room temperature, showing retention of the protein secondary structure after the bioconjugation process; b) Emission fluorescence spectra of native BSA and BSA-PEEP conjugates at room temperature, showing the dependence on the protein tertiary structure from the polymer grafting. . Adapted with permission from: Reversible Bioconjugation: Biodegradable Poly(phosphate)-Protein Conjugates, T. Steinbach, G. Becker, A. Spiegel, T. Figueiredo, D. Russo, F.R. Wurm, Macromol. Biosci. (2017) [43]. Copyright © 2016. The authors published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Figure 12: TEM images of Pseudomonas Aeruginosa treated with antimicrobial agents. 20k magnification, sale bar 0.5 µm. From left to right: no treatment; treatment with 4 µg/mL of silver acetate; treatment with 2 µg/mL of minocycline; treatment with 4 µg/mL of silver acetate + 2 µg/mL of minocycline. Adapted with permission from: Minocycline and Silver Dual-Loaded Polyphosphoester-Based Nanoparticles for Treatment of Resistant Pseudomonas aeruginosa, Q. Chen, K.N. Shah, F. Zhang, A.J. Salazar, P.N. Shah, R. Li, J.C.

Sacchettini, K.L. Wooley, C.L. Cannon, Mol. Pharm. 16 (2019) 1606–1619 [78]. *Copyright © 2019, American Chemical Society.*

Figure 13: a) Chemical structure of pH/reduction dual-responsive polymeric prodrug synthesized by Dong et al. b) Fluorescence images of HeLa cells incubated with the prodrug, for different incubation times, stained by DOX The (scale bars: 50 µm). Adapted with permission from: Multifunctional Polymeric Prodrug with Simultaneous Conjugating Camptothecin and Doxorubicin for pH/Reduction Dual-Responsive Drug Delivery, S. Dong, Y. Sun, J. Liu, L. Li, J. He, M. Zhang, P. Ni, ACS Appl. Mater. Interfaces. 11 (2019) 8740–8748 [48]. *Copyright © 2019, American Chemical Society.*

Figure 14: PPE-based branched copolymers used for biomedical applications. a) Hyperbranched phosphate functionalized with spiropyran molecules. Adapted from Reversibly light-responsive micelles constructed via a simple modification of hyperbranched polymers with chromophores, C.J. Chen, Q. Jin, G.Y. Liu, D.D. Li, J.L. Wang, J. Ji, Polymer (Guildf). 53 (2012), 3695–3703 [108]. Copyright © 2012, with permission from Elsevier; b) Amphiphilic hyperbranched PPEs with phosphate bond as the branching point. Adapted with permission from: Enzyme Degradable Hyperbranched Polyphosphoester Micellar Nanomedicines for NIR imaging-guided Chemo-Photothermal Therapy of Drug-Resistant Cancers, M. Yao, Y. Ma, H. Liu, M.I. Khan, S. Shen, S. Li, Y. Zhao, Y. Liu, G. Zhang, X. Li, F. Zhong, W. Jiang, Y. Wang Biomacromolecules. 19 (2018) 1130–1141 [97]. Copyright © 2018, American Chemical Society; c) Multi-arm block co-polymer, namely a poly(amido amine) core with arms formed by block copolymers conjugated with the folic acid. Adapted with permission from: Supramolecular micellar drug delivery system based on multi-arm block copolymer for highly effective encapsulation and sustained-release chemotherapy, L. Zhang, D. Shi, C. Shi, T. Kaneko, M. Chen, J. Mater. Chem. B. 7 (2019) 5677–5687 [47]. Copyright © 2019, with permission from Royal Society of Chemistry.

Figure 15: Chemical structure of the tri-block co-polymer mPEG-PCL-PPEEA, representative illustration of micelleplex formation and the subsequent loading of paclitaxel and siRNA. Adapted with permission from: Simultaneous delivery of siRNA and paclitaxel via a "two-in-one" micelleplex promotes synergistic tumour suppression, T.M. Sun, J.Z. Du, Y.D. Yao, C.Q. Mao, S. Dou, S.Y. Huang, P.Z. Zhang, K.W. Leong, E.W. Song, J. Wang, ACS Nano. 5 (2011) 1483–1494 [163]. *Copyright © 2011, American Chemical Society.*

Figure 16: Schematic illustration of the hydrogel network proposed by Wang et al., with a focus on the interaction formed between the nucleobases. Adapted with permission from: DNA-Inspired Adhesive Hydrogels Based on the Biodegradable Polyphosphoesters Tackified by a Nucleobase, W. Wang, S. Liu, B. Chen, X. Yan, S. Li, X. Ma, X. Yu, Biomacromolecules 20 (2019), 3672–3683 [169]. *Copyright © 2019, American Chemical Society.*

Figure 17: Composition and synthetic route for the formation of polyphosphate copolymers, able to cross-link in a second moment through UV irradiation, obtaining an hydrogel network. Adapted with permission from: Design of Degradable Polyphosphoester Networks with Tailor-Made Stiffness and Hydrophilicity as Scaffolds for Tissue Engineering, R. Riva, U. Shah, J. Thomassin, Z. Yilmaz, A. Lecat, A. Colige, C. Jerome, Biomacromolecules 21 (2020) 349–355 [173]*. Copyright © 2020, American Chemical Society.*

Tables.

Table 1 and 2: Comparison between PEG and promising potential alternatives: water-soluble PPEs, HMPA, POxs, and PG.

Table 3: Monomers that homopolymerize with ROP mechanism to form water-soluble main-chain PPEs.

Table 4: Main-chain degradation by hydrolysis of main-chain water-soluble PPEs, calculated by ^{31}P NMR or GPC.

Table 5: Cytocompatibility of PPE-containing (co)polymers.

Table 6: Activity of PPEylated protein-polymer conjugates and their PEGylated analogues.