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Poly(organo)phosphazenes: recent progress in the synthesis and applications in tissue engineering and drug delivery

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It is a highly desirable goal of researchers to develop effective biomaterials with minimum recovery time and affordable treatment expense for tissue engineering and drug delivery. In this scenario, numerous synthetic and natural polymers have been used. Among those synthetic polymers, polyorganophosphazenes (POPs) have got much attention as highly promising candidates for applications in tissue engineering and drug delivery. Polyorganophosphazenes are hybrid polymers containing inorganic backbone consisting of alternating nitrogen and phosphorus atoms with two organic side groups. POPs possess a wide range of unique properties, *i.e.*, synthetic flexibility, biocompatibility, osteocompatibility, osteoinductivity, sustainability and degradability into harmless end products with predictable degradation rate and adjustable mechanical strength. Moreover, their tunable hydrophilic/hydrophobic and stimuli responsive properties add extra points to their use in biomedical applications. In addition, their various polymeric forms, *i.e.*, microspheres, nano/microfibres, micelles, membranes, polymersomes, hydrogels and nano-conjugate linear polymers provide different carriers to efficiently deliver various hydrophilic/hydrophobic therapeutic agents both *in vitro* and *in vivo*. This review focuses on the most recent progress that has been made in the synthesis and applications of POPs in tissue engineering and their different polymeric forms used for drug delivery. Moreover, we have also summarized the effect of different side groups on the overall efficiency of POPs.
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1. Introduction

Biomaterials (*e.g.*, polymers, metals or ceramics) have characteristic features to provide proper combination of physical, chemical, biological and mechanical properties for various biomedical applications, such as orthopaedics,[1] implants,[2] controlled drug delivery,[3] medicine,[4] biosensors,[5] carriers of cells,[6] immobilized enzymes,[7] bio-adhesives,[8] dental materials,[9] surface modifiers,[10] components of diagnostic assays[11] and tissue engineering.[12] During the last twenty years, various biomaterials such as metals, ceramics and polymeric materials have been explored for the applications in tissue engineering and drug delivery.[13] The biodegradable polymers are the most prominent materials, which have attracted enormous interest as instant substrates for tissue regeneration and drug delivery due to facile processability, reasonable price and easy availability with adjustable mechanical, physicochemical, interfacial and biomimetic characteristics.[14] In addition, polymeric biomaterials can easily be shaped in different forms (membranes, films, fibres, gels, sheets, hydrogels, *etc.*) for biomedical applications.[15] Polyorganophosphazenes (POPs) are hybrid polymers containing inorganic backbone of alternating nitrogen and phosphorus atoms with repeating single and double bonds having two organic side groups linked to each phosphorus atom. Their synthetic flexibility, biocompatibility, biodegradability, tunable mechanical and hydrolytic properties make them attractive as biomaterials for applications ranging from artificial heart valves[16] to immunoadjuvants,[17,18] anti-inflammatory agents,[19] antibacterial agents,[20] vaccine delivery,[21,22] gene delivery,[23–25] protein delivery,[10,26] enzyme immobilization[27,28] and so on. Different researchers have synthesized POPs using different substituents as side groups, including amino acid esters,[29] antioxidants,[30] vitamins[31] and antibiotics.[32–36] Nowadays, a lot of progress has been made in the field of tissue engineering and drug delivery. Several reviews and monographs have been published.[37–39] Allcock published a detailed overview about the chemistry and applications of POPs produced up to 2003.[40] Gleria and Jaeger published a comprehensive overview on the applicative aspects of POPs up to 2004.[41] Andrianov presented a detailed monograph covering a broad range of polyphosphazenes for biomedical applications up to 2009.[42] Teasdale and Brüggemann published a monograph providing detailed information on the synthesis and use of phosphazenes for biomedical applications up to 2014.[43] A review of Rothemund and Teasdale is mainly focused on recent development on the synthesis of POPs.[44] However, review literature concerning the recent progress in the synthesis and applications of POP-based biomaterials for tissue engineering and drug delivery is rather scarce. The objective of this review is, first, to provide an overview on the most recent progress that has been made in the synthesis to get POPs with desired properties for intended applications. Second, it is focused on the latest developments in the applications of POPs for tissue engineering and drug delivery. This review is helpful for the researchers to choose appropriate substituents and proper synthetic method to synthesize new POP-based multifunctional biomaterials for desired applications.

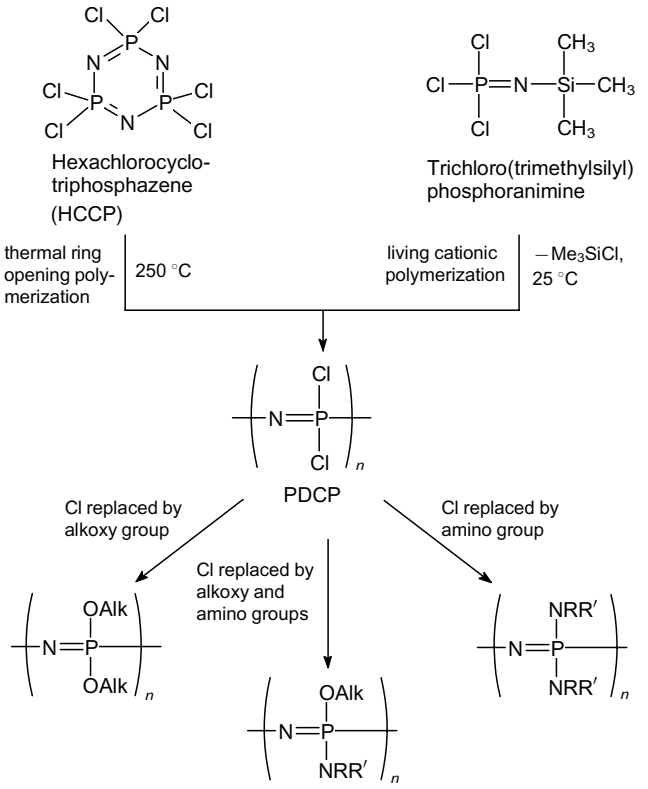
2. Synthesis of polyorganophosphazenes

Many efforts have been made to synthesize a reactive intermediate, polydichlorophosphazene (PDCP),[33] which provides a platform for the synthesis of POPs with wide range of properties. Thermal ring opening and living cationic polymerization are two important methods for the synthesis of PDCP (Scheme 1).[45]

2.1. Thermal ring opening polymerization (TROP)

Generally, POPs are synthesized by thermal ring opening polymerization (TROP) of the cyclic trimer known as hexachlorocyclotriphosphazene (HCCP) to give PDCP with a linear structure, followed by macromolecular nucleophilic substitution reaction with a wide range of organic nucleophiles.[46–49] In 1965, Allcock and Kugel synthesized for the first time linear PDCP from HCCP by TROP.[50] Nowadays, this classical method is fully developed and widely used in both research laboratories and industrial plants.[51] Several mechanistic approaches have been reported to explain the synthesis of PDCP from HCCP including radical, anionic and cationic polymerizations.[52] Based on the available evidences, a few mechanisms may be operative depending on the nature of catalyst and other reaction conditions.[52] A cationic polymerization mechanism is widely accepted.[53,54] The mechanism of cationic ring opening polymerization is shown in Scheme 2. At high temperature (250 °C), HCCP is converted to PDCP through several steps. The first step is heterolytic cleavage of the P–Cl bond of HCCP by $AlCl_3$ to form a cationic reactive intermediate (phosphazenium species) (step 1). This reactive intermediate initiates the chain by reacting with an

Scheme 1



unreacted HCCP molecule (step 2). This process is repeated and chain propagation occurs, resulting in the formation of linear polymer, *i.e.*, PDCP (step 3). Finally, chain termination is induced by lowering the reaction temperature when the contents of polymerization tube has ceased to flow (step 4). Traces of impurities and branching have also been reported to terminative the chain growth.[51] For successful formation of linear PDCP, careful control of the temperature and time is very important. The above mechanism also explains the branching and cross-linking commonly observed during the TROP of HCCP. Low temperature leads to slow polymerization, while a temperature above 250 °C leads to the formation of branched (step 5) and ultimately cross-linked rubbery material (step 6) (see Scheme 2).[55] The PDCP produced by this method is often a high-molecular-weight polymer ($\sim 10^6$), but with broad polydispersity (PDI) (~ 10).[56] Moreover, to get control over the molecular weight and PDI, various catalysts like BCl_3, PPh_3, $PhCO_3H$ and $R_3Si(CHB_{11}X_{11})$ have been used.[57–59] Recently, a new solution method of ring opening polymerization (ROP) using silylium ion ($SiEt_3^+$) as a catalyst showed complete conversion of HCCP to PDCP with a molecular weight (M_n) of 1.12×10^5 g mol^{-1} and PDI of 1.83 under optimized conditions even at room temperature.[60] The above-mentioned cationic mechanism is also supported by a recent study of the TROP of HCCP at ambient temperature catalyzed by $[Et_3Si(N_3P_3Cl_6)][CHB_{11}H_5Br_6]$.[61] The drawbacks associated with this method are uncontrolled molecular weight polymers with broad PDI and low yield. In this method, if polymerization is not terminated in appropriate time, then cross-linking takes place and PDCP is formed as a rubber like material which has no further use.[62]

The formation of PDCP in the absence of catalysts *via* TROP follows second-order kinetics in the temperature range from 240 to 320 °C and the activation energy is in the range of 42 to 57 kcal mol^{-1}.[63] In the presence of benzoic acid as a catalyst, TROP followed first-order kinetics and the activation energy observed was 24.3 kcal mol^{-1}. The straight lines in Fig. 1 *a*, *b* show the reasonably second- and first-order kinetics for the uncatalyzed and catalyzed polymerization. The polymerization rate was increased with increasing temperature or catalyst concentration for both catalyzed and uncatalyzed reactions. *i.e.*, within the suitable temperature range (240 to 255 °C), an increase in temperature increased the rate of polymerization. At 255 °C, conversion to the polymer was almost 30% within 6 h, but at 240 °C, the same polymerization proceeded by less than 15% even after 8 h. Figure 1 *c* shows that an increase in catalyst concentration resulted in an increase in the polymerization rate, while percentage polymerization as a function of time decreased. The relative effect of two different catalysts, *i.e.*, benzoic acid and sodium benzoate, on polymerization kinetics at 235 °C was found to be similar.[63]

The ROP for conversion of HCCP to PDCP carried out at ambient temperature with $[Et_3Si(N_3P_3Cl_6)][CHB_{11}H_5Br_6]$ as the precatalyst showed that increase in the catalyst

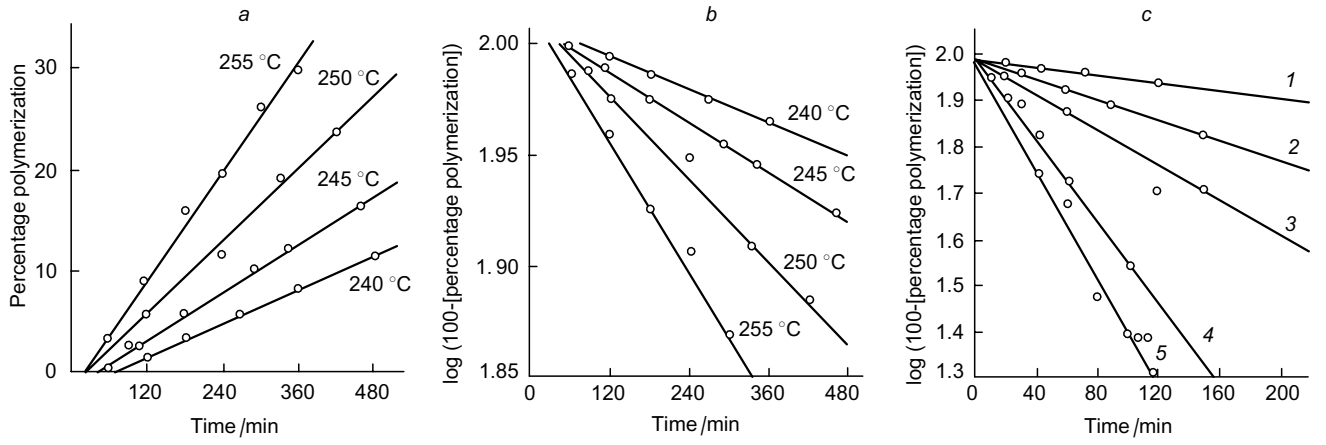
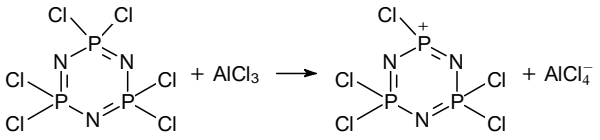


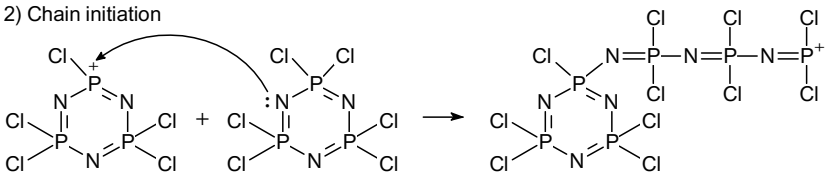
Figure 1. Kinetic studies of TROP; percentage polymerization of uncatalyzed reaction (*a*), catalyzed reaction (*b*) and effect of the catalyst concentration (*c*): (*1*) 10.1, (*2*) 15.4, (*3*) 20.2, (*4*) 30.0 and (*5*) 35.1 mg g^{-1} at 250 °C.[63]

Scheme 2

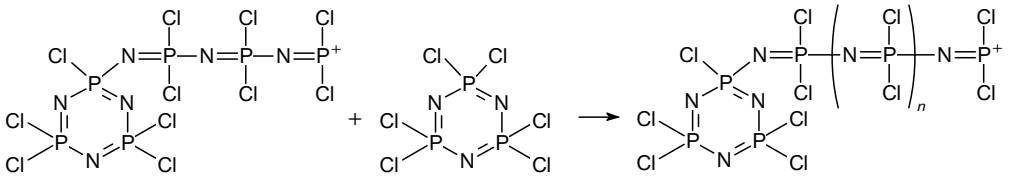
1) Initiator formation



2) Chain initiation

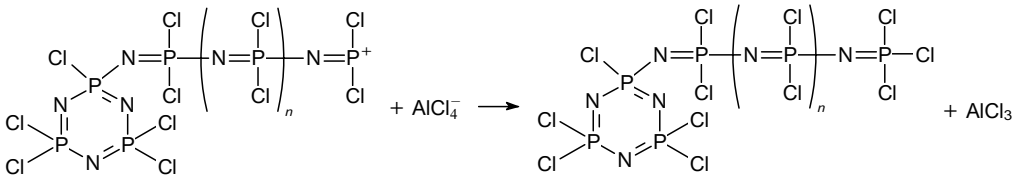


3) Propagation

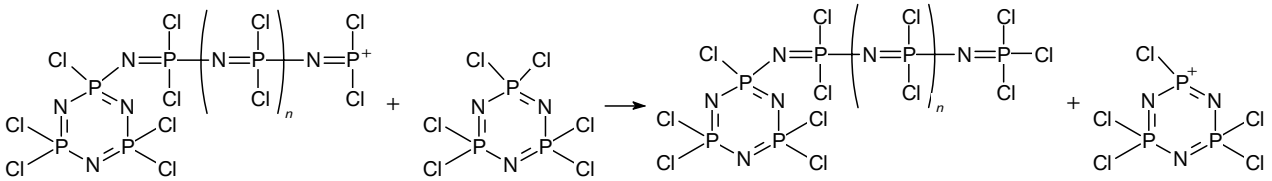


4) Chain termination

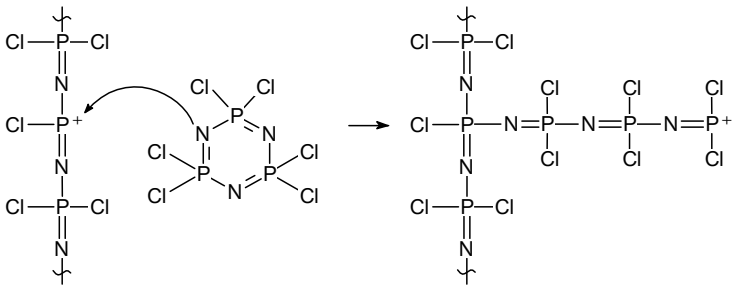
Termination by catalyst



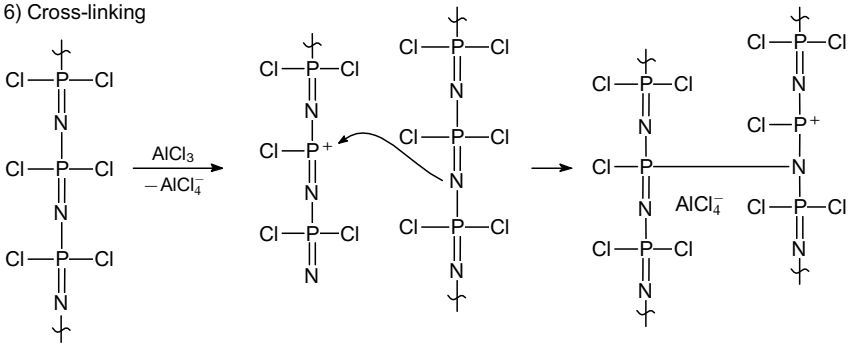
Termination by monomer



5) Branching



6) Cross-linking

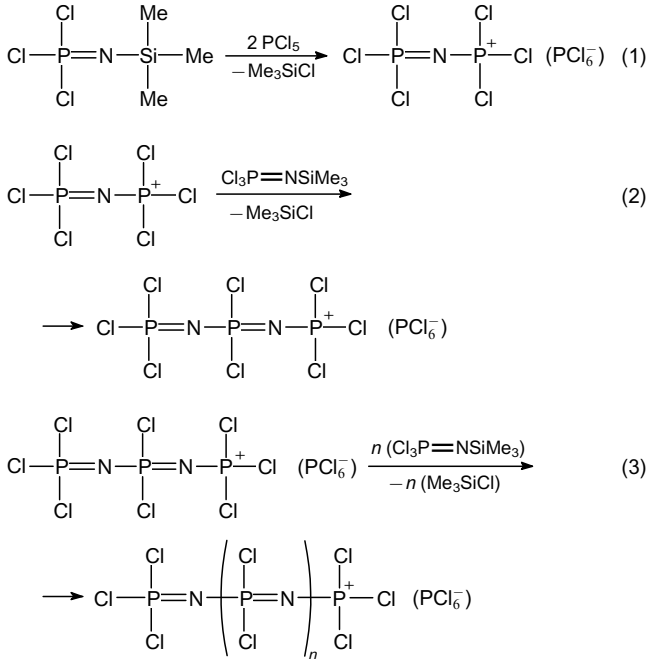


amount increased the polymerization rate.[61] Apart from experimental investigations, computational/theoretical studies have also been carried out to determine the kinetics of TROP of HCCP to PDCP. First-principles simulations (using the Car-Parrinello molecular dynamics approach) results are in agreement with experiments, clarifying the formation of phosphazenium cation *via* heterolytic cleavage of the P – Cl bond in HCCP and its activation energy.[64]

2.2. Living cationic polymerization

Modification and functionalities are possible by using living cationic polymerization in bulk or solution to obtain POPs with a desired molecular weight and narrow PDI with a controlled pattern of substitutions of the side groups.[65, 66] Often, PDCP is prepared by living cationic polymerization of trichloro(trimethylsilyl)phosphoranimine ($Cl_3PNSiMe_3$) in solution at room temperature; $Cl_3PNSiMe_3$ reacts with a twofold molar amount of PCl_5, which provides both a cationic species and a counter-anion to give $[Cl_3PNPCl_3]^+$ and $[PCl_6]^-$, which initiates polymerization (step 1). The subsequent addition of $Cl_3PNSiMe_3$ results in polymer chain growth with a 'living' cationic end group,[67] with one equivalent of $ClSiMe_3$ being formed as a side product upon the addition of every monomer molecule to the chain (steps 2 and 3). The general mechanism of PDCP synthesis *via* living cationic polymerization is shown in Scheme 3.[68] The living cationic polymerization provides a significant control over molecular weight for short polymer chain lengths up to approximately 50 repeating units and a narrow PDI, while in the case of longer polymer chain length, there is less control over molecular weight and a slightly broader PDI as compared to short chains.[69] Recently, POPs of controlled molecular weight (M_n) of 2.5×10^4 Da and PDI of 1.13 have been prepared by varying the initiator (H_2O) to monomer ratios at 125 °C.[70] It has been reported that a delay in halogen replacement in PDCP results in increased molecular weights due to macrocondensation of two PDCP chains at room temperature.[66] Recent investigations showed that the counter-anion produced during PCl_5-initiated living cationic polymerization has significant effect on the chain growth. The Cl^- anion can initiate polymerization even in the absence of active cationic initiator ($[Cl_3P{=}N{=}PCl_3]^+$). However, the active

Scheme 3



$[Cl_3P{=}N{=}PCl_3]^+$ cation considerably increases the reaction rate as well as allows the control over the molecular weight and PDI. According to GPC analysis, M_n of the polymer was 6.4×10^4 g mol^{-1} and the PDI was 1.36.[67]

The kinetics of living cation polymerization have also been investigated in detail. It was found that polymerization followed a linear relationship between $\ln[M]/[M_0]$ and time, which suggested that this polymerization follows first-order kinetics. The rate constants obtained from the reported linear regressions for living cationic polymerization are in agreement with the Arrhenius equation. The activation energy was found to be 56.6 kcal mol^{-1}.[66, 71] The effect of temperature was investigated in the range of 15 to 35 °C and confirmed by NMR spectroscopy (^{1}H and ^{31}P) in dichloromethane (20 : 1 ratio of monomer to the initiator). Within the given range, the temperature followed an inverse relationship with the reaction time. At 35 °C, the polymerization was completed within 35 min, while at 15 °C, the

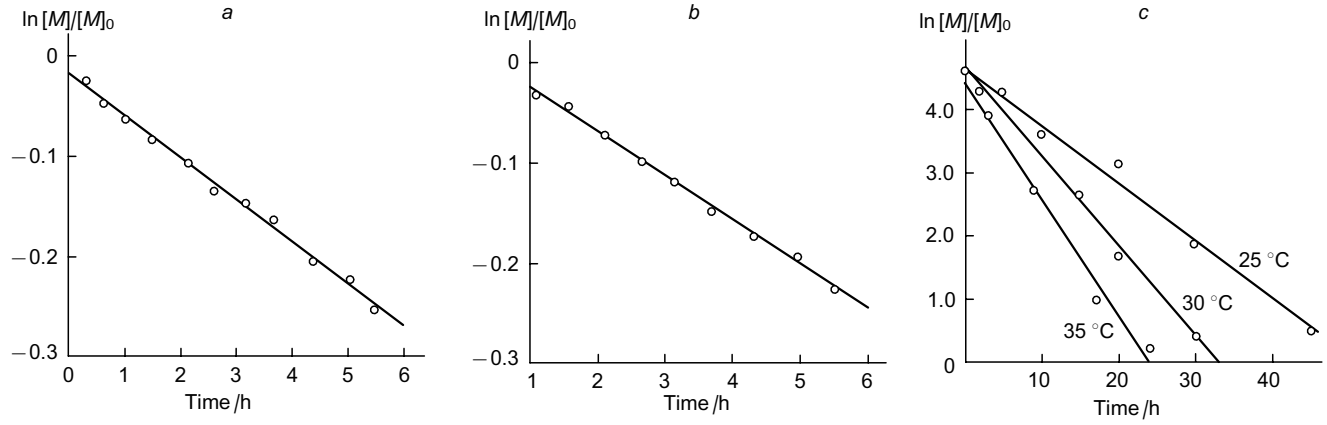


Figure 2. Kinetics of living cationic polymerization using PCl_5 as the initiator confirmed by ^{1}H NMR: $Cl_3PNSiMe_3 : PCl_5$ of 93 : 1 (*a*), $Cl_3PNSiMe_3 : PCl_5$ of 20 : 1 (*b*) and $Cl_3PNSiMe_3 : PCl_5$ (20 : 1) reaction in dichloromethane at various temperatures (*c*). $[M_0]$ is initial monomer concentration, $[M]$ is monomer concentration for time t.[66]

same polymerization reaction was completed within 120 min. Lowering the temperature increased the time needed for completion of the polymerization, while as temperature was increased, the polymerization time decreased (Fig. 2).[66] In another set of experiments, similar results were obtained, in particular, a temperature rise caused an increase in the reaction rate (decrease in the reaction completion time) within temperature range from 10 to 50 °C. Furthermore, with increase in temperature, increase in the molecular weight was also observed.[72]

2.3. Nucleophilic substitution reactions in linear PDCP

Due to extremely reactive nature of PDCP, it is either stored under anhydrous conditions or immediately processed *via* nucleophilic substitution reactions to synthesize stable POPs. A widely used route is a macromolecular nucleophilic substitution reaction to synthesize POPs *via* precursor PDCP. After successful synthesis of linear PDCP, the second step is the replacement of reactive chlorine atoms of PDCP with various nucleophiles *via* macromolecular nucleophilic substitution reactions (Scheme 4).[37] The extent of replacement of chlorine atoms of PDCP by nucleophiles is much more dependent on temperature, reaction time, chemical nature of the nucleophiles and polarity of the reaction medium.[73]

Single substituent-POPs are synthesized by the addition of a single substituent to the reaction mixture and simultaneous replacement of all chlorine atoms of PDCP, while in the case of synthesis of co-substituted POPs, simple stepwise addition of substituents is carried out. The less reactive group is added first and the more reactive group is added subsequently for complete substitution.[74] Moreover, sequential substitution of two or more substituents is carried out, *i.e.*, the bulky group is allowed to react first followed by reaction of the smaller reactive group.[37,47]

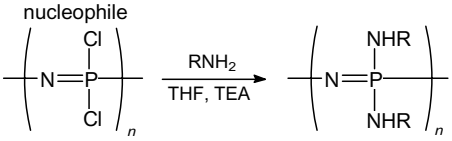
Two strategies are used to overcome the restrictions caused by steric hindrance of bulky groups in complete replacement of chlorine atoms of PDCP: the use of a spacer (trimethylene) to minimize the steric hindrance of bulky groups at the reaction site or the use of two or more nucleophiles as substituents, one bulky and one smaller nucleophile being added in a sequential manner, *i.e.*, the initial substitution of chlorine atoms by bulky groups is followed by replacement of the remaining chlorine atoms by smaller side groups.[38] In co-substituted POPs, the two active chlorine atoms at backbone phosphorus atom of each repeating unit are replaced by substituents, which are conventionally enclosed in brackets with subscripts *x*, *y* and so on to represent the average molar ratio of the substituents attached to the POP backbone. We often get three different types of POPs in terms of the linkages between the backbone phosphorus and substituents, namely, (*a*) P–O linkages, (*b*) P–N linkages and (*c*) both P–O and P–N linkages at the same time (see Scheme 1). Living cationic polymerization is also used to get POPs with one more, the fourth type of linkages, namely, P–C linkages between the backbone and side groups, which are of special interest because they can be modified by reactions with organometallic reagents without significant cleavage of the backbone.[65,75]

Macromolecular nucleophilic substitution reactions are advantageous for the synthesis of new series of bioactive POPs by replacing all the chlorine atoms simultaneously by either one type of substituent or partly by one substituent and then completely by another substituent taken in a desired molar ratio *via* sequential addition of multiple substituents. Harry Allcock reported the first synthesis of hydrolytically stable, soluble and high-molecular-weight POPs using macromolecular nucleophilic substitution reactions to replace chlorine atoms of PDCP with different organic substituents, *i.e.*, alkoxides or aryl oxides,[76] primary or secondary amines[77] and organometallic reagents.[78] Nowadays, different kinds of nucleophiles such as organic, inorganic or organometallic species have been used.[79–84]

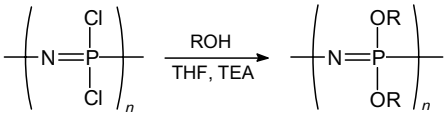
For therapeutic delivery, polymers of the desired molecular weight are required to ensure a specific circulation time in the blood. High molecular weight polymers are significantly important for the enhanced pharmacokinetics.[85] Non-biodegradable and high molecular weight polymers above renal clearance limit (30 ~ 50 kDa) accumulate in the body.[85,86] Meanwhile, non-biodegradable low molecular weight polymers (below the renal clearance limit) induce lysosomal disorders.[78] Hence, polymers used for drug delivery and tissue engineering must be degraded to sufficiently small molecules, instead of being degraded to oligomeric parts just below the renal clearance limit.[85] The effect of molecular weight and PDI on biocompatibility and cytotoxicity of POP-based materials has been studied using a biodegradable POP, poly(2-dimethylamino ethylamino)-phosphazene[24] employed for *in vivo* gene delivery to tumour

Scheme 4

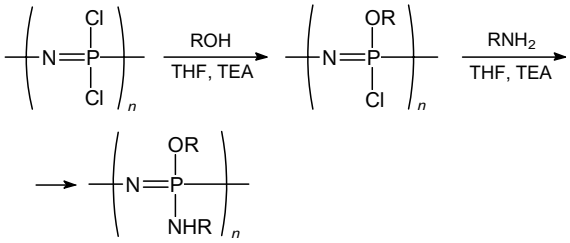
1) Simultaneous replacement of chlorine atoms by one type of nucleophile



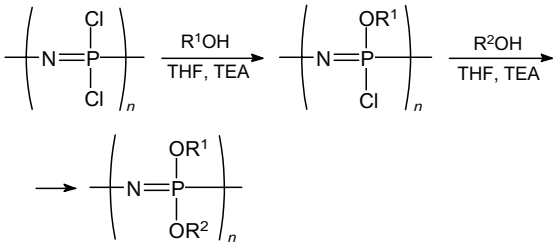
2) Simultaneous replacement of chlorine atoms by one type of nucleophile



3) Stepwise/sequential substitution of chlorine atoms by different nucleophiles



4) Stepwise/sequential replacement of chlorine atoms by different nucleophiles



sites.[87] This POP was found to be tumour selective with lower transfection efficiency and lower toxicity as compared with the well-known non-biodegradable linear polyethylimine (PEI).[23] POPs with broad molecular weight distributions were separated into four fractions of different molecular weights and relatively narrow PDI *via* size exclusion chromatography. Detailed *in vitro* and *in vivo* tumour cell transfection and induced toxicity studies of these fractions of different molecular weights have indicated that polyplexes based on high molecular weight POP fractions showed significantly high cytotoxicity, whereas low molecular weight POP fractions showed low cytotoxicity.[88] This has demonstrated that many important aspects including tumour cell transfection/toxicity are markedly affected by changing molecular weight and PDI of POPs, which is in agreement with reported findings.[89, 90] The enhanced cytotoxicity of high molecular weight fractions of POP may be attributed to initiation of cell membrane damage.[90, 91]

3. Properties of polyorganophosphazenes

Various synthetic and natural polymers have been used for tissue engineering and drug delivery because of their significant properties. Natural polymers have advantage of biocompatibility and enhanced cells adhesion, but associated with unpredictable enzymatic degradation and poor mechanical properties. For instance, natural polymers, *i.e.*, proteins (collagen) and polysaccharides (chitosan) are biocompatible with low inflammatory response, but their fast biodegradability and poor mechanical properties restrict their use for biomedical applications. Among synthetic polymers, polycaprolactone (PCL), polylactic acid-co-glycolic acid (PLGA) and polymethyl methacrylate (PMMA) are the most commonly used biomaterials. But PCL and PLGA have drawbacks of bulk degradation and acidic end products, respectively, while PMMA is not biodegradable. Moreover, the activity profile of the growth factors is significantly changed by these acidic degradation products [92, 93] as well as inflammation or fibrous encapsulation are induced.[94, 95] Most of the organic polymers are sensitive to light and fire and have low oxidation stability. On the other hand, inorganic polymers show high stability to light and fire and oxidation resistance, along with high solvent resistance, but their low processability and brittleness limit their applications.[40] Polyorganophosphazenes provide the best opportunity for these purposes, having hybrid nature, *i.e.*, the inorganic backbone composed of alternating phosphorus and nitrogen atoms with repeated double and single bonds, bearing two organic side groups attached to each phosphorus atom.[96] The selection of appropriate side groups is very important to tune their physical, chemical and biological properties for tissue engineering applications and drug delivery.[29, 97 – 100] Moreover, the availability of wide range of side groups and their easy attachment to the backbone make POPs even more attractive. Amino acid based-POPs are considered as excellent materials for tissue engineering and drug delivery due to their biodegradability, biocompatibility, porous scaffolds with enhanced cell adhesion and non-toxic end products.[101 – 103] Moreover, functionalization of side groups *via* indirect attachment of macromolecules to the POP backbone provides additional opportunity to obtain block copolymers.[104] Amino acid-based substituted POPs form composites with calcium hydroxyapatite, which can be effectively used for regeneration of cardiac muscle, cartilage, nerve conduits and retina, for vascular tissue engineering, for treating tympanic membrane perforations, for targeted drug delivery and as tissue adhesive materials.[105] Similarly, blends of POPs with PLGA showed enhanced properties of bone formation [106] with an opportunity to control the degradation rate and to avoid the limitations associated with acidic end product of PLGA.[107, 108] Hence, to get the combination of properties, POPs-based blend systems have been widely used for different applications.[109 – 113] There are different ways to modify the properties of POPs, *i.e.*, changing side groups, either the same (homopolymer) or different side groups (copolymers), changing the ratios of the different side groups (copolymers) and functionality of the side groups (copolymers) as well as formation of their blends and composites.[114, 115] The data on some important side groups and their respective properties of POPs with these groups are summarized in Table 1.[31, 34, 40, 102, 116]

To consider the suitability of POPs for tissue engineering and drug delivery, their properties must be well-matched with body environment [101, 117] and with natural tissues.[96] Some significant properties of POPs related to their applications in tissue engineering and drug delivery are discussed below.

Table 1. Various side groups and their effect on polyorganophosphazene properties.[31, 34, 40, 102, 116]

Side groups used	Required properties of POPs
OCH_2CF_3, OC_6H_5, OC_6H_4R	For fibres and films
OCH_3, OC_2H_5, $OCH_2CH_2OCH_3$, $OC_6H_5OCH_3$	For elastomers
$NHCH_3$, OCH_3, OC_6H_4COONa, $OC_6H_4SO_3Na$	For solubility in water and hydrogel
OC_6H_4COOH, $OC_6H_4SO_3H$	For microsphere and micelle formation
OCH_2CF_3, $OCH_2CF_2CF_2H$, $CH_2Si(CH_3)_3$	For bio-stability
Glucosyl, Glyceryl, OC_2H_5	For bio-erosion
OCH_2CF_3, OC_6H_5, $CH_2Si(CH_3)_3$	For surface hydrophobicity
$OCH_2CH_2OCH_2CH_2OCH_3$, OC_6H_4COOH, $OC_6H_4SO_3Na$	For surface hydrophobicity
$OCH_2CH_2OCH_2CH_2OCH_3$, $OC_6H_4SO_3Na$, OC_6H_5, $OC_6H_4SO_3H$	For solid ionic conductivity
$OC_6H_4NH_2$, OC_6H_4COOH, $OCH_2CH_2OCH_2CH_2NH_2$,	For coupling to other molecules
$NHCH_2CHCH_2$, $C_{10}H_9O_4$, citronellol	For cross-linking
OC_6H_5, $OC_6H_5OCH_3$, $OC_6H_4C_6H_5$	For mechanical strength
Amino acid ester	For osteoinductivity and osteoconductivity
Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$	For Ca deposition and biocompatibility
NHC_6H_4, $NHC_6H_4NHC_6H_4NHC_6H_4$, NHC_6H_5	For conductance
Citronellol	For anti-inflammatory activity

3.1. Biocompatibility

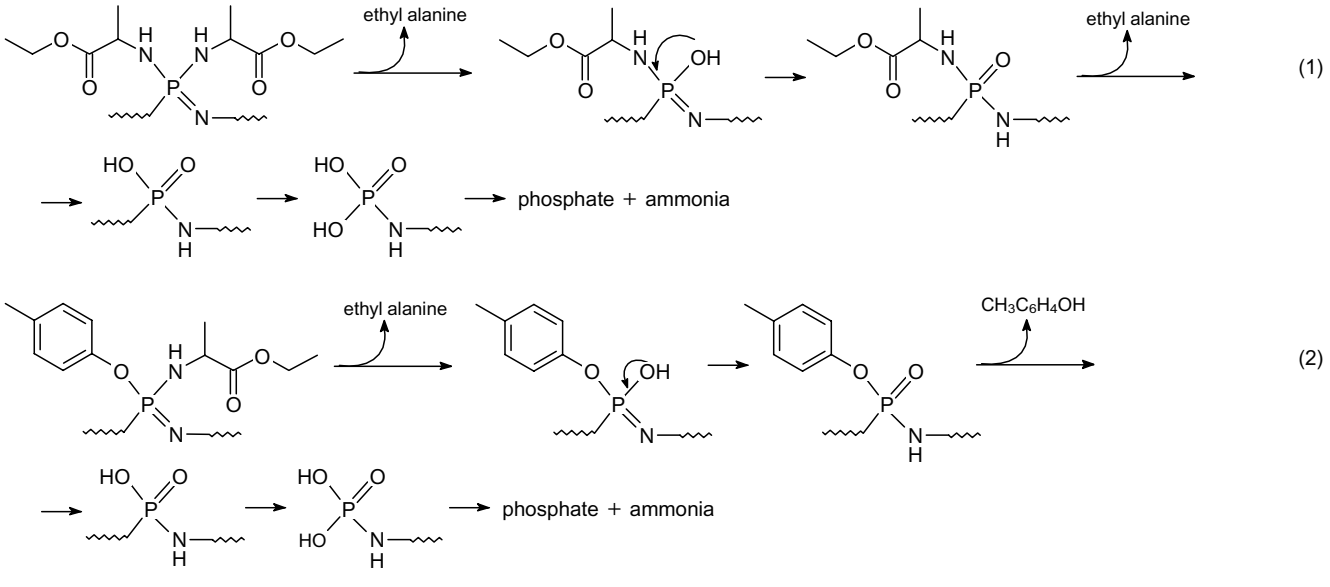
Many biomedical polymers have been synthesized to investigate their specific properties that are essential for their use as biomaterials in tissue engineering and drug delivery. These polymers should be biocompatible (non-toxic, non-inflammatory, non-carcinogenic, non-pyrogenic and non-allergic response), which is an important criterion for tissue engineering and drug delivery. The cellular functions, which are essential for tissue generation, are affected by biocompatibility of the polymeric scaffolds. According to David Williams, 'the biocompatibility of scaffold or matrix for tissue engineering products means capability to achieve a substrate that facilitates appropriate cellular activity together with the acceleration of molecular and mechanical signal systems'.[118] Amino acid ester-substituted POPs show biocompatibility and biodegradability and have been established as appropriate biomaterials for tissue engineering and drug delivery.[119] The *in vivo* biodegradability studies of amino acid ester-based POPs, namly poly[(ethyl alanato)(ethyl oxybenzoate)phosphazene] (PNEAEOB) and poly[(ethyl alanato)(propyl oxybenzoate)phosphazene] (PNEAPOB) showed potential for various biomedical applications including the development of reinforcing agents for hard tissue engineering.[106] Poly[bis(ethyl 4-aminobutyro)phosphazene] was found to have enhanced proliferation and biocompatibility.[120]

3.2. Degradability

Polyorganophosphazenes for tissue engineering and therapeutic delivery should be either biostable or biodegradable into non-toxic end products. Bioerodible non-toxic biomaterials are usually preferred, as *via* degradation, these materials limit the long term risk of immune response and possible adverse effect on natural tissues.[121 – 123] Important characteristics of POPs for various applications are controlled over their degradation, which is crucial for biomedical purposes. When degradation occurs, the degraded products and degradation kinetics affect the physical and chemical properties of these POPs, which, in turn, affects their efficiency for specific application. For therapeutic delivery purposes, degradation rate of POPs ultimately affects the release pattern of therapeutic agents, *i.e.*, rapid degradation results in burst release of therapeutics, while slow degradation causes slow release. The nature, composition and ratio of side groups determine the degradation pattern of the POPs. It is important to determine the effect of POPs on cellular properties and cell biocompatibility prior to their use as scaffold biomaterials in tissue engineering.[124, 125] Biodegradability and the rate of degradation are important factors, which margin their potential applications. Moreover, it is important that the degradation rate of the scaffold material should be similar to the rate of tissue growth or desired therapeutic release rate. For tissue engineering, if the scaffold material degrades too quickly, there will be insufficient support for the growing tissue, which results in mechanical weakness. If the material degrades too slowly or not at all, it must be surgically removed which in turn damage the new tissue.[126, 127]

On hydrolytic decomposition, amino acid ester-based POPs give the corresponding amino acids, the alcohol of the ester, ammonia and phosphates, which act as a buffer system and control variation in pH.[128] It is important to know about their degradation mechanism, as this will help to confirm the toxicity profiles of the intermediates or end products. The degradation mechanism of POPs mainly depends on the nature of the attached side groups. Several pathways have been suggested to explain the degradation routes of POPs.[129, 130] All the suggested pathways have common features. In the first step, water attacks the amino acid esters on these polymers. Thus, the backbone of POPs becomes sensitive to hydrolytic degradation. Basically, there are three active sites, which are exposed to the attack of water, resulting in hydrolytic cleavage. One possibility is that the ester linkage breaks and subsequently produces carboxylic acid, which attacks either the same or neighbouring POP chain to cause the phosphorus – nitrogen skeletal degradation. According to the second possibility, water attacks the POP backbone and the ester functionality itself to release an amino acid ester and then eventually cleave to give ammonia and phosphate upon further hydrolysis. In the third option, the amino acid ester units attached to the POP backbone are separated *via* cleavage of the bond with the polymer backbone. Scheme 5 shows the possible mechanism of hydrolysis of POPs substituted by amino acid

Scheme 5



esters (1) [130] and POPs co-substituted with methylphenoxy groups and amino acid esters (2).[129, 130]

This cleavage leaves hydroxyl groups connected to phosphorus in the backbone of the polymer and results in self-hydrolysis of amino acid ester to amino acid. The end products can be confirmed by ninhydrin test and ^{1}H NMR spectroscopy.[129, 130] Usually, the POP backbone is protected *via* shielding against the direct attack of water by linking phenylalanine, valine or tyrosine esters, that is, esters of amino acids having large hydrophobic substituents as pendant groups at the α-position, to the POP backbone.[97, 131] The attachment of hydrophilic side groups, that is, amino acid glycolate, glucosyl, lactate, glyceryl or imidazole makes the POP backbone sensitive toward hydrolytic degradation. On the other hand, attachment of hydrophobic side groups such as aryloxy, fluoroalkoxy and higher alkoxy units endows these polymers with stability to hydrolytic degradation.[132] The variation of the ratio of side groups of POPs having both hydrolytically sensitive and hydrolytically retarding groups provides significant opportunities to modify the degradation rate of POPs in a controlled manner to give non-toxic end products.[40, 45, 133] Moreover, hydrophobic, fluorinated, organosilicon, bulky, aromatic and metal-containing groups make POPs hydrolytically stable, whereas hydrolytically sensitive, small and bioactive side groups make POPs hydrolytically unstable (Table 2).

3.3. Thermal behaviour

Usually, glass transition temperature depends on the size and nature of side groups attached to the backbone of POPs. In the case of bulky side groups, high glass transition temperature was observed. For example, POPs substituted with amino acid esters, such as leucine, valine and phenylalanine ethyl esters, show high glass transition temperatures. The amino acid ester-substituted POPs with smaller side groups have lower glass transition temperature. In particular, POPs with identical side groups represented by ethyl esters of glycine, alanine, leucine, valine and phenylalanine have glass transition temperatures of −40, −10, 15.4, 24.8 and 41.6 °C, respectively, while POPs with ethyl esters of glycine, alanine, leucine, valine and phenylalanine as side groups co-substituted with ferulic acid have glass transition temperatures in the range of 51 – 54 °C. It has

Table 2. Summary of *in vitro* degradation studies of polyorganophosphazenes containing various side groups.

Side group 1	Side group 2	Study duration	Ref.
Glycine methyl ester	Glycine methyl ester	5 – 17 weeks	99, 131, 134
Glycine ethyl ester	Glycine ethyl ester	7 weeks	106
Glycine ethyl ester	Alanine ethyl ester (50%)	7 weeks	106
Glycine ethyl ester	*p*-Methyl phenoxy (25% – 90%)	7 weeks	135
Glycine ethyl ester (25%)	Phenylalanine ethyl ester (70%)	17 weeks	99
Glycine *tert*-butyl ester (25%)	Phenylalanine ethyl ester (70%)	5 weeks	131
Glycine benzyl ester (25%)	Phenylalanine ethyl ester (70%)	5 weeks	131
Glycine methyl ester	Phenylalanine ethyl ester (70%)	5 weeks	131
Alanine ethyl ester	*p*-Methyl phenoxy (50%)	7 weeks	106
Alanine ethyl ester	*p*-Phenyl phenoxy (50%)	7 weeks	106
Alanine benzyl ester	*p*-Phenyl phenoxy (50%)	7 weeks	134
Valine methyl ester	Valine ethyl ester	5 weeks	134
Phenylalanine ethyl ester	Phenylalanine ethyl ester	5 weeks	131
Glycine ethyl ester	Ferulic acid	8 weeks (∼ 25% weight loss)	30
Alanine ethyl ester	Ferulic acid	8 weeks (∼ 15% weight loss)	30
Valine ethyl ester	Ferulic acid	8 weeks (∼ 10% weight loss)	30
Phenylalanine ethyl ester	Ferulic acid	8 weeks (∼ 5% weight loss)	30
Ethoxy	Tri-fluoroethoxy	12 weeks (∼ 10% weight loss)	133
Ethoxy	Phenoxy	12 weeks (∼ 10% weight loss)	133
Ethoxy	*p*-Methyl phenoxy	12 weeks (∼ 10% weight loss)	133
Glycine ethyl ester (66.7%)	Allyl amine (33.3%)	3 weeks (∼ 22% and 6% weight loss) [a]	136
Glycine ethyl ester (33.3%)	Allyl amine (66.7%)	3 weeks (∼ 12% and 6% weight loss) [a]	136
Glycine citronellol ester	Glycine citronellol ester	12 weeks (∼ 28% weight loss)	137
Alanine citronellol ester	Alanine citronellol ester	12 weeks (∼ 25% weight loss)	137
Valine citronellol ester	Valine citronellol ester	12 weeks (22% weight loss)	137
Phenylalanine citronellol ester	Phenylalanine citronellol ester	12 weeks (∼20% weight loss)	137

[a] The weight loss before and after cross-linking.

Table 3. Glass transitions temperature of homo- and co-substituted polyorganophosphazenes.

Side group 1	Side group 2	Glass transition temperature (T_g) /°C	Ref.
Glycine ethyl ester	Glycine ethyl ester	−40	138
Alanine ethyl ester	Alanine ethyl ester	−10	138
Leucine ethyl ester	Leucine ethyl ester	15.4 ± 0.6	138
Valine ethyl ester	Valine ethyl ester	24.8 ± 0.9	138
Phenylalanine ethyl ester	Phenylalanine ethyl ester	41.6 ± 1	138
Glycine ethyl ester	*p*-Methylphenoxy	−40	138
Glycine ethyl ester	*p*-Phenylphenoxy	35.2	138
Glycine ethyl ester	Ferulic acid	52.1	30
Alanine ethyl ester	Ferulic acid	51.0	30
Valine ethyl ester	Ferulic acid	54.0	30
Phenylalanine ethyl ester	Ferulic acid	52.1	30
Glycine citronellol ester	Glycine citronellol ester	−62.7	137
Alanine citronellol ester	Alanine citronellol ester	−58.2	137
Valine citronellol ester	Valine citronellol ester	−23.0	137
Phenylalanine citronellol ester	Phenylalanine citronellol ester	−10.5	137
Glycyl-glycine ethyl ester	Glycyl-glycine ethyl ester	52.86	110

been found that POPs substituted with phenylalanine ethyl ester and co-substituted with amino acid esters and ferulic acid exhibited glass transition temperatures above the physiological temperature. Hence, these compounds can be used as scaffold materials for bone tissue engineering.[138] The detailed effect of various side groups on the thermal properties of POPs is summarized in Table 3.

3.4. Mechanical properties

Biomaterials which need to be implanted inside the body must be able to to tolerate and recover from repetitive changes due to different mechanical environments to allow for material/tissue integration without causing any harmful response to nearby tissues. The mechanical properties of the natural bone are different in different parts of the body with respect to function of inborn bone tissue. For successful implantation of scaffolds for bone tissue engineering, particular consideration must be given to the materials for multi-functions, such as tissue regeneration and drug delivery. The mechanical properties should be suitable and closely resemble those of the natural bone to avoid incompatibility problems.[139–141] A match between the mechanical properties of POPs such as compressive and tensile strength and those of native tissue lead to successful tissue-engineered constructs.[142–144] Sethuraman *et al.* synthesized alanine-based POPs and investigated their mechanical properties for bone tissue engineering.[145] Comparative analysis was carried out with the currently used bone tissue engineering standard, PLGA. The alanine-based polyorganophosphazene $PNEA_{50}PhPh_{50}$ {poly[(50% ethyl alanato)(50% phenyl phenoxy)phosphazene]} showed a higher compressive strength compared to PLGA due to the presence of large aromatic groups. It was found that more bulky groups increase the steric hindrance, which, in turn, results in increase of both tensile strength and elasticity of POPs. Moreover, an increase in the molar proportion of bulky side groups significantly increased the compressive strength of POPs (Fig. 3).[145]

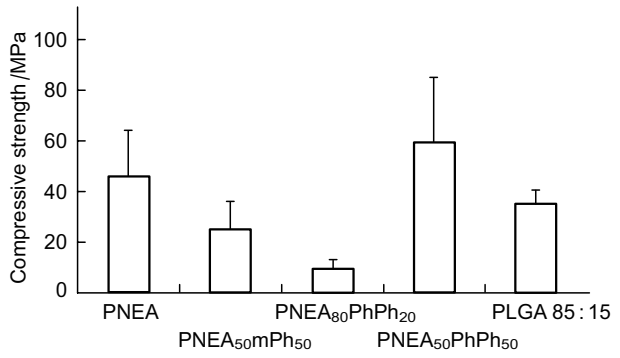


Figure 3. Side group effect on the mechanical properties of polyorganophosphazenes.[145]
$PNEA_{50}mPh_{50}$ is poly[(50% ethyl alanato)(50% methyl phenoxy) phosphazene].

Table 4 shows that the poor mechanical properties of glycine ethyl ester (GlyEE)-substituted POP can be increased up to 13–17-fold *via* introduction of 2-hydroxyethyl methacrylate (HEMA), in order to meet requirements for tissue engineering applications. Also, the mechanical properties of POPs are significantly enhanced *via* blending with HEMA or acrylic acid (AA) as cross-linking agents.[146]

Functionalized POPs are able to act as compatibilizers in melt-compounded polyetherimide (PEI)/thermotropic liquid crystalline polymer (TLCP) blend with enhanced thermal stability and tensile properties. In a study, POPs substituted with different side groups were used as compatibilizers and showed improved tensile strength as shown in Table 5.[35]

Table 4. Mechanical properties of POPs.[146]

Polymer	GlyEE	HEMA	Pendant ethenyl/HEMA/AA	Modulus of elasticity /kPa	Falilure stress /kPa	Failure stress, strain ($\Delta L/L$)
1	2	–	–	17.2 ± 2.6	23.5 ± 2.9	2.15 ± 0.32
2	1.8	0.2	0.2/0.2/0	212.8 ± 13.9	248.0 ± 30.6	3.08 ± 0.27
3	1.6	0.4	0.4/0.2/0	287.5 33.7	294.5 ± 39.6	3.90 ± 0.25
4	1.8	0.2	0.2/0/0.2	191.3 ± 10.9	229.6 ± 17.3	2.97 ± 0.35
5	1.6	0.4	0.4/0/0.2	239.6 ± 27.6	242.5 ± 18.9	3.12 ± 0.2

Table 5. Blend formulation and mechanical properties.[35]

Sample code	PEI (wt.%)	TLCP (wt.%)	POPs 2.5 (wt.%)	Tensile strength /MPa	Elongation at break (%)
A	75	25	Without POP	29.64	12.5
B	75	22.5	Amino-phenol phenol-substituted POP	42.85	15.2
C	75	22.5	Allyl-phenol phenol-substituted POP	33.39	12.2
D	75	22.5	Di(diethyl ether)-substituted POP	45.98	9.1

3.5. Suitable porosity

Polyorganophosphazenes are very significant materials for tissue engineering and drug delivery applications due to their controlled degradability depending on side group nature and composition. The porosity and pore interconnectivity, biocompatibility and mechanical properties of POP scaffolds can be tuned to particular applications. Currently, calcium phosphate hydroxyapatite and bioactive glass as ceramic materials are the most commonly used biomaterials for bone tissue engineering[128, 147] and are considered as significant scaffold materials due to their osteoconductivity. To make them biocompatible for bone regeneration, pore size should be optimized to improve the regeneration efficiency *via* macroporosity.[148, 149] The surfaces with different type porosity are shown in Fig. 4.[148] An ideal scaffold material must possess characteristic properties of biodegradability, macroporosity, mechanical stability and osteoconductivity. A significant property of POP scaffolds to make them suitable for regeneration purposes is the interconnectivity, resembling that of a web, rather than isolated porosity. Hence, in addition to biodegradability and mechanical stability, POP scaffolds have also interconnectivity, which provides a feasible environment and proper platform for the transportation of growth factors/cells for regeneration of new tissues to replace the damaged or injured parts of the body.[150 – 152]

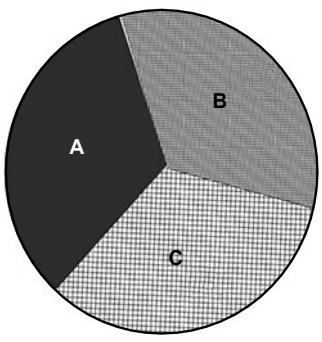


Figure 4. Surfaces with different type of porosity; A is non-porous, B is microporous and C is macroporous.[148]

3.6. Osteocompatibility and osteoinductivity

Osteocompatibility and osteoinductivity of materials play a significant role in regeneration of lost or damaged parts of the body. Bone tissue engineering mainly focuses on the development of biomaterials which have the ability to imitate the natural bone and induce bone formation process. A large number of POP platforms have been developed and established as osteocompatible materials by varying the side groups.[106, 135, 153] To check the osteocompatibility for bone regeneration, the cell adhesion, proliferation and mineralization of primary rat osteoblast (PRO) cells were carried out on the surface of PNEA and $PNEA_{50}mPh_{50}$. In this study, the adhesion, growth of PRO cells and expression of osteoblastic genes showed the osteocompatibility of the alanine-based POPs, namely, PNEA, $PNEA_{50}mPh_{50}$ and $PNEA_{50}PhPh_{50}$ (Fig. 5).[145]

3.7. Stimuli responsive behaviour

Over the past 30 years, stimuli-responsive polymers have been investigated for numerous biomedical applications, especially for drug delivery and tissue engineering. To get desired properties, stimuli-responsive polymers have been combined with a wide range of bioactive molecules, such as proteins and peptides, nucleic acids, small organic molecules and carbohydrates, using a vaiety of methods. These methods include physical mixing, chemical conjugation or complexation. A numbers of POP-based stimuli responsive materials have been synthesized for tissue engineering and drug delivery.[154 – 159] The important stimuli for biomedical applications are temperature, ultrasound, light, pH, ionic strength and enzymes.[160] Hence, the properties of POPs for a particular application can be fine-tuned by careful selec-

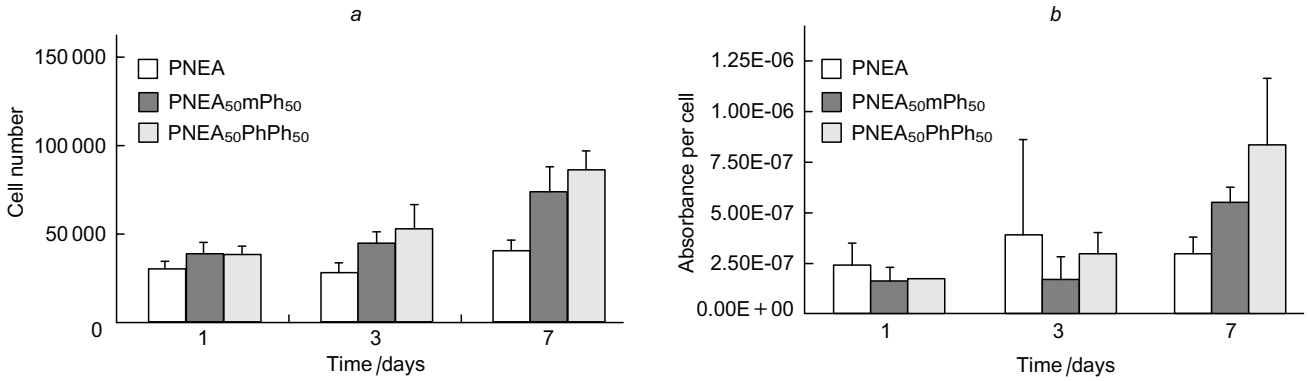


Figure 5. Cell adhesion studies (*a*) and cell proliferation studies (*b*).[145]

tion of suitable side groups or combinations of different groups as well as by varying the the molar ratio of side groups.[40]

4. Applications of polyorganophosphazenes

Polyorganophosphazenes have been investigated for wide range of biomedical applications. Here, we have described the applications of POPs for tissue engineering and drug delivery.

4.1. Tissue engineering

During daily life activities, unwanted situations may happen, resulting in injury or loss of some parts of the body. Various techniques, *i.e.*, allografts, autografts, tissue engineering, *etc.*, have been developed to restore, regenerate the injured or lost tissues or organs. Each technique is associated with particular advantages and disadvantages. Among them, tissue engineering is a multidisciplinary (involving chemistry, biology and engineering) field, newly developed as an alternative approach to restore or replace the damaged or missing tissues or organs of the body using biomaterials, cells and growth factors either alone or in combination. This technique overcomes the limitations associated with traditional treatment techniques.[127] Various biomaterials and especially biodegradable polymers are used as temporary substrates to provide media for tissue regeneration.[127, 161 – 163] Polyorganophosphazenes have beneficial properties for applications in different types of tissue engineering.[6, 12, 164, 165] Here we will describe the important POPs reported for different types of tissue engineering applications.

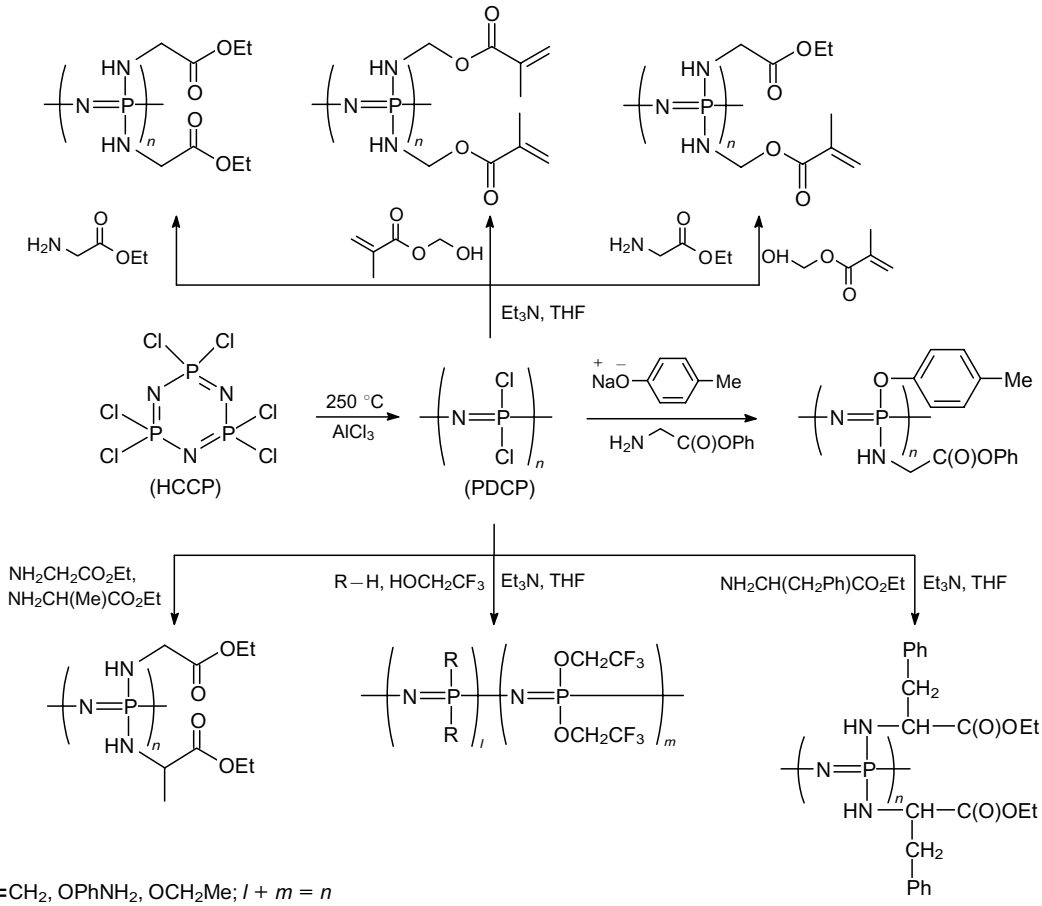
4.1.1. Bone and skeleton tissue engineering

A number of mechanical, biological and chemical properties, *i.e.*, mechanical support, shielding, storage of healing cells and mineral ion homeostasis, are dependent on the bone structure. It is mandatory to know the bone components, their categorized structural phases and their structural relationships with various functions. Usually, a bone consists of mineral, collagen and non-collagenous proteins. Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is the major portion of bone, which is about 70% by weight. Hence, various techniques have been used to increase the binding efficiency of POPs to hydroxyapatite, which will enhance the bone formation process. Most of the research in the field of tissue engineering using POPs as biomaterials has been carried out for bone and skeletal tissue regeneration. Poly[(imidazolyl)(methylphenoxy)phosphazenes] and poly[(ethyl glycinato) (methylphenoxy)phosphazenes] were both found to facilitate the growth of MC3T3-E1 cells.[135] Three-dimensional amino acid-based POPs as scaffold materials stimulate osteoblast adhesion and proliferation, which shows that POPs are suitable candidates for bone tissue engineering.[153] Polyorganophosphazene microspheres and films prepared from POP substituted with phenylalanine ethyl ester showed an improved bone formation process.[166] Poly[(ethyl glycinato)(*p*-methylphenoxy)phosphazene] – hydroxyapatite composite was found to be a promising material for bone tissue engineering as compared with 50 : 50 poly(lactide-co-glycolide) – hydroxyapatite composite.[36] Various techniques have been adapted to overcome the limitations of fast hydrolysis and poor mechanical properties associated with the linear POPs, for example, the formation of blends [110, 111] or composites [167 – 172] or cross-linking.[146]

Surface functionalization plays a key role in cell adhesion and adsorption of biomolecules. Heyde *et al.*[100] have synthesized a novel co-substituted POP by introducing three different side groups to the PDCP backbone for different functions, in particular, amino acid ester, PEG and galactose to fine-tune the degradation, biocompatibility and cell adhesive properties, respectively. Surface characterization of this POP scaffold by various advanced techniques confirmed that the surface is enriched with PEG and galactose. This indicates its enhanced biocompatibility and cell adhesive properties, respectively.[100] Scheme 6 shows synthetic routes to POPs containing various organic substituents.[34 – 36, 138, 146] Leucine, valine and phenylalanine ethyl ester-based POP – nanohydroxyapatite composite microspheres were found to be suitable candidates for bone tissue engineering scaffolds, because of good osteoblast cell attachment, proliferation and alkaline phosphatase expression.[138] Functionalized POP-aided polyetherimide (PEI) – liquid crystalline polymer (LCP) blend showed improved mechanical properties (see Table 5).[35]

Polyorganophosphazenes containing choline chloride co-substituted with glycine ethyl ester, alanine ethyl ester, valine ethyl ester, or phenylalanine ethyl ester have also been synthesized [109] (Scheme 7). The blends of these POPs with PLGA in the weight percent ratios of 50 : 50 and 85 : 15 were found to be hydrolytically and thermally stable. The *in vitro* studies of choline-based POP blends revealed enhanced osteocompatibility as compared to the polyester.[109] Morozowich *et al.*[31] synthesized POPs using vita-

Scheme 6



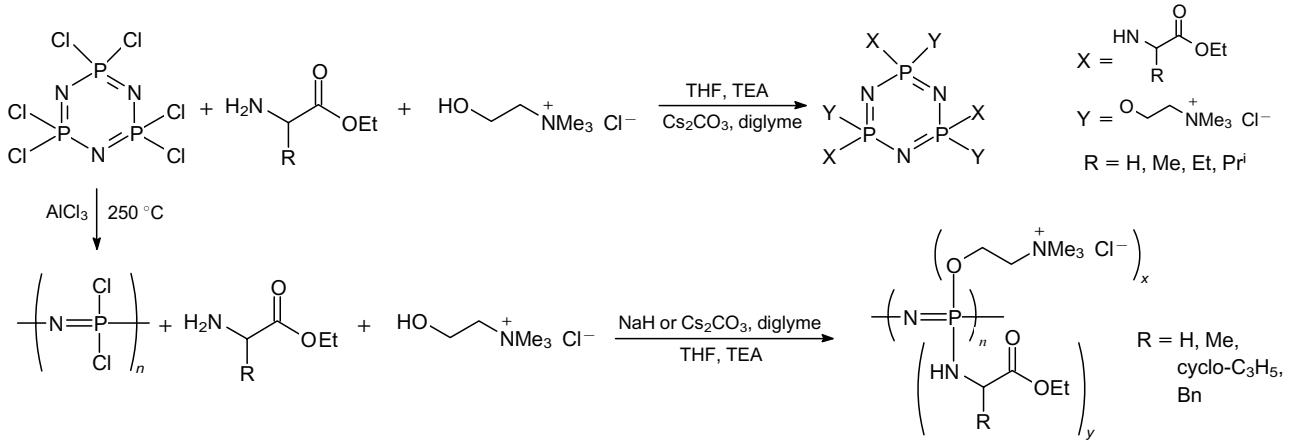
R = OPhCH=CH$_2$, OPhNH$_2$, OCH$_2$Me; $l + m = n$

mins and amino acid esters as side groups to enhance the bone formation and mechanical properties of bone tissues (Scheme 8).[31] The bound biomolecules provided a platform to guide incoming cells, while being released during scaffold degradation, thus providing a significant long-term effect. Another series of hydrophobic and hydrophilic POPs containing ferulic acid ester (antioxidant) co-substituted with various amino acid esters with different lengths of side groups have been synthesized [30, 31, 173] (see Scheme 8).These POPs were specially obtained for hard tissue engineering and were characterized by enhanced mechanical properties and well-regulated hydrolytic degradation due to UV-induced cross-linking of ferulic acid. Moreover, the end products of degradation were formed at about the physiological pH.[30] Further studies of these POPs in a simulated body fluid (SBF) solution showed better mineralization with enhanced bone regeneration process.[173]

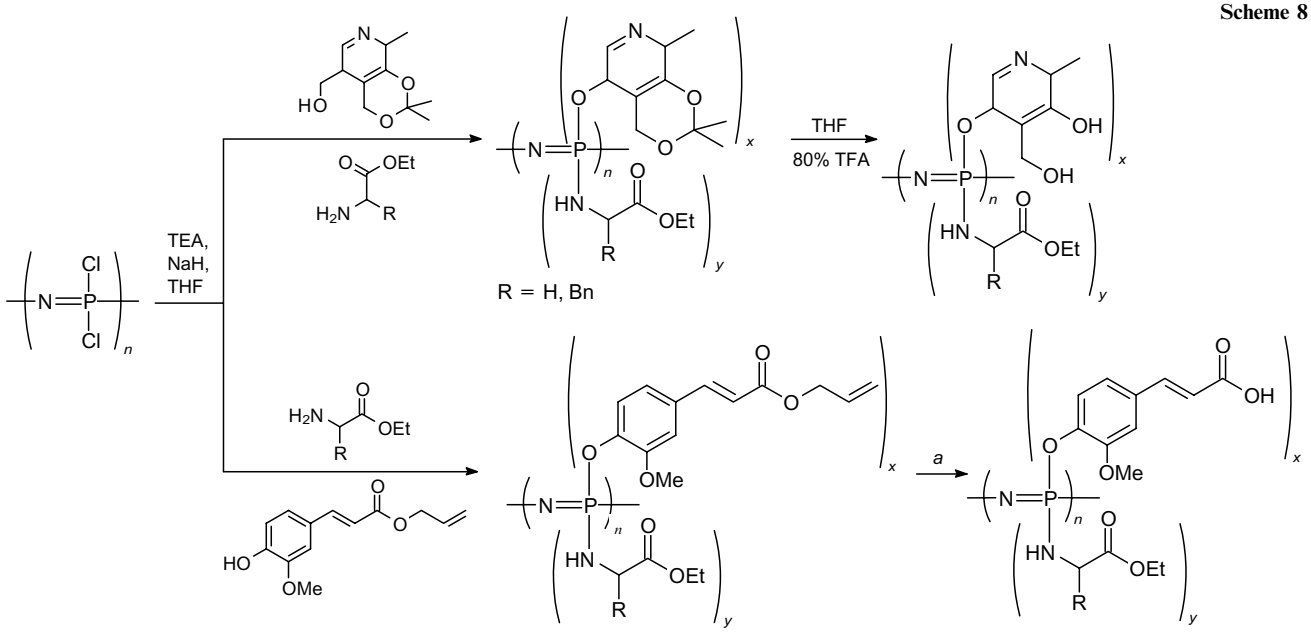
Morozowich *et al.*[174,175] synthesized POPs co-substituted with amino acid ester and a multilinker (Boc-tyramine) (Scheme 9).

The binding ability of these functionalized POPs with hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] showed enhanced natural bone healing process and hydrolytic sensitivity. Thus, these POPs were confirmed as promising candidates for implantable bone grafts (Fig. 6).[174] Further detailed studies of hydroxyapatite (HAp) mineralization by these POPs were

Scheme 7



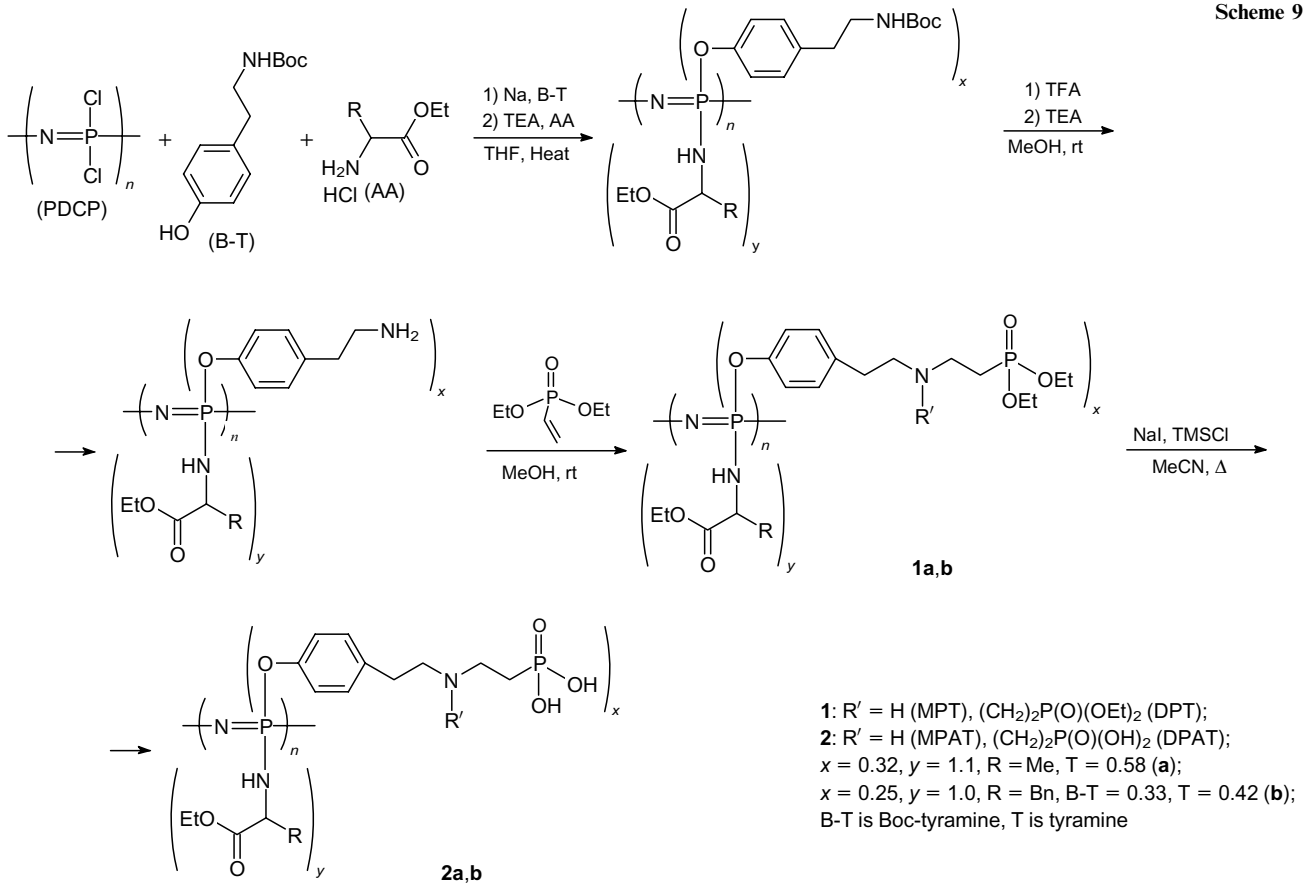
Scheme 8



(*a*) $Pd(PPh_3)_4$, morpholine, THF; $x = 2$, $y = 0$; $x = 1.5$, $y = 0.5$: R = H, Me; $x = 1.6$, $y = 0.4$: R = Pr^i, Bn; $x = 1.2$, $y = 0.6$: R = Pr^i; $x = 1.0$, $y = 0.5$: R = H, or Pr^i

carried out in simulated body fluid (SBF) to meet the requirements for tissue engineering. The initial mineralization process was found in all these polymers, but the amount and time of deposition were dependent on the chemistry of the side groups. Phosphonic acid-containing POPs (PAA and PAPA) exhibited a higher rate of mineralization than POPs with phosphoester units (PEA and PEPA) in an experiment carried out in SBF during four weeks. The mineralization mechanism and time dependence of the deposition are shown in Fig. 7.[175]

Scheme 9



1: R′ = H (MPT), $(CH_2)_2P(O)(OEt)_2$ (DPT);
2: R′ = H (MPAT), $(CH_2)_2P(O)(OH)_2$ (DPAT);
$x = 0.32$, $y = 1.1$, R = Me, T = 0.58 (**a**);
$x = 0.25$, $y = 1.0$, R = Bn, B-T = 0.33, T = 0.42 (**b**);
B-T is Boc-tyramine, T is tyramine

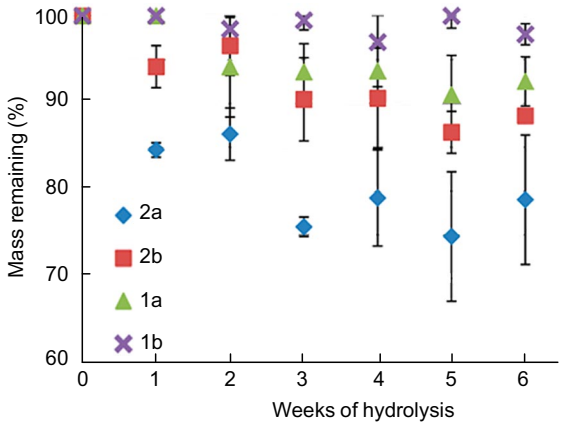


Figure 6. Percentage of mass remaining for POPs **1a**,**b** and **2a**,**b**.[174]

Honeycomb-shaped or flat films of POPs containing glycine ethyl ester and alanine ethyl ester side groups (PAGP) showed improved protein adsorption and apatite deposition in simulated body fluid and osteogenous differentiation with the increase in surface roughness due to increased phosphorus content. These POPs represent good options for applications in bone tissue regeneration.[34] However, although these POPs have biodegradable nature and osteoconductivity due to high phosphorus content in the backbone, they show poor cell adhesion and proliferation in contrast to other commonly known polymers, such as biodegradable aliphatic polyesters. Surface modified POPs with an aqueous solution of dopamine showed enhanced cell adhesion and proliferation in relation to MC3T3-E1 osteoblasts. However, a lower osteogenous differentiation was observed in contrast to that with pure PAGP nanofibres due to the use of poly(dopamine) coating on the nanofibre surface, which reduces the phosphorus content. Thus, it was found that poly(dopamine)-modified POPs are the scaffold materials of choice with enhanced cell adhesion and proliferation.[169] Greish *et al.* prepared a nanofibrous composite scaffold using alanine-substituted POPs loaded with different proportions of Ca-deficient apatite precursors.[176] The hydrolysis of these biodegradable POP nanofibres was found to be controlled by addition of Ca-deficient apatite precursors. Moreover, pH of the hydrolysis medium was within the range of physiological pH values. Hence, more studies are needed to establish the potential use of POP-based composite scaffolds in bone tissue engineering.[176]

In 2015, Modzelewski *et al.* synthesized a series of POPs using negatively charged side groups like γ-amino butyric acid (GABA) and β-alanine (β-Ala) to bind with calcium ions in order to enhance mineralization process upon exposure to SBF solution (Scheme 10).[177] All these POPs were hydrolytically sensitive and exhibited a significantly

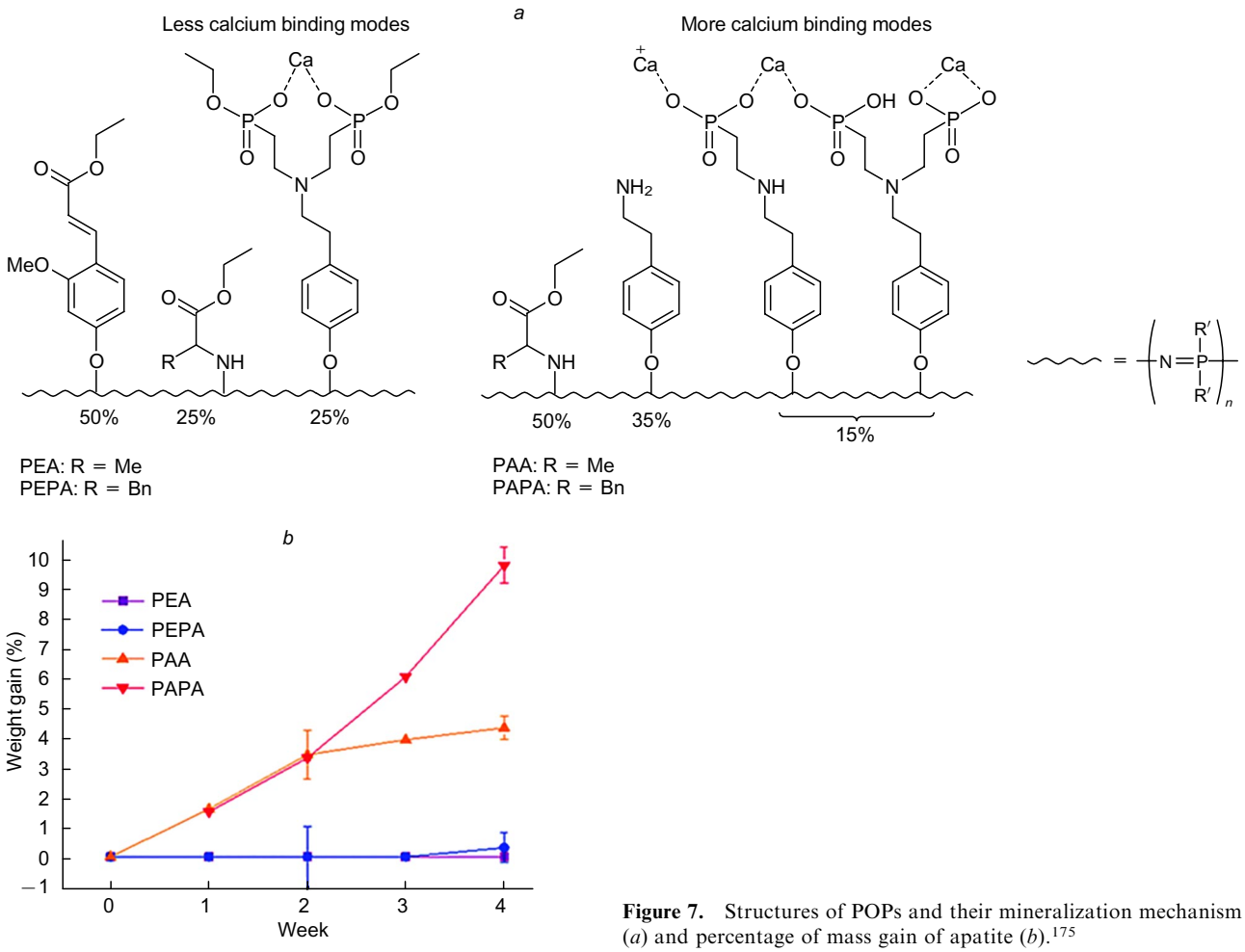


Figure 7. Structures of POPs and their mineralization mechanism (*a*) and percentage of mass gain of apatite (*b*).[175]

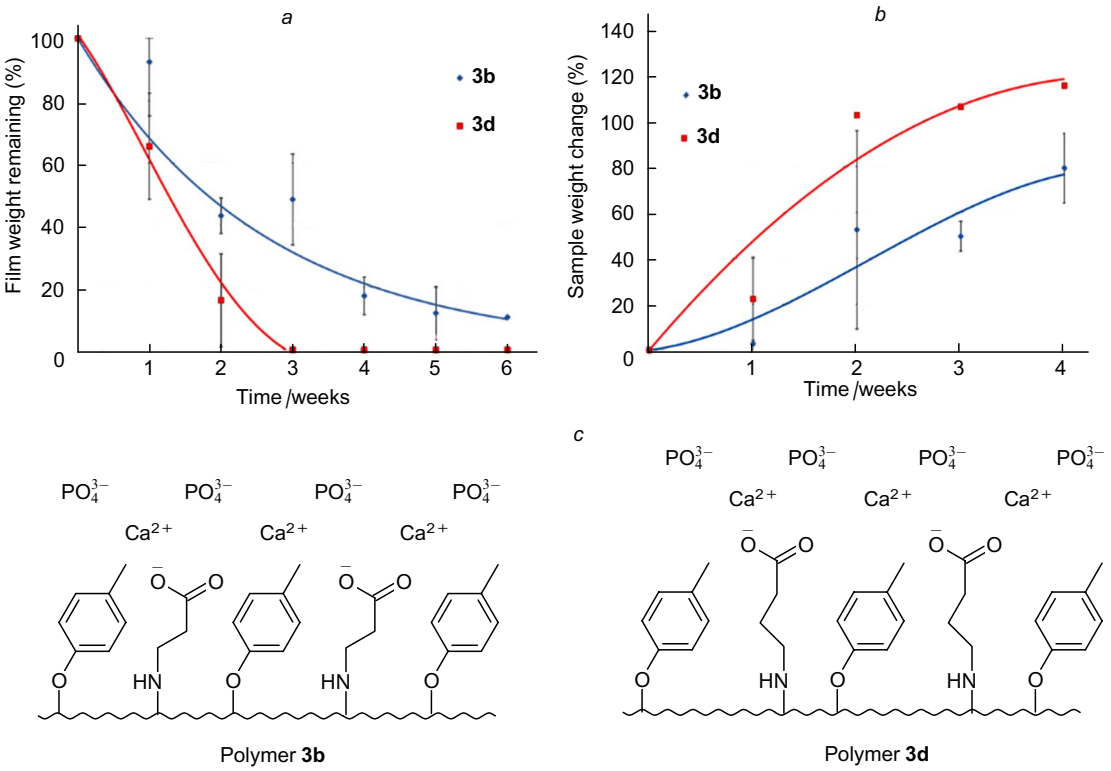
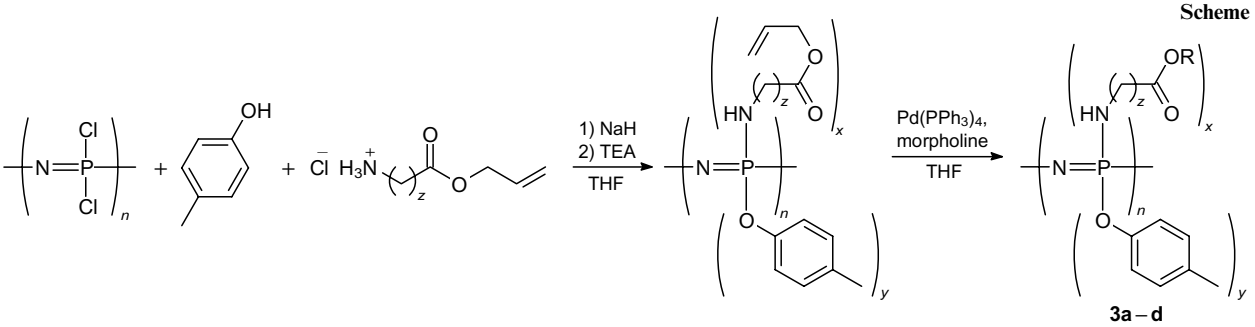


Figure 8. Hydrolytic degradation (*a*), mineralization (*b*) and mineralization mechanism of POPs (*c*).[177]

high and uniform hydroxyapatite deposition across the whole surface (Fig. 8).[177]

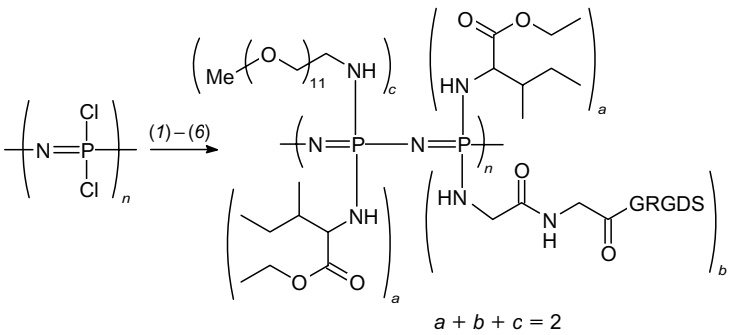
Rothemund *et al*. prepared a biocompatible glycine allyl ester-substituted POP and combined it with trifunctional thiol and adipic acid divinyl ester in different ratios; the products were subjected to thiol-ene photo-polymerization to get highly cross-linked porous scaffolds of 100 to 200 μm pore sizes.[12] It was found that using this new route, photo-polymerizable multifunctional scaffolds with desired properties, *i.e*., mechanical properties, cell adhesion and cell

Scheme 10



x = 1.54, *y* = 0.46, *z* = 2, R = H (91%) and All (9%) (**a**); *x* = 1.0, *y* = 1.0, *z* = 2, R = H (**b**); *x* = 1.56, *y* = 0.44, *z* = 3, R = H (92%) and All (8%) (**c**); *x* = 1.0, *y* = 1.0, *z* = 3, R = H (**d**)

Scheme 11



(*1*) , THF, TEA, from −60 to 25 °C, 48 h;

(*2*) , THF, TEA, 25 °C, 8 h;

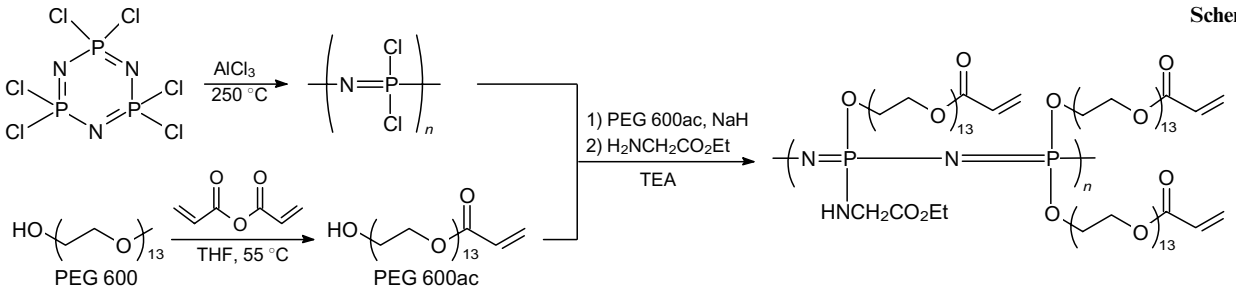
(*3*) , THF, TEA, from −60 to 25 °C, 48 h

(*4*) $(PPh_3)_4Pd^0$, morpholine, THF;

(*5*) THF, TEA, under N_2, IBCF (isobutyl chloroformate), 0 °C, 1 h;

(*6*) GRGDS, H_2O, pH 8.5, rt, overnight.

Scheme 12



wettability, can easily be obtained for tissue engineering and other biomedical applications.[12]

Polyorganophosphazene-based hydrogels have been investigated for various biomedical applications, especially for hard tissue engineering and drug delivery. Chun *et al.* synthesized POP-Gly-Arg-Gly-Asp-Ser (GRGDS) conjugates in order to develop injectable, thermosensitive and biodegradable hydrogels to improve their function toward differentiation conditions of mesenchymal stem cells (MSCs) for bone regeneration *via* cell adhesion through

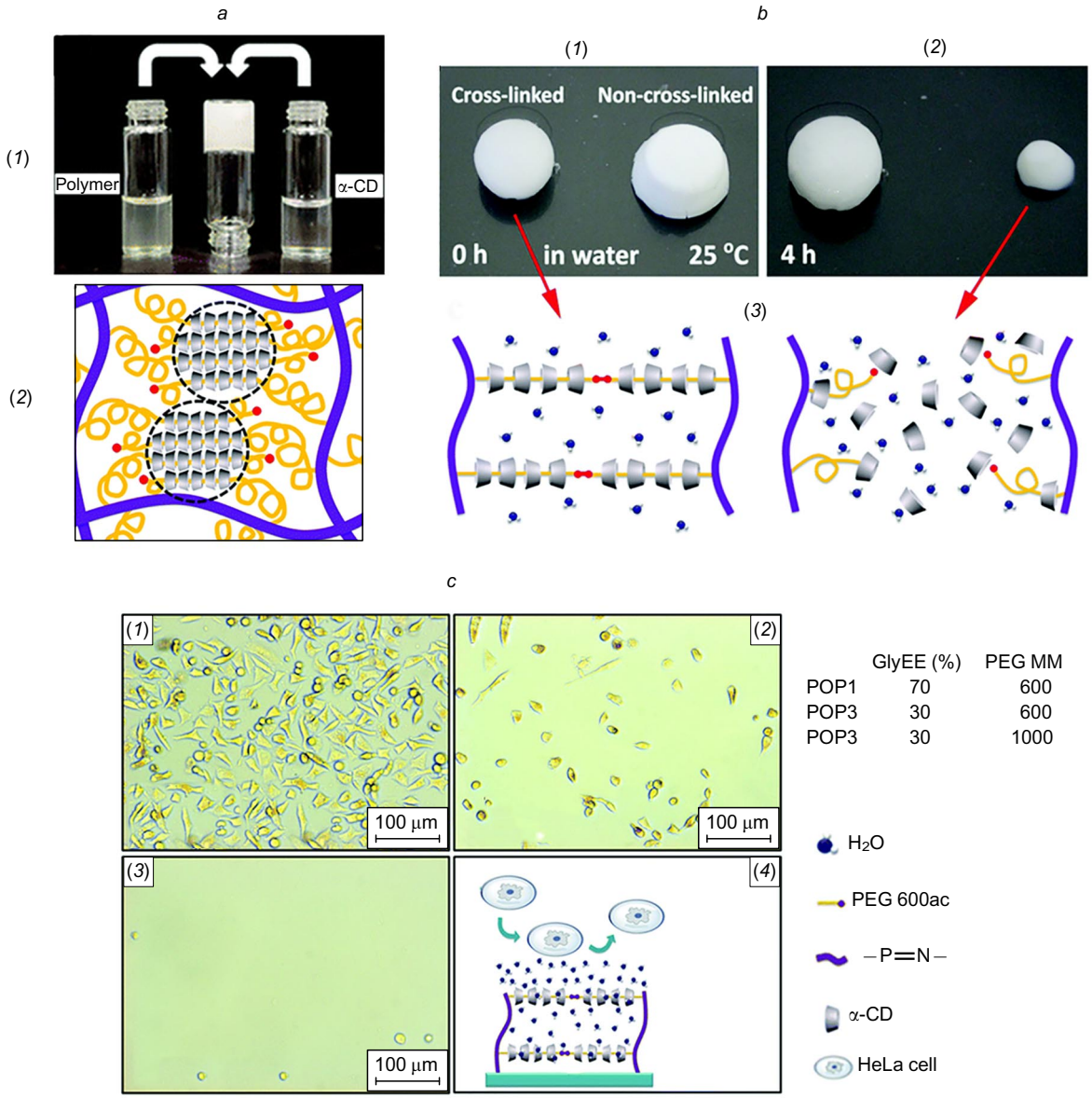


Figure 9. Hydrogel consisting of POP3CD inclusions (*a*), (*1*) gelation process of POPs and αCD; (*2*) illustration of the self-assembly structure of the hydrogel; (*b*) anti-water solubility test and images of cross-linked and non-cross-linked POP2CD before (*1*) and after (*2*) 4 h in water and UV irradiation (*2*); photo-cross-linking and possible dissolution mechanism (*3*); (*c*) effect of UV irradiation and GlyEE content on the cellular adhesion of (*1*) POP1CD, (*2*) POP2CD and (*3*) POP3CD, (*4*) drawing of the cell-phobic mechanism.[179]

amide bond formation between a cell and carboxylic acid-terminated POP-RGD. They synthesized POPs co-substituted with three different side groups, namely, L-isoleucine ethyl ester (IleOEt), glycylglycine (GlyGlyOH) and amino-methoxy-poly(ethylene glycol) (AMPEG) using the nucleophilic substitution reactions. Then an aqueous solution of GRGDS was added to the activated POP solutions in order to get three POP-GRGDS conjugates with different molar ratios of side groups and GRGDS (Scheme 11).[178]

Aqueous solutions of synthesized POP-GRGDS conjugates were liquid at room temperature and instantly changed into gels at a physiological temperature. Particularly, enhanced osteogenic differentiation process was observed in four weeks. It is noteworthy that high calcium deposition was observed, *i.e.*, mineralization actively took place. The *in vivo* degradation studies carried out in mice for two weeks showed faster degradation rate than the degradation rate of POPs alone. This evidence indicates that POP-GRGDS conjugates can be used for bone tissue engineering due to their bio-functionality to improve osteogenic differentiation of MSCs for fast bone regeneration process.[178]

Usually, injectable hydrogels have poor mechanical properties, which limit their use for various biomedical applications. The mechanical properties of injectable hydrogels can be enhanced *via* addition of reinforcing agents, but these agents make the materials non-biodegradable, which ultimately restricts their medical applications. Huang *et al.* used GlyEE and monoacrylic-terminated AMPEG as side groups to synthesize three different POPs by changing the PEG group length and the molar ratio of the side groups (Scheme 12).[179]

Injectable and cross-linkable hydrogels were prepared from these biodegradable co-substituted POPs and natural organic α-cyclodextrin (αCD) molecules by host–guest inclusion (POPCD). Their gelation time was found to be directly related to their precursor concentration. Then these hydrogels were transformed into the desired solid shape by photo-cross-linking. Their mechanical strength and anti-dissolving ability were carefully tuned by adjusting the extent of photo-cross-linking for the intended biomedical applications. Moreover, the surface of the cured hydrogel can be easily changed from cell-philic to cell-phobic by changing the ratio of two side groups pendant to the POP backbone. It was found that hydrogel containing a high content of GlyEE showed an enhanced adhesion (Fig. 9).[179]

Biodegradable and photo-polymerizable POPs have been reported, in which amino acid derivatives were linked to the polyphosphazene backbone *via* the amino group of amino acids and the allylic group linked to the amino acid *via* a spacer.[180] These photo-cross-linkable POPs were cross-linked to get the desired mechanical and degradation properties required for tissue engineering.[180]

Huang *et al.* developed photo-luminescent biodegradable scaffolds from POPs co-substituted with a fluorescent compound, namely, 5-oxo-2,3-dihydro-5*H*-thiazolo[3,2-α]-pyridine-7-carboxylic acid (TPCA) and alanine ethyl ester.[181] Both *in vitro* and *in vivo* results showed their biocompatibility, non-toxicity and controlled biodegradability with photo-stability. The development of these POP-based scaffolds provided a new route for tissue engineering based on theranostics applications.[181] Seo *et al.* synthesized POPs using polyethylene glycol, IleOEt and carboxylic acid moiety as substituents to the PDCP backbone to impart amphiphilicity and hydrophobic and ionic interactions with morphogenetic protein-2 (BMP-2).[182] It was found that BMP-2-loaded nanocomplex hydrogel released the loaded BMP-2 in a sustained manner within three weeks, providing enhanced bone generation ability.[182] Furthermore, this BMP-2-loaded nanocomplex hydrogel showed enhanced vertical bone regeneration with a higher osseointegration level.[183] Seo *et al.* developed a new series of POP-based thermosensitive hydrogel systems using co-substituted POPs with three cyclic anhydrides.[184] These POP-based hydrogel systems showed a potential for enhanced bone regeneration or controlled drug delivery.[184]

4.1.2. Nerve tissue engineering

Various efforts have been made to investigate POP-based materials for nerve tissue regeneration. Poly[(ethyl alanato)$_{1.4}$(imidazolyl)$_{0.6}$phosphazene] (PEIP) tubular nerve guides were shown in *in vivo* experiments to be biodegradable into non-toxic end products.[185] Poly[bis(ethyl alanato)phosphazene] tubes were tested as guides for the regeneration of nerve tissue in an experimental animal model. The POP channels may be considered as effective guides for nerve regeneration, mainly in view of the possible use of the polymer matrix as a carrier for neurite-promoting factors.[186] The POP (PGAP) synthesized by using GlyEE and the parent aniline pentamer (PAP) as side groups has biodegradable and electroactive properties. Its electrical conductivity was found in a protonic-doped experiment to be approximately 2×10^{-5} S cm^{-1} in the semiconducting region. Moreover, this POP showed *in vitro* degradation and improved cell adhesion and proliferation properties using RSC96 Schwann cells. Hence, this new polymer may serve as a candidate for scaffold applications in neuronal and cardiovascular tissue engineering.[187] Later on, this POP was investigated for application in nerve regeneration because of the electrically active nature and biodegradable properties (Fig. 10).[188]

4.1.3 Vascular tissue engineering

Many researchers have tried to explore polymeric materials for vascular tissue engineering. In this scenario, different natural and synthetic polymers have been investigated as scaffold materials for regeneration of vascular tissue. The flat or tube-shaped matrices obtained from biocompatible poly[(ethyl phenylalanato)$_{1.4}$(ethyl glycinato)$_{0.6}$phosphazene] showed the characteristics to support regeneration of human tissues, such as vessels or cardiac valves.[189] Conconi *et al.* synthesized poly[bis(ethyl alanate)phosphazene] and poly[(ethyl phenylalanate)$_{0.8}$(ethyl alanate)$_{0.8}$(ethyl glycinate)$_{0.4}$phosphazene] (PPAGP) and checked their capability as small fibres to facilitate rat neuromicrovascular endothelial cell (EC) for cell adhesion and cell growth using poly(D,L-lactic acid) as reference material.[190] These POPs were found to possess high cell adhesion and cell growth properties as compared to polystyrene. High endothelial cell proliferation was observed for PPAGP. Hence, PPAGP can be considered to be a promising scaffold material for regeneration of blood vessels, as it stimulates adhesion, growth and organization of endothelial cells into capillary-like shape.[190]

4.1.4 Ligament and tendon tissue engineering

A number of POPs have been investigated as scaffolds for ligament and tendon tissue regeneration.

Nichol *et al.* synthesized a series of amino acid-substituted POPs with different alkyl group length. Alanine and

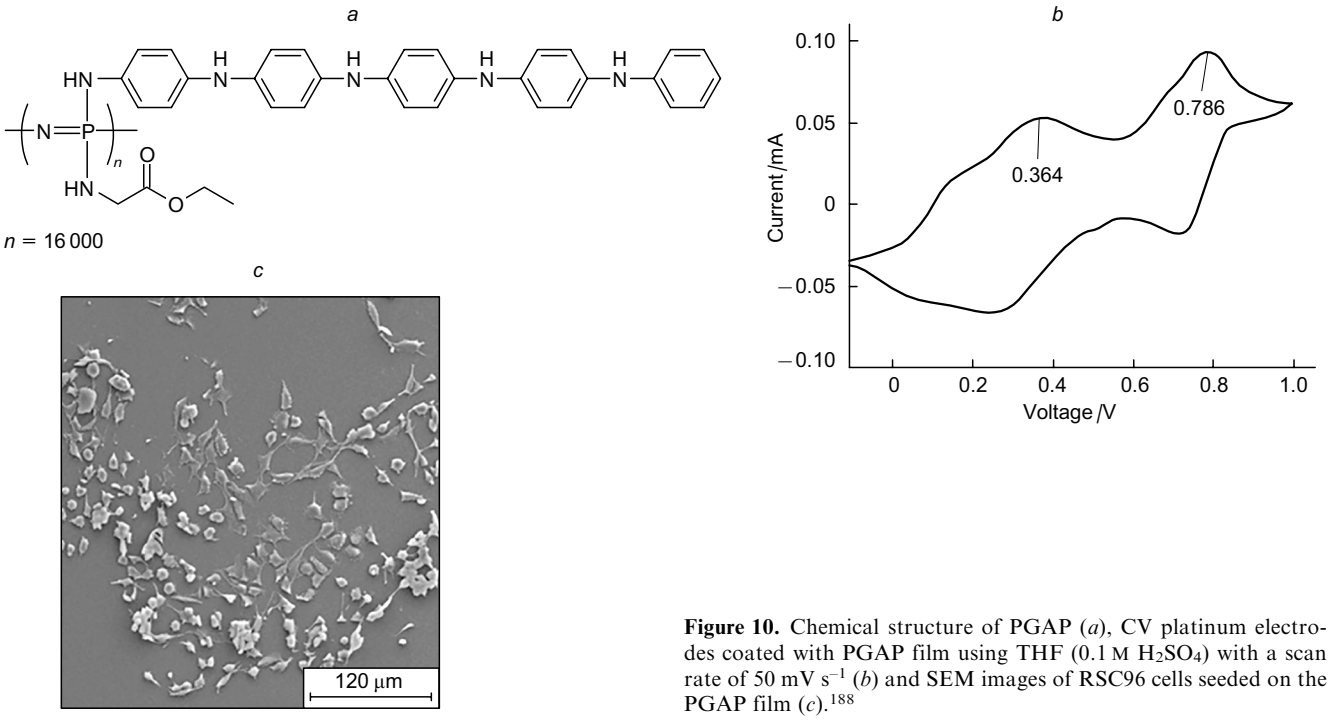


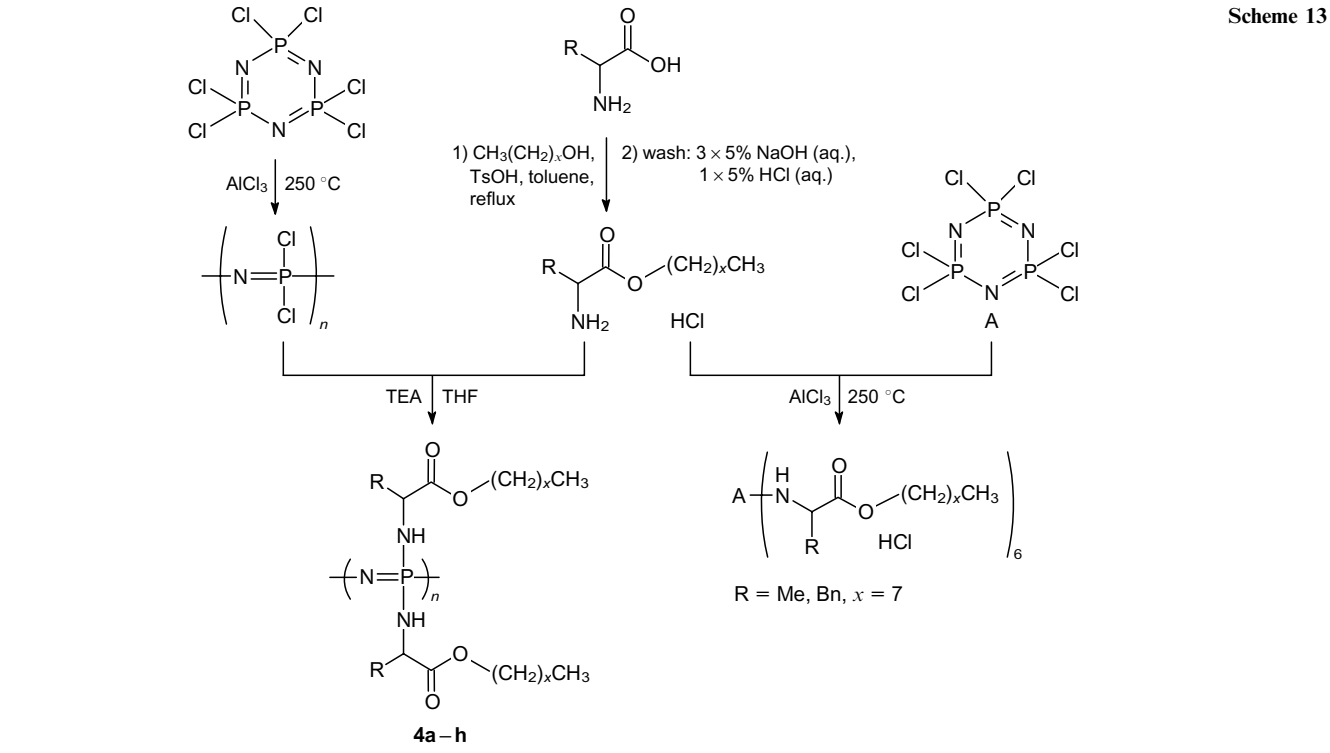
Figure 10. Chemical structure of PGAP (*a*), CV platinum electrodes coated with PGAP film using THF (0.1 M H_2SO_4) with a scan rate of 50 mV s^{-1} (*b*) and SEM images of RSC96 cells seeded on the PGAP film (*c*).[188]

phenylalanine substitution are shown in Scheme 13.[191] These POPs exhibited controlled degradation depending on steric hindrance of the amino acid and alkyl group length (Fig. 11). Almost all these POPs were shown to be promising as ligament and tendon tissue engineering scaffold materials. Phenylalanine-substituted POPs were considered as the best candidates for ligament and tendon engineering, because of film formation behaviour and slow hydrolysis.[191]

Recently, direct and indirect linking of citronellol to the POP backbone has been studied. Citronellol is an anti-inflammatory molecule with a double bond, which can be easily cross-linked on exposure to UV radiation and has a potential for tissue engineering. Direct[192] and indirect[137] binding of citronellol to the backbone of POPs have been reported. The synthetic routes to POPs substituted with amino acid citronellol esters are shown in Scheme 14.[137]

The indirect binding was found to be more beneficial to adjust mechanical and hydrolytic properties, as the max-

Scheme 13



R = Me: x = 4 – 7 (**4a** – **d**); R = Bn: x = 4 – 7 (**4e** – **h**)

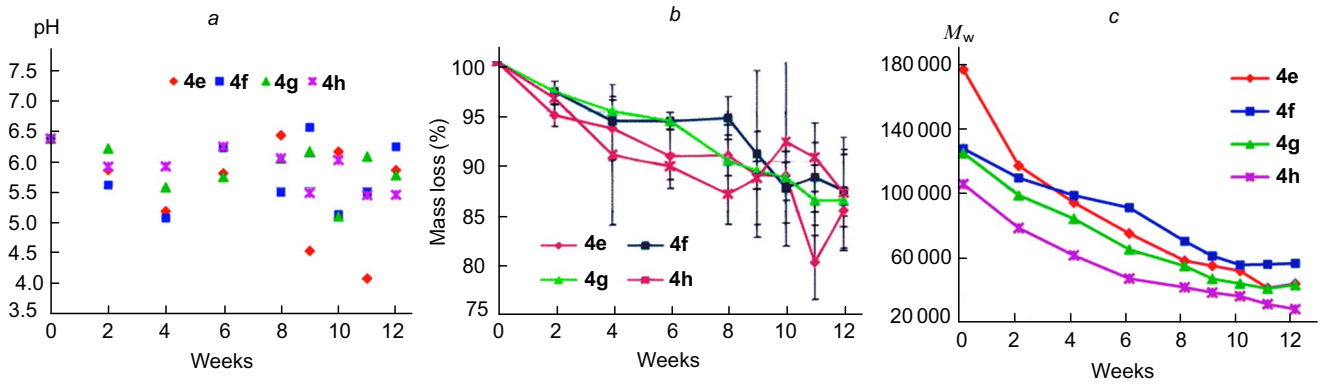
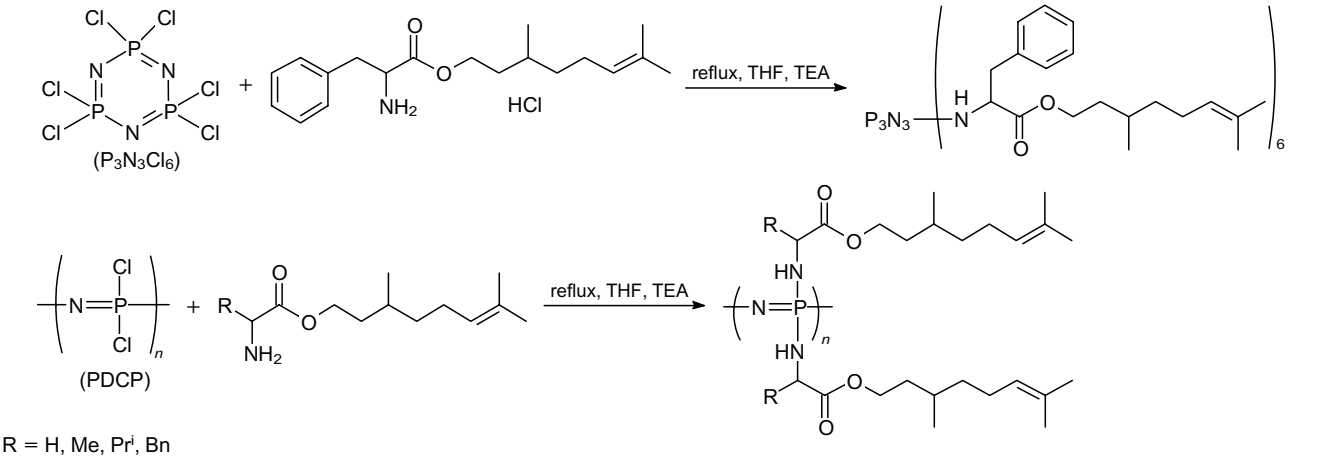


Figure 11. pH-Dependent hydrolysis studies (*a*); percent of mass loss upon hydrolysis (*b*) and decline of M_w for POPs **4e**–**h**, respectively (*c*).[191]

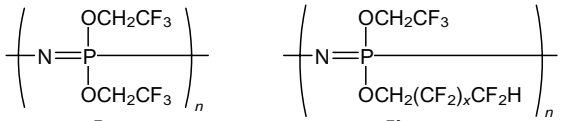
Scheme 14



R = H, Me, Pr^i, Bn

imum citronellol content can be attached *via* carboxylic acid group of the amino acid esters (glycine, alanine, valine, and phenylalanine) linked to the polymer backbone. The rate of hydrolysis and mechanical properties were adjusted by steric hindrance of amino acid esters and mutual cross-linking of citronellol moieties on exposure to UV radiation (see Scheme 14).[150] These POPs were found to be promising materials for ligament and tendon tissue engineering.[137]

Structures 5a,b



5a **5b**

4.1.5. Cardiac tissue engineering

Usually, polymeric materials having properties like high hydrophobicity, hydrolytic stability and UV- and γ-ray resistance are used for cardiac tissue engineering. Fluoroalkoxy-substituted POPs have elastomeric properties. For instance, poly[bis(trifluoroethoxy)phosphazene] (**5a**) is highly hydrophobic, but has low stability toward some organic solvents.[50,76] The micro- and nanofibres formed from this POP by electrospinning were found to possess super-hydrophobic properties with about 160° water contact angle.[32,193] Moreover, its fibres and films can be obtained with excellent surface modification for various applications.[194] In addition, this POP was found to be stable towards UV and γ-rays as well as hydrolytically stable in physiological media.[195]

Furthermore, different pendant fluoroalkoxy side groups were used to change the polymer properties from a thermoplastic microcrystalline material to a rubbery material,[16] providing a starting material for manufacturing process. A POP with fuoroalkoxy side groups (**5b**), exhibiting elastomeric and antimicrobial properties, has been approved as a dental liner material. POP (**5b**) is also used as a material for artificial heart valves famous with names like PN-F or Eypel-F. Hence, POPs co-substituted with different fluoroalkoxy side groups have excellent potentialities for cardiac tissue engineering.[16]

4.1.6. Polymeric scaffolds for tissue engineering

The scaffold's function is to provide a temporary supporting media for cells, biomolecules and growth factors in order to maintain and facilitate the growing cells so as to repair, restore and regenerate the missing or damaged tissue of the body. A number of materials, *i.e.*, PLGA, PCL, polyhydroxyalkanoates, polyurethanes, polyphosphazenes, collagen, fibrin, hyaluronic acid (HA), bioactive ceramics, such as calcium phosphates and bioactive glasses (BAGs)

and hydroxyapatite have been investigated for tissue engineering applications.[196, 197] Recently, more attention has been given to the design of scaffolds with biocompatibility,[165, 198] biodegradability,[164] mechanical strength,[198, 199] osteoconductivity and osteoinductivity,[128, 200] sufficient porosity,[201] interconnectivity,[202] easy fabrication and reproducibility.[203] Polyorganophosphazenes are attractive biomaterials to get scaffolds with desired functionalities for tissue engineering (see the Properties section).

4.2. Drug delivery: matrix fabrication and drug release kinetics

In treatment of various diseases, polymeric materials have a significant role and have been used for the delivery of therapeutic agents. Especially, biocompatible and biodegradable polymers have been commonly used for drug delivery. Among these biodegradable polymers, POPs have excellent potentialities for drug delivery. Usually, in the drug delivery matrices, biodegradable polyphosphazenes have been used as pellets or films. Due to better solubility of POPs in various solvents, their pills or films are generally fabricated by solvent casting technique.[134, 135, 204] Spray drying, solvent evaporation and emulsion solvent evaporation methods such as micro-encapsulation are also applied. Various POP forms are used for drug delivery, such as microspheres, nanofibres, micelles, polymersomes, hydrogel nano-conjugates and dendrimers (Fig. 12). Veronese *et al.*[205] synthesized POPs with phenylalanine ethyl ester co-substituted with imidazole and prepared its microspheres of various size distributions with 2 to 70 μm average diameters using three different techniques: spray drying, emulsion solvent evaporation and emulsion solvent evaporation – extraction. The microspheres produced by emulsion technique were highly porous because of the removal of water as microdrops of the double emulsion from the inner area during strengthening of the microspheres. Thus, the microspheres fabricated in this process provide a route for controlled drug release.[205]

An important condition of colon-specific targeted drug delivery is stability of polymeric structures in acidic media. For this purpose, azo-based POP – anticancer drug conjugates stable in an acidic buffer (pH = 1.2) have been prepared.[206] The synthesis of azo-based POP – drug conjugates containing methotrexate and gemcitabine is presented in Scheme 15. The *ex vivo* release experiments carried out in the presence and absence of rat cecal content showed a more than 89% release of methotrexate and gemcitabine.[206] The *in vitro* cytotoxicity study against human colorectal cancer cell lines (HT-29 and COLO 320 DM) showed activity of the drug conjugates. These azo-containing POP – drug conjugates of methotrexate and gemcitabine were found to be promising candidates for colon targeted drug delivery system with slight adverse side effects.[206]

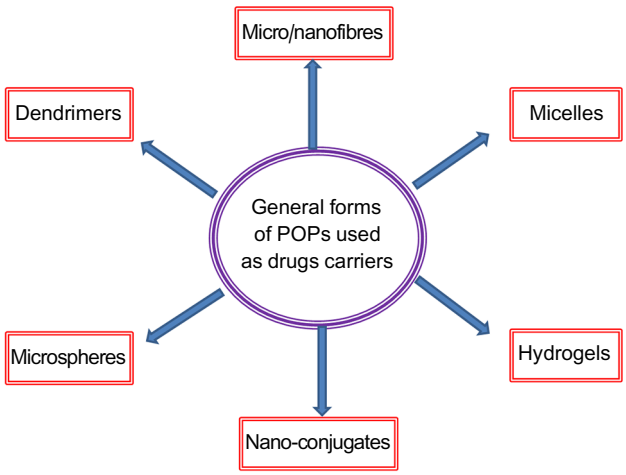
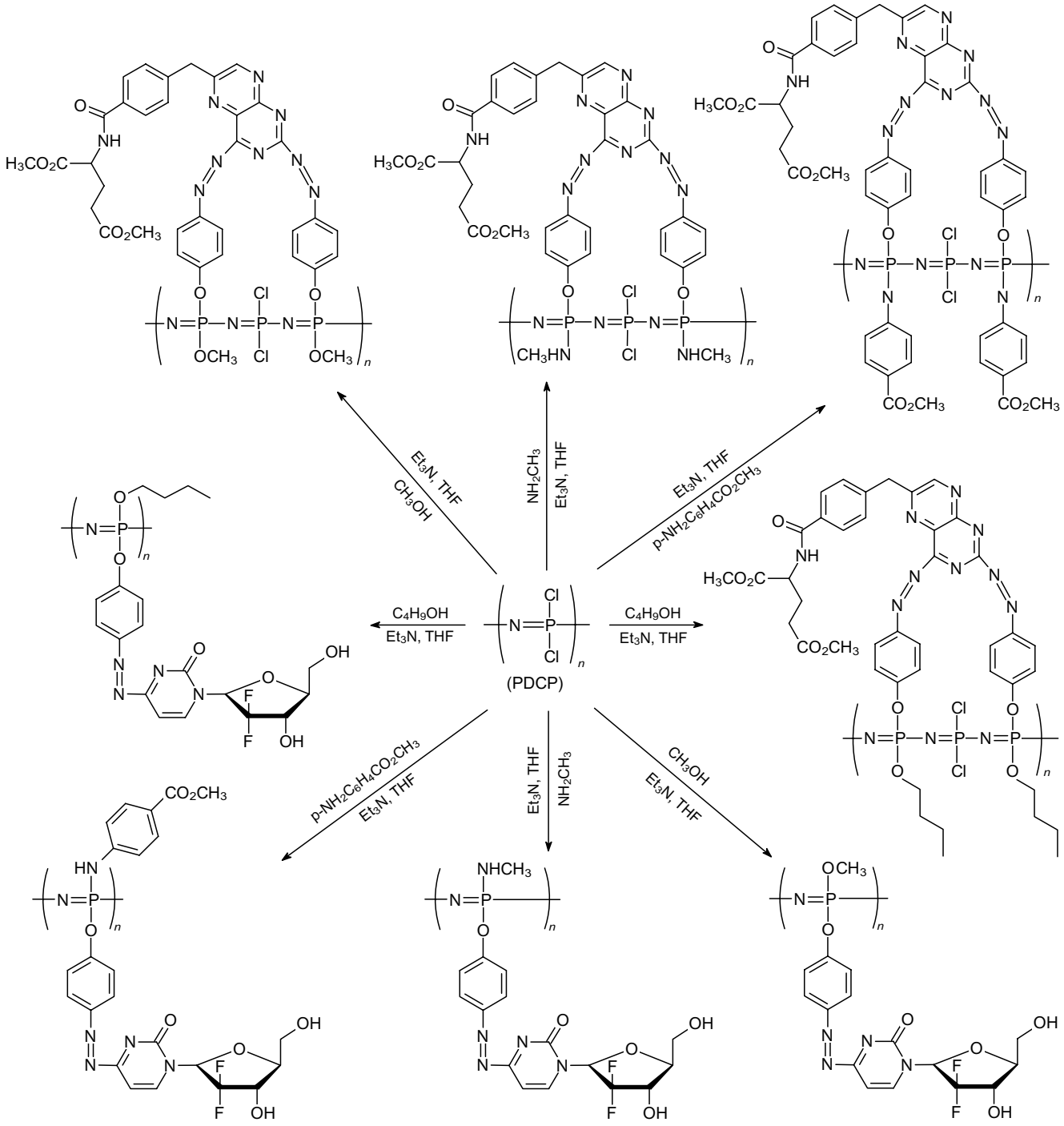


Figure 12. Various forms of POPs used as drug carriers.

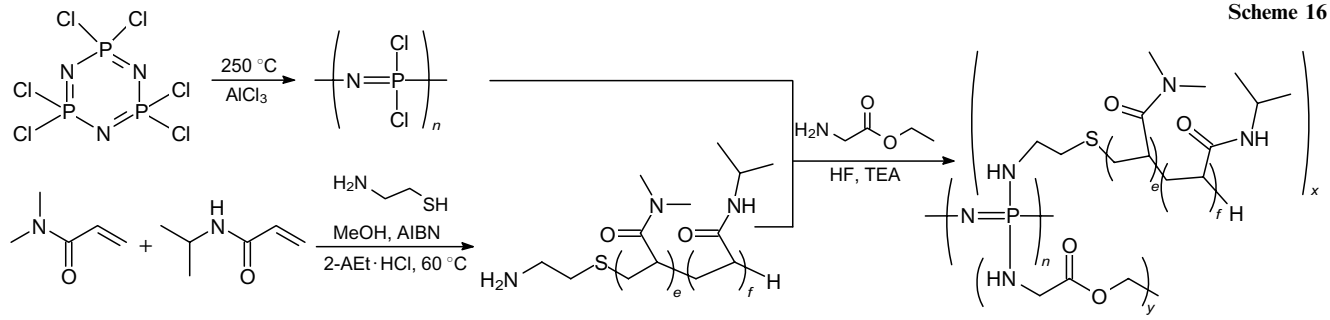
Qiu *et al.*[207] synthesized a series of amphiphilic graft POPs (PNDGPs) using hydrophilic and hydrophobic side groups. Scheme 16 shows the synthetic routes to N-isopropylacrylamide- and GlyEE-co-substituted POPs.[207] Their micelles loaded with hydrophobic anti-tumour doxorubicin drug were evaluated for the treatment of cancer. It was found that encapsulation of doxorubicin into the micelle increased with increasing hydrophobic content of the polymer. In addition, these micelles were found to be more efficient than the free drug.[207]

Various POP-based hydrogels have been extensively studied for biomedical applications. Bae Lee *et al.* synthesized thermosensitive POPs using AMPEG and hydrophobic IleOEt as side groups.[208] The hydrophobic interaction between the side-chain fragments [$-CH(CH_3)CH_2CH_3$] of IleOEt act as a physical connection in POP aqueous solutions. These POPs were found to exhibit four phase transitions with increasing temperature in an aqueous solution, in particular, transparent sol, transparent gel, opaque gel and turbid sol. Moreover, gelation properties were dependent on various factors including composition, the length of side groups and the concentration of POP solutions. A more hydrophilic composition results in a higher gelation temperature.[208] Later on, Lee *et al.* synthesized another series of thermosensitive POPs using AMPEG, some hydrophobic amino acid esters and depsipeptide ethyl ester [ethyl 2-(*O*-glycyl)glycolate] as a hydrolysis-sensitive moiety.[209] These POPs were found to be degradable under the physiological conditions, with the hydrolysis rate being dependent on the composition.[209] Kang *et al.* synthesized POPs using both hydrophobic and hydrophilic groups with or without depsipeptides.[210] These POP hydrogels exhibited thermo-reversible sol – gel transition behaviour and were relatively stronger at low polymer concentrations. A sustained *in vitro* release over a period of two weeks was observed for loaded water-soluble model drugs such as FITC-dextran and human serum albumin. It was shown that higher POP concentrations result in smaller pore size and ultimately cause a slower release of the loaded drug. Hence, these POPs can be promising candidates for the manufacture of injectable hydrogels for the delivery of bioactive protein drugs such as erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF) and human growth hormone (hGH).[210] Hydrophobic doxorubicin was loaded, and considerably sustained release was observed for three weeks, mainly deponding on the POP gel strength. Thus, drug release can be controlled *via* the strength of the gel. The anticancer efficacy was also investigated using mouse lymphoblast cell line of P388D1 and the efficacy of doxorubicin was observed to be sustained for a period of more than a month. Hence, it was established that the release of doxorubicin was constant for a long time without an initial burst release.[211] Lee *et al.* synthesized a thermosensitive POP hydrogel using hydrophobic IleOEt and hydrophilic AMPEG side groups with and without hydrolysis-sensitive glycyl-lactate ethyl ester or functional glycylglycine.[212] Their aqueous solutions were in the liquid states

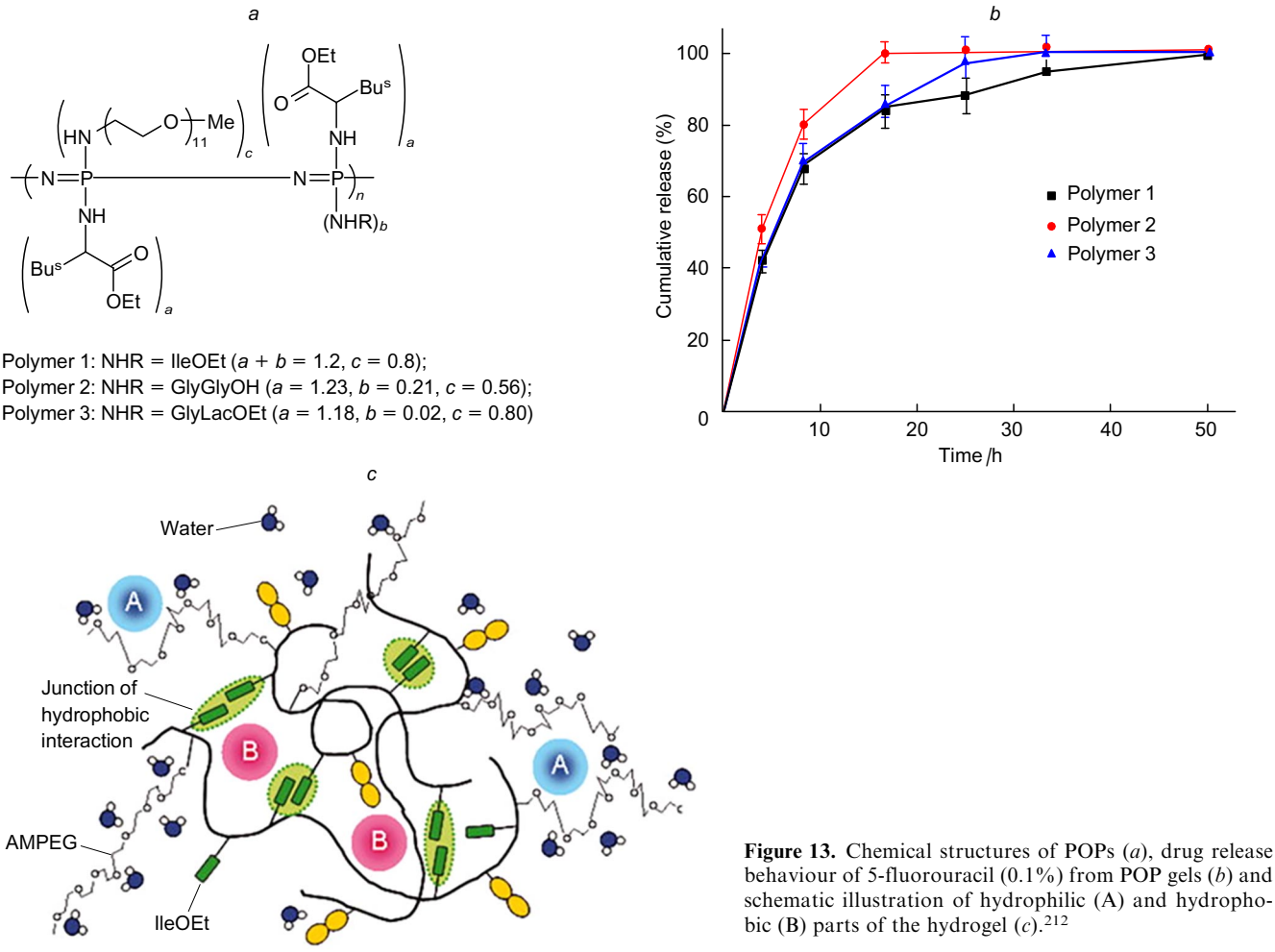
Scheme 15



Scheme 16



2-AEt·HCl is 2-aminoethanethiol hydrochloride



Polymer 1: NHR = IleOEt ($a + b = 1.2$, $c = 0.8$);
Polymer 2: NHR = GlyGlyOH ($a = 1.23$, $b = 0.21$, $c = 0.56$);
Polymer 3: NHR = GlyLacOEt ($a = 1.18$, $b = 0.02$, $c = 0.80$)

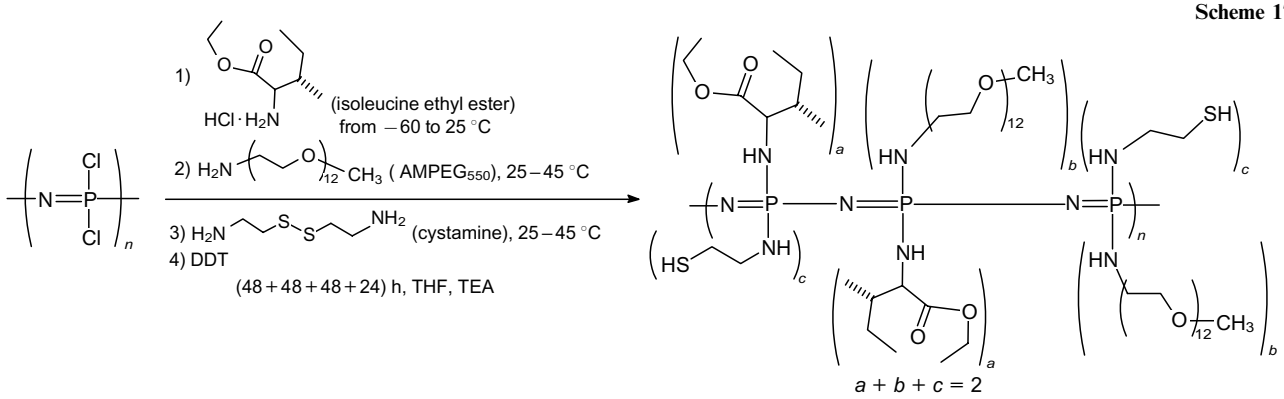
Figure 13. Chemical structures of POPs (*a*), drug release behaviour of 5-fluorouracil (0.1%) from POP gels (*b*) and schematic illustration of hydrophilic (A) and hydrophobic (B) parts of the hydrogel (*c*).[212]

and were transformed into a transparent gel at the physiological temperature. 5-Fluorouracil (5-FU), a model anticancer drug, was loaded. The solubility of 5-FU drug in a POPs solution was found to be ten times (34.26 mg ml^{-1}) higher than that in phosphate-buffered saline (3.39 mg ml^{-1}) at pH 7.4 (Fig. 13).[212] The *in vitro* drug release experiments showed diffusion-controlled, relatively sustained and localized release at target sites with no side effects.

Potta *et al.* synthesized a series of chemically cross-linkable and thermosensitive POPs as injectable biomaterial by using thiol (−SH), hydrophobic IleOEt and hydrophilic AMPEG side groups (Scheme 17).[213]

At the body temperature, their aqueous solutions were transformed into hydrogels with a desired gel strength as a result of hydrophobic interactions. It was found that under physiological conditions, cross-linking of thiol groups took place with cross-linkers, divinyl sulfone (VS) and PEG divinyl sulfone (PEGVS), which further enhanced the mechanical properties. This dual cross-linking nature can be used to control the degradation pattern and the mechanical strength of the hydrogel. A hydrogel was developed *via* physical and chemical cross-linkings upon mixing of stoichiometric amounts of thiolated POPs and the cross-linking reagent separately dissolved in PBS solutions at low temperature, which was then increased to the body temperature. The cross-linking with divinyl sulfone was found to be

Scheme 17



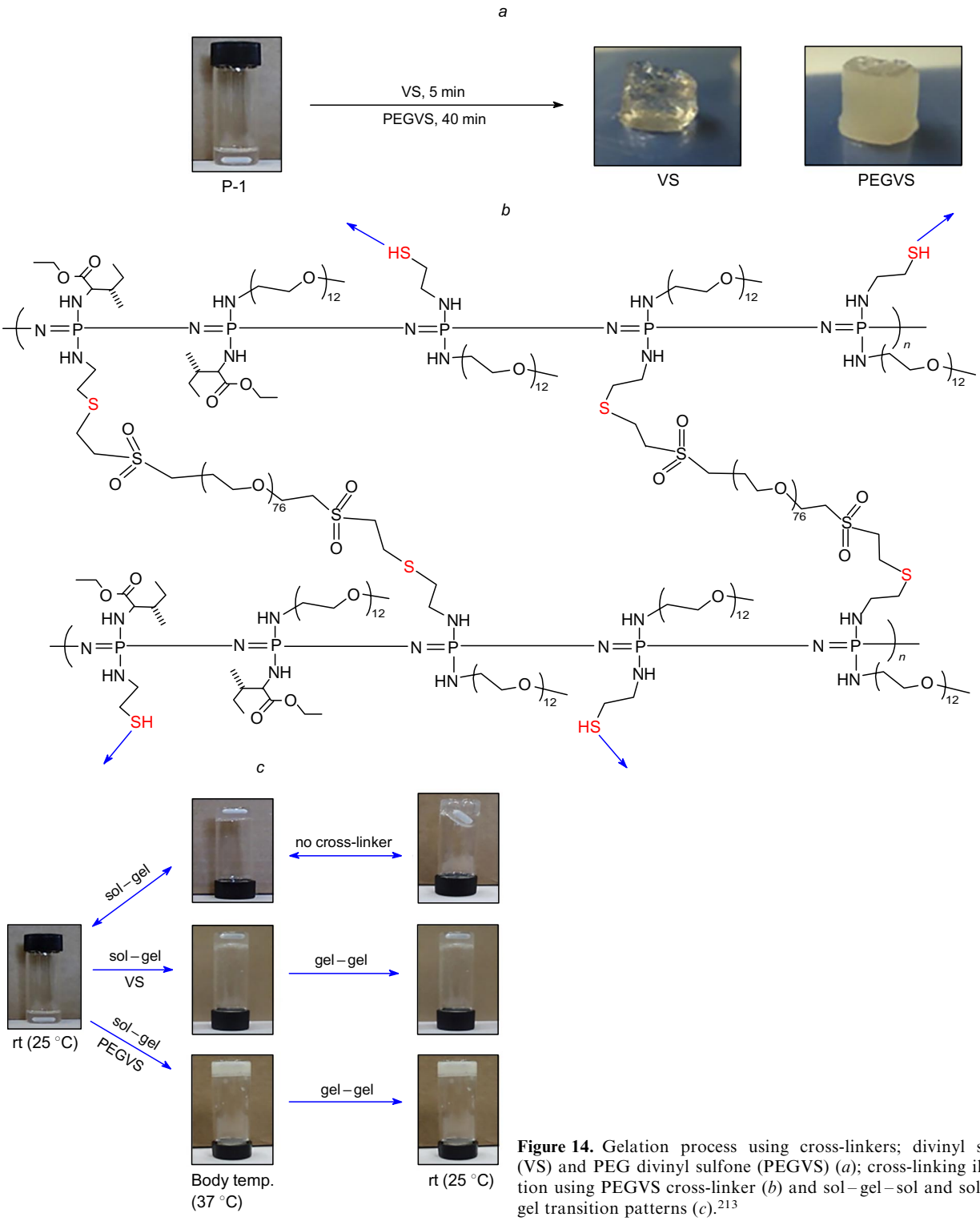
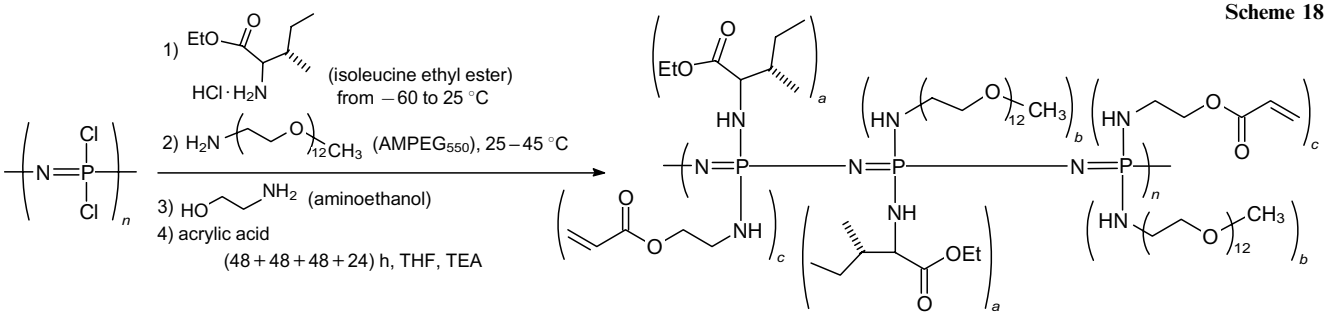


Figure 14. Gelation process using cross-linkers; divinyl sulfone (VS) and PEG divinyl sulfone (PEGVS) (*a*); cross-linking illustration using PEGVS cross-linker (*b*) and sol–gel–sol and sol–gel–gel transition patterns (*c*).[213]

accomplished in 5 min, while cross-linking of PEG divinyl sulfone was found to be completed in 40 min. The extent of cross-linking depended on the degree of cysteamine substitution and the amount and nature of the cross-linker. The *in vivo* and *in vitro* cross-linking behaviours were almost similar (Fig. 14).[213]

Later on, Potta *et al*. introduced a new functionality to already prepared POPs. Briefly, first, chlorine atoms of PDCP were partially replaced by IleOEt hydrochloride to produce the intermediate, then chlorine atoms were further replaced with AMPEG 550 to yield another intermediate, which was finally treated with aminoethanol for complete replacement of the remaining chlorine atoms to obtain the POPs **6** (Scheme 18).[214] Aqueous solutions of these new thiolated and acrylated POPs were liquid at low temperature and transformed into hydrogels at physiological temperature. The mechanical strength was further improved by the chemical cross-linking of thiol groups with acrylate groups in the polymer network under physiological conditions (Fig. 15). Their solutions were in the liquid state at

Scheme 18



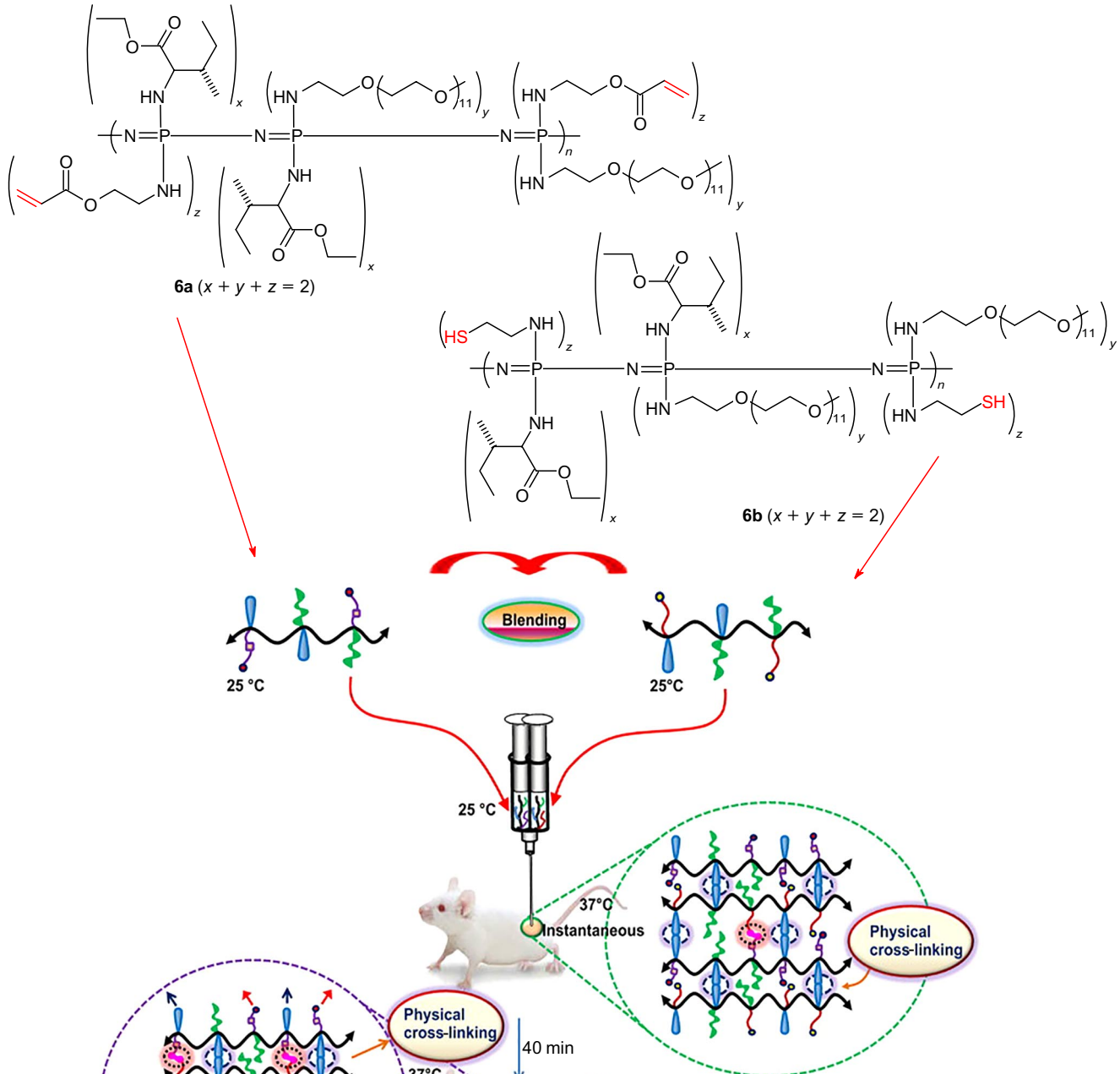


Figure 15. POP blend hydrogel system showing physical and chemical cross-linking.[214]

ambient temperature and rapidly transformed into transparent gels due to development of physical cross-linking, ultimately accompanied by chemical cross-linking *via* Michael addition within 40 min. This doubly cross-linked hydrogel showed good mechanical properties and swelling behaviour. *In vivo* experiments were also carried out by injecting solutions of equimolar polymer blends to nude mice. This injectable, self-cross-linkable and thermosensitive hydrogel system has advantages over the previously developed injectable carriers and overcomes many barriers associated with the development of injectable carriers such as the use of monomeric cross-linkers, catalysts, oxidants, pH adjustment, initiators, UV light, heat production and organic solvents. This injectable hydrogel with tunable degradation and mechanical properties can be designed using the biodegradable, biocompatible and thermo-sensitive POPs.[214]

Park *et al.* designed an injectable, biodegradable and thermosensitive hydrogel by combination of POPs and a polyelectrolyte complex (PEC) for a controlled and sustained delivery of hGH. Two POPs were synthesized using IleOEt, ethyl 2-(*O*-glycyl)lactate and AMPEG side groups.[215] The hGH-loaded PEC complex was prepared by mixing solutions of polycations and hGH. The complex formation of poly-L-arginine (PLA) with hGH took place even at a small weight ratio, in contrast to that of poly-L-lysine (PLL). *In vitro* and *in vivo* release profiles for all PEC-loaded POP hydrogels showed a sustained release of hGH for longer periods of time with no burst release, unlike the hGH-loaded POP-based hydrogels exhibiting an initial burst release. The detailed release mechanism is shown in Fig. 16.[215]

Later, Park *et al.* designed another injectable, biodegradable and thermosensitive hydrogel by combination of POPs and PEC.[216] They synthesized two different POPs, particularly, anionic and neutral POPs using three different side groups, IleOEt and AMPEG common for both POPs, while the third side group was glycylglycine allyl ester (GlyGlyOAll) for the anionic polymer and ethyl 2-(*O*-glycyl)lactate (GlyLacOEt) for the neutral polymer.[216] The

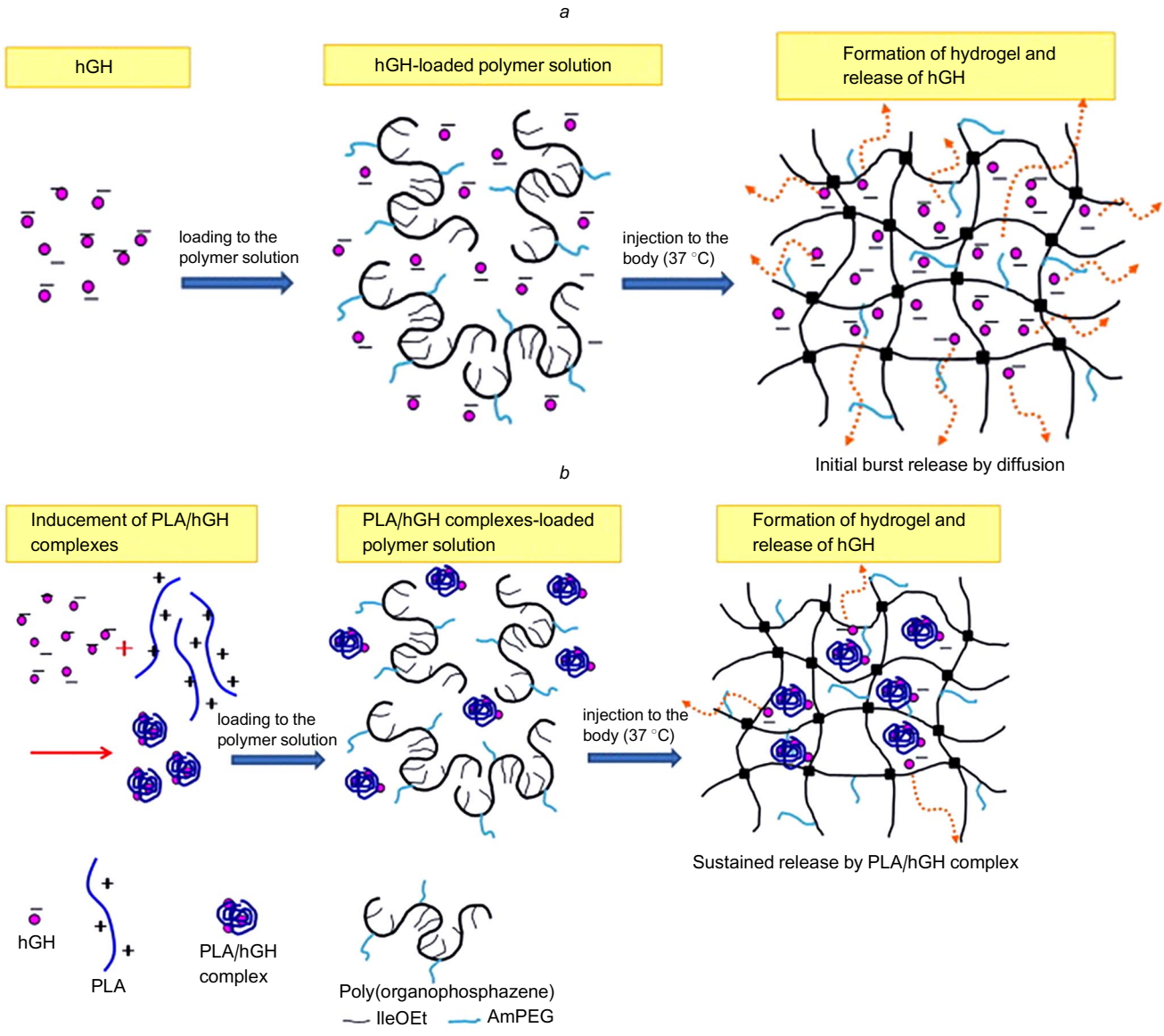


Figure 16. Schematic illustration of hydrogels; hGH/POP complex hydrogel (*a*) and hGH/PLA/POP complex-loaded hydrogel (*b*).[215]

anionic hydrogel was used to ensure the availability and sustained release of hGH. The first electrostatic interaction was due to the anionic hGH and the cationic protamine sulfate (PS) and PEC. The second electrostatic association was caused by the interaction of PEC and anionic thermosensitive POPs. This dual ionic system-based hydrogel occurred in the 'sol' form at low temperature and in the 'gel' form at the body temperature (Fig. 17). The PEC-loaded hydrogels showed sustained *in vitro* and *in vivo* release of hGH for long time periods without any burst release. It was also found that single injection of PEC anionic hydrogel loaded with hGH was more efficient than

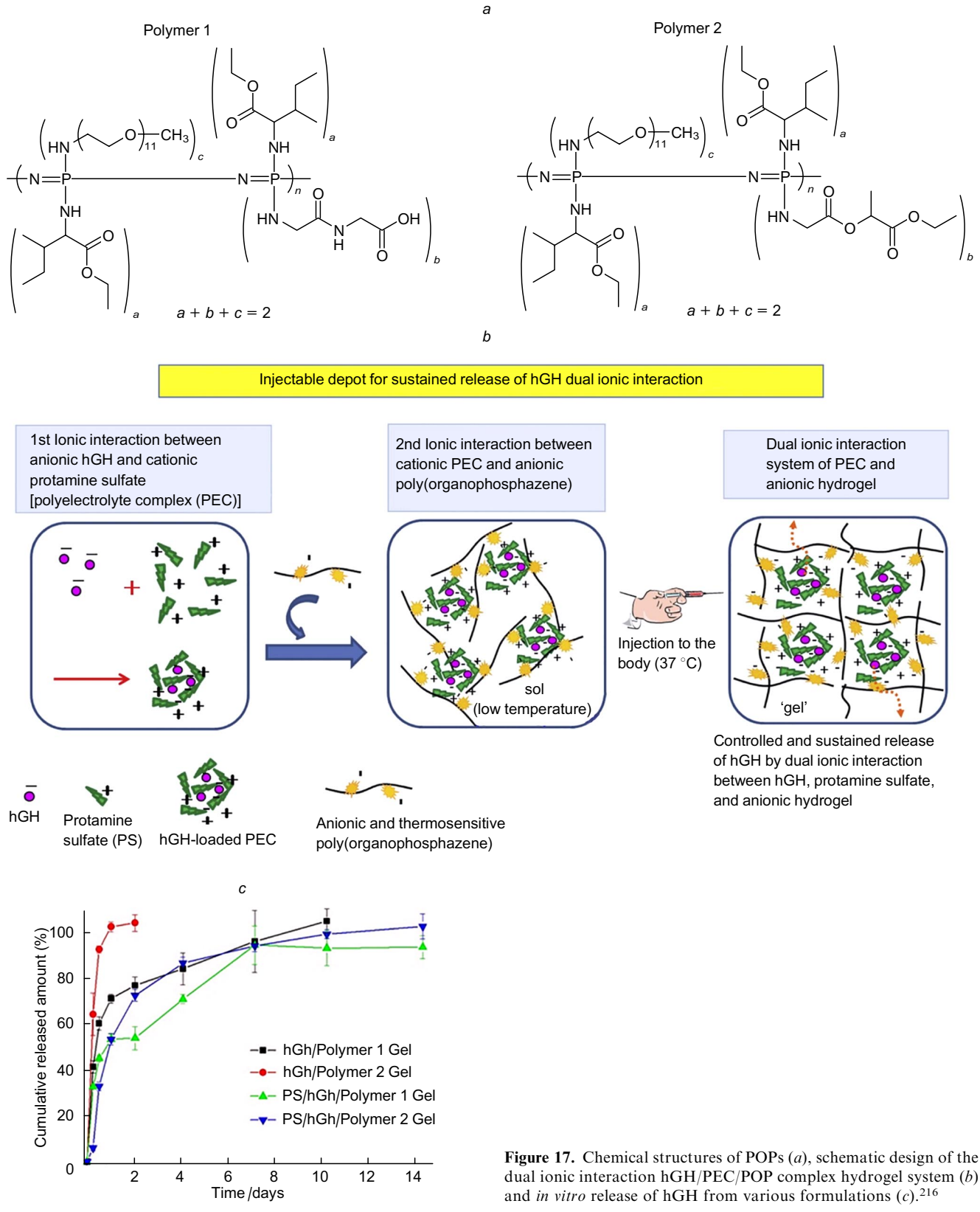
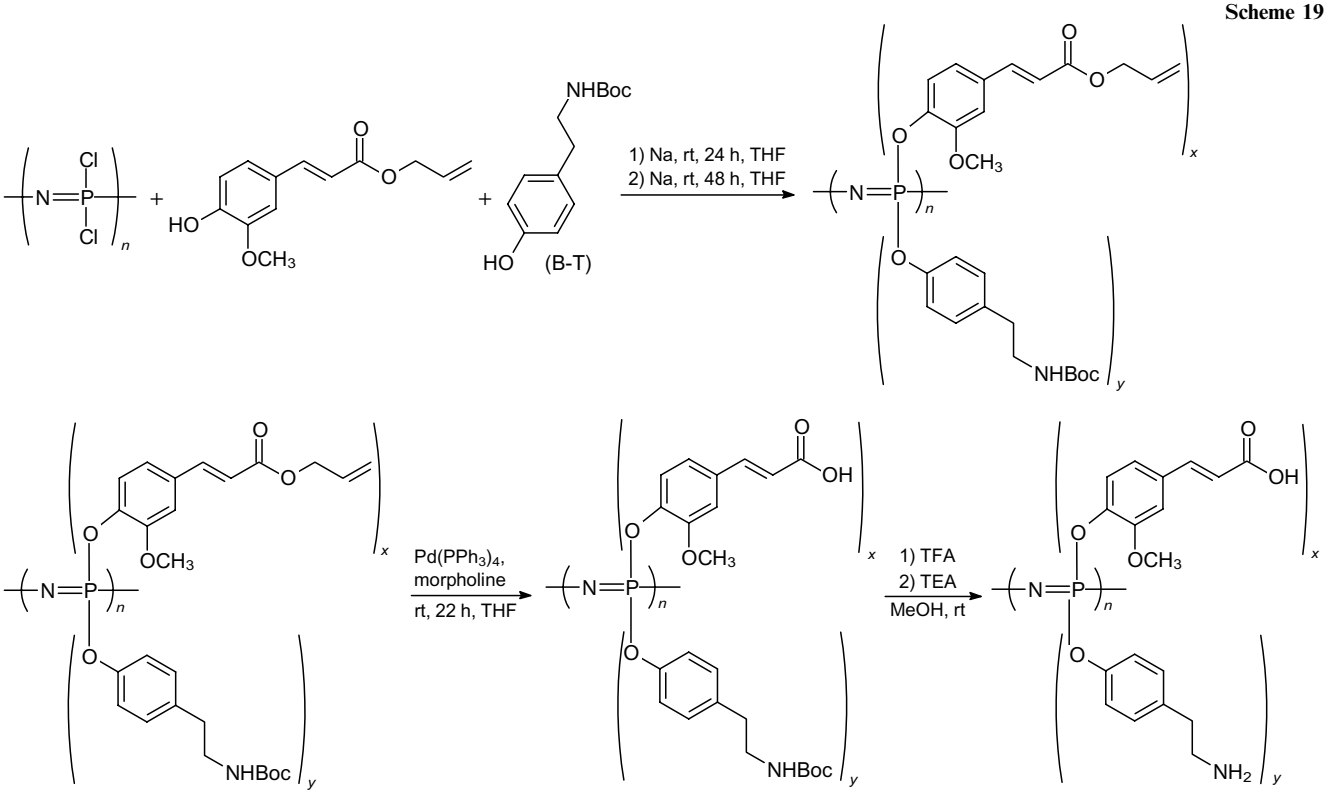


Figure 17. Chemical structures of POPs (*a*), schematic design of the dual ionic interaction hGH/PEC/POP complex hydrogel system (*b*) and *in vitro* release of hGH from various formulations (*c*).[216]

the daily injection of hGH solution for one week. Hence, these hGH-loaded hydrogels based on dual ionic interactions provided efficient delivery of hGH with enhanced bioavailability.[216]

Another new series of hydrogels was developed by Morozowich *et al. via* interaction of two different types of hydrophilic polymers.[217] The researchers used amino-functionalized POP containing tyramine co-substituted with ferulic acid side groups (Scheme 19).[217] The other component of hydrogels was aldehyde-modified dextran with biocompatible, hydrophilic and non-toxic properties. In addition, it was flexible toward different levels of aldehyde loading. Mixing of aqueous solutions of POP with aldehyde functionalized-dextrans resulted in the formation of a gel at room temperature *via* Schiff base chemistry, *i.e.*, the amino and aldehyde functionalities of the POP and dextran, respectively, reacted to form the imine bond at the body temperature. Hydrogels **A** (POP + 25% ox-Ald-Dex) and **B** (POP + 98% ox-Ald-Dex) have different storage moduli, average pore sizes and cross-linking densities, depending on the aldehyde content. The Cryo-SEM and ESEM studies of hydrogels **A** and **B** showed lower and higher cross-linking densities with average pore size of 114 and 26 μm, respectively (Fig. 18). These hydrogels provide appealing oppor-

Scheme 19



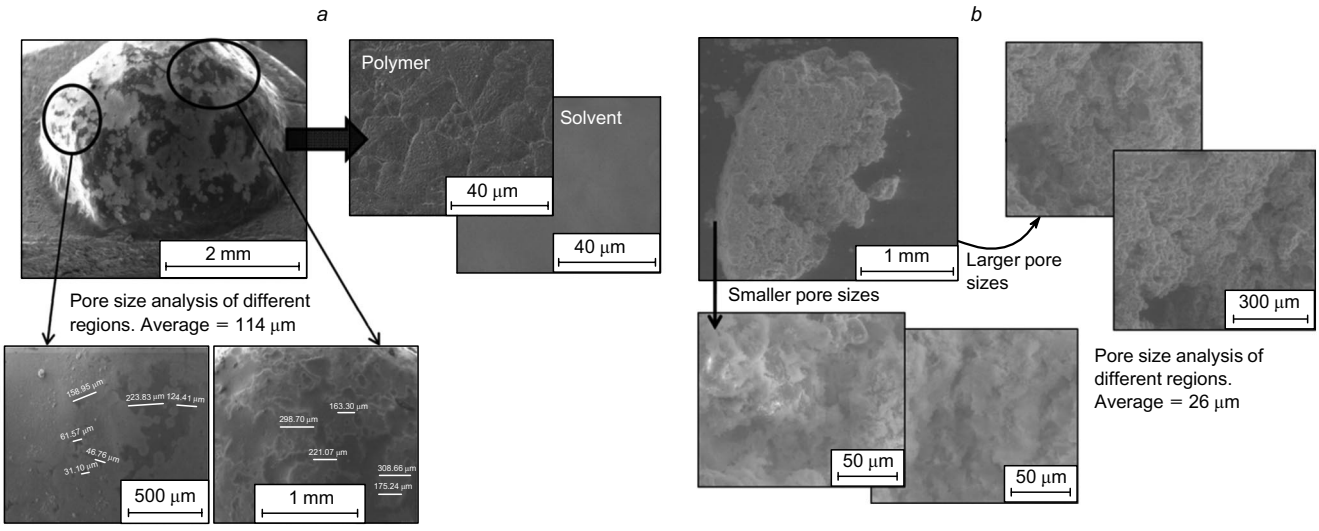
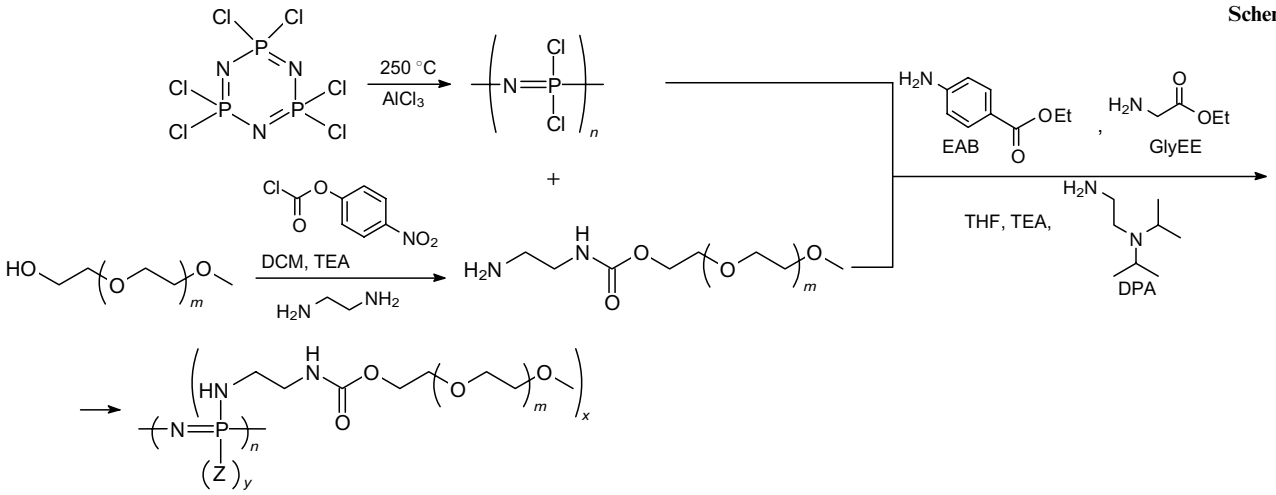


Figure 18. Cryo-SEM images of frozen hydrogel **A** (*a*) and ESEM images of hydrogel **B** (*b*).[217]

tunities for biomedical applications because the unbound aldehyde groups can react with living tissue to form strong adhesive linkages, while maintaining the imine cross-linking of the hydrogel. Moreover, more complex and bioerodible hydrogels based on POPs co-substituted with amino acid ester can be synthesized *via* this route.[217]

Scheme 20



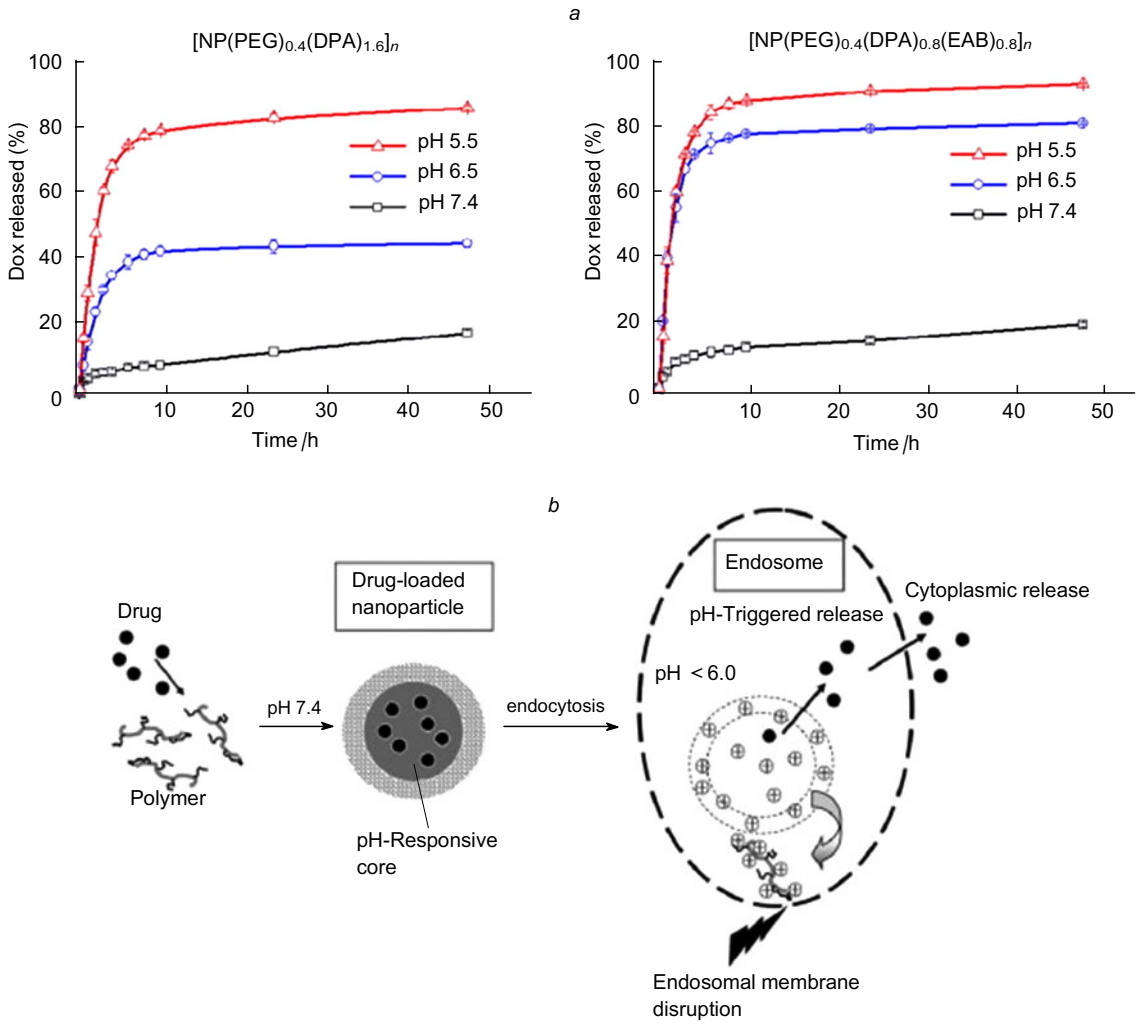


Figure 19. DOX release of POP nanoparticles at pH 5.5, 6.5 and 7.4 (*a*) and schematic representation of the release of loaded drug with rupturing of endosomal membranes (*b*).[221]

Zheng *et al.* synthesized a series of amphiphilic graft POPs using AMPEG and GlyEE side groups (Scheme 20).[218] Their polymeric micelles were loaded with doxorubicin (DOX). It was found that micelle morphology was markedly changed upon drug loading. Drug-free micelles from rod-like and spherical structures were changed into spheres and vesicles upon drug loading, respectively. *In vitro* experiments showed *s*ustained release, enhanced cytotoxicity against adriamycin-resistant human breast cancer MCF-7 cells and effective suppression of cell proliferation.[218] Zheng *et al.* synthesized another series of amphiphilic graft POPs using AMPEG and ethyl *p*-aminobenzoate (EAB) side groups (see Scheme 20).[219] It was found that a high AMPEG content resulted in the formation of spherical micelles, while a decrease in the PEG content resulted in vesicle-like structures (polymersomes). Water-soluble anticancer drug (DOX HCl) was successfully loaded into the core of polymersomes. It was also found that polymersome-based delivery provided enhanced cytotoxicity of DOX against the HepG2 cells.[219]

Another series of POPs was synthesized by linking *N*,*N*-diisopropylethylenediamine (DPA) with and without EAB to a backbone of POPs grafted with AMPEG (see Scheme 20).[220, 221] These amphiphilic POPs were self-assembled into nanoparticles. These nanoparticles showed pH-responsive drug release with the ability to disrupt endosomal membranes with better *in vitro* efficacy as compared to free doxorubicin. The burst release of loaded drug into the cytoplasm is caused by pH-sensitive rupturing of the endosomal membranes of compartments, which helps to minimize the chance of drug efflux *via* exocytosis and reversal of drug resistance of cancer cells. These pH-responsive POP nanoparticles are promising for the treatment of tumours (Fig. 19).[221]

Afterward, a further detailed study of *in vivo* biodistribution and anti-cancer effect of drug-loaded polymersomes was carried out.[222] Since the polymersomes have both hydrophilic and hydrophilic chambers for water-soluble and water-insoluble drugs, hydrophilic DOX·HCl or hydrophobic DOX can both be successfully encapsulated into the self-assembled POP polymersomes using dialysis (Fig. 20). This study showed that DOX·HCl-loaded POP polymersomes exhibited pH-sensitive drug release, enhanced blood circulation time and therapeutic efficacy. Moreover, this study also revealed that POP-based polymersomes loaded with hydrophilic and hydrophobic drugs have advantages over the conventional micelles and liposomes as drug carries (see Scheme 20).[222]

Xu *et al.* loaded DOX·HCl or DOX into POP-based polymersomes (EAB/AMPEG-POPs; see Scheme 20) with chloroquine phosphate (CQ) for drug resistance-reversal in the weight ratios of 1 : 1 or 2 : 1 through common dialysis process.[223] Their cytotoxicity study on DOX-resistant MCF-7/Adr breast cancer and HL60/Adr leukemia cells showed enhanced toxicity. Besides, an *in vivo* tumour inhibition study performed on zebrafish xenograft model also revealed significantly improved sensitivity of the MCF-7/Adr breast cancer to chemotherapeutics (Fig. 21).

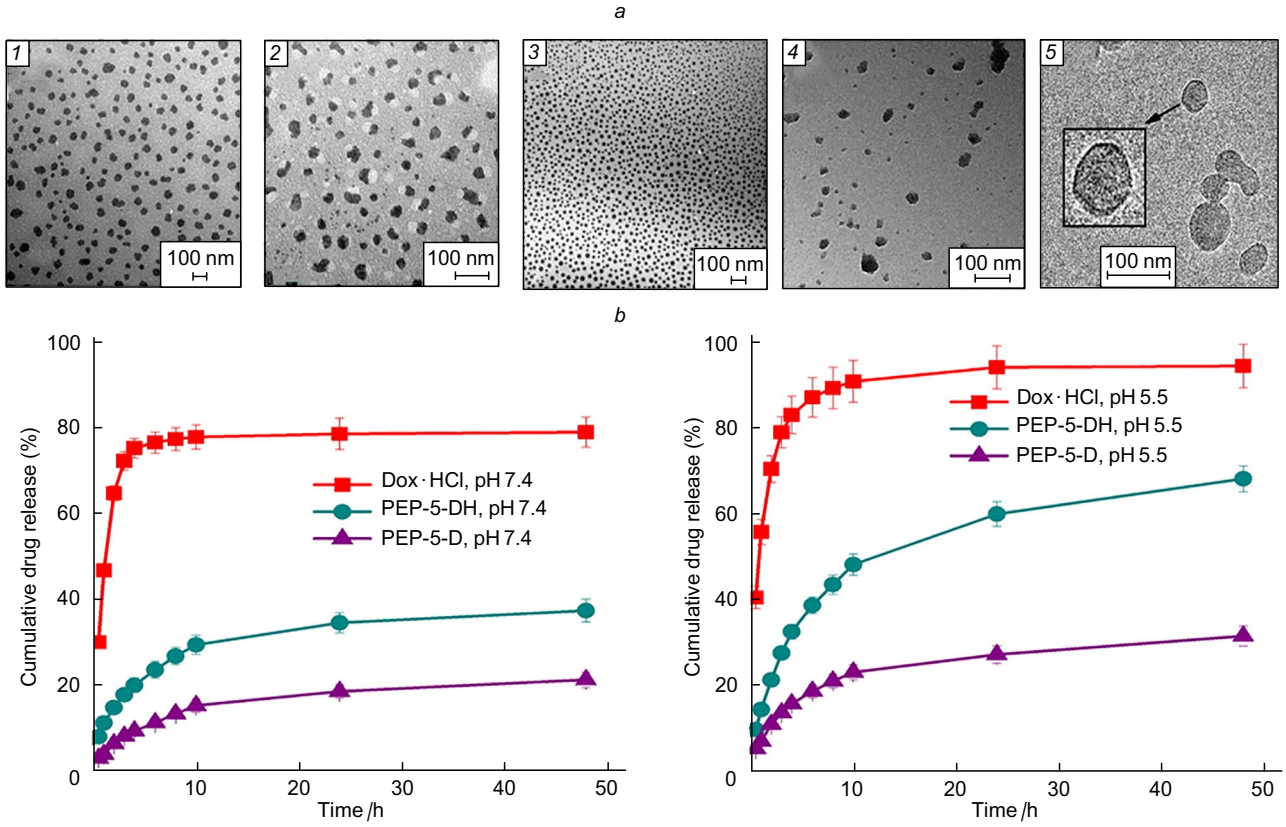


Figure 20. (*a*) TEM image of (*1*) blank PEP-1, (*2*) blank PEP-2, (*3*) PEP-5-DH, (*4*) PEP-5-D and (*5*) cryo-TEM image of blank PEP-5; the inset displays the enlarged image of PEP-5; (*b*) *in vitro* drug release behaviours of free DOX·HCl, PEP-5-DH and PEP-5-D at 37 °C in pH 7.4 PBS and in pH 5.5 PBS. PEP is poly[methoxy-poly(ethylene glycol)(ethyl *p*-aminobenzoate)phosphazenes]; in the series PEP-1, PEP-2 and PEP-5, the percentage of EAB increases.[222]

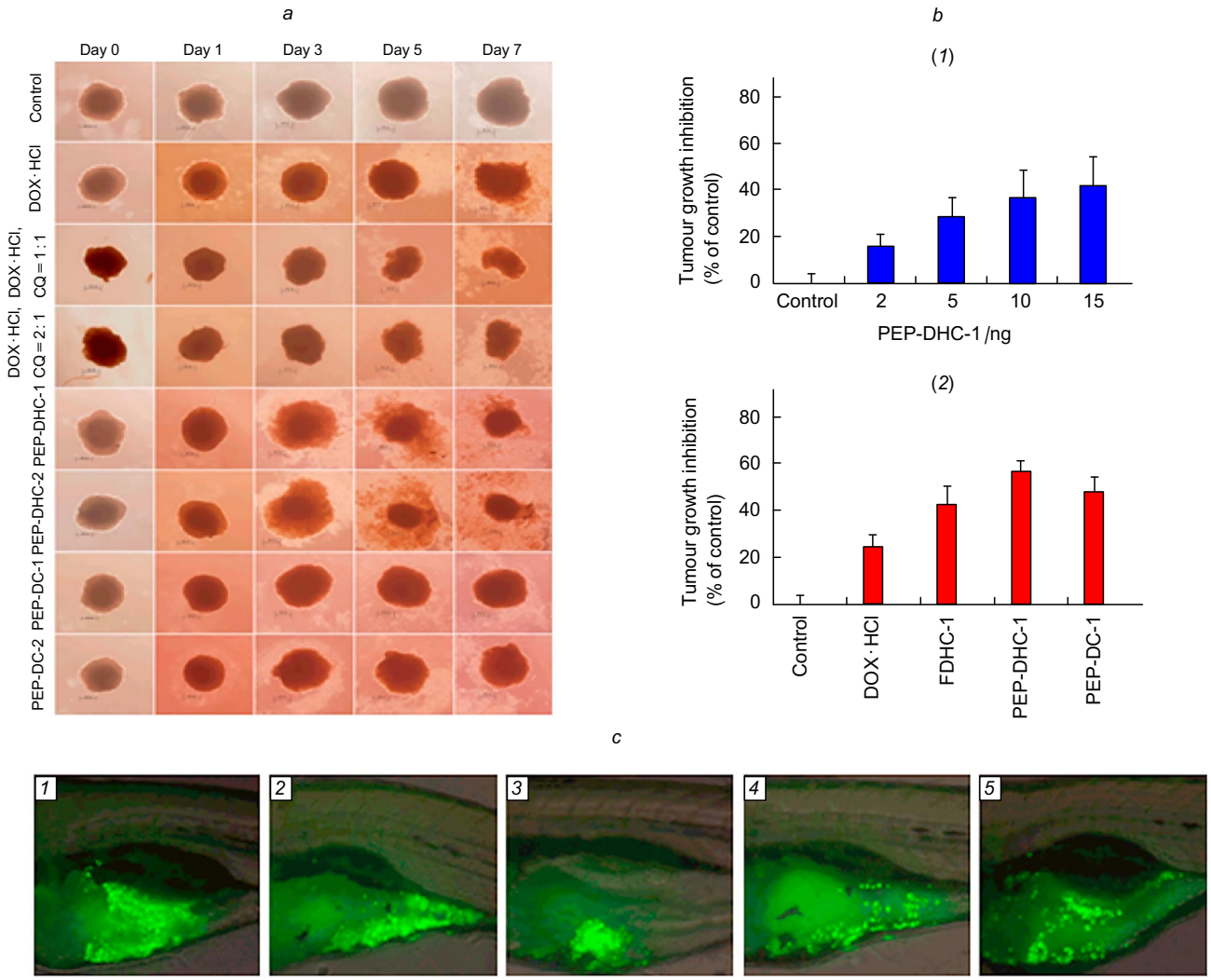


Figure 21. Growth inhibition assay; images of untreated (control) and treated (for one week) tumour spheroids (*a*) and drug treatment effect in tumour inhibition of zebrafish (*b*): (*1*) quantitative effect comparative to control; (*2*) qualitative effect relative to saline control and DOX·HCl, FDHC-1, PEP-DHC-1, PEP-DC-1 in equivalent of 15 ng DOX·HCl/DOX content and (*c*) fluorescence images of zebrafish after treatment with: (*1*) control, (*2*) DOX·HCl, (*3*) FDHC-1, (*4*) PEP-DHC-1 and (*5*) PEP-DC-1. FDHC-1 and FDHC-2 are mixtures of free DOX·HCl and CQ in 1 : 1 and 1 : 2 ratios; DHC = DOX·HCl, DC = DOX.[223]

Hence, this study showed that DOX·HCl and CQ co-delivery system based on POP polymersomes might be advantageous for drug resistance reversal of cancer therapy with enhanced anti-cancer efficacy.[223]

The POP-based polymersomes (DPA/AMPEG-POPs) were loaded with recombinant murine IL-12 plasmid (pmIL-12) for its systemic delivery to avoid the severe toxicity associated with its local administration.[26] These polymersomes were found to be biocompatible and quite stable, despite the activities of serum protein and DNase. A significant *in vivo* suppression of tumour growth was observed after intravenous injection of the pmIL-12 polymersomes in mice bearing CT-26 colon carcinoma.[26] Zhu *et al.* synthesized co-substituted POPs (EAB/AMPEG-POPs).[224] These POPs were further functionalized by reacting with tris(2-aminoethyl)amine (TAA) to form amino-functionalized POPs. These functionalized POP-based polymersomes showed significant potential for carboplatin delivery in cancer therapy.[224] Fu *et al.* introduced gold nanoparticles in these functionalized POP-based polymersomes for dense packing of their membrane to minimize the premature leakage of hydrophilic drug.[158] It was found that these hybrid polymersomes showed prolonged blood circulation time and less premature leakage of the hydrophilic drug at pH 7.4 as well as enhanced antitumour efficacy as compared with free drug or drug-loaded POPs (Fig. 22).[158]

Li *et al.* developed POP-based polymersomes (AMPEG/EAB-POPs) anchored with a target functional group for enhanced cellular uptake of hydrophilic drugs for the targeted tumour therapy.[225] The researchers found that polymersome-based targeted therapy is a significant achievement for cancer therapy by hydrophilic drugs.[225] Peng *et al.* prepared electroneutral composite polymersomes by using two types of amphiphilic POPs, AMPEG/EAB-POP and DPA/AMPEG-POP, and successfully loaded a well-known paclitaxel resistance inhibitor (miR-200c) with high loading efficiency.[226] These POP-based composite polymersomes loaded with miR-200c exhibited enhanced anticancer efficacy even at low doses as compared with nanoparticles of PEEP or POPs individually. Moreover,

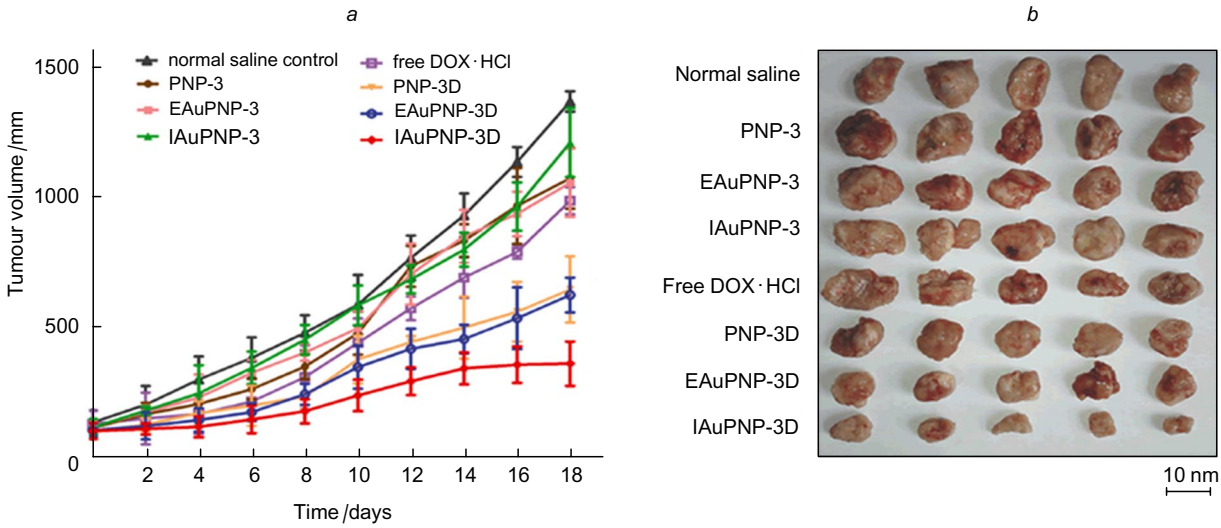
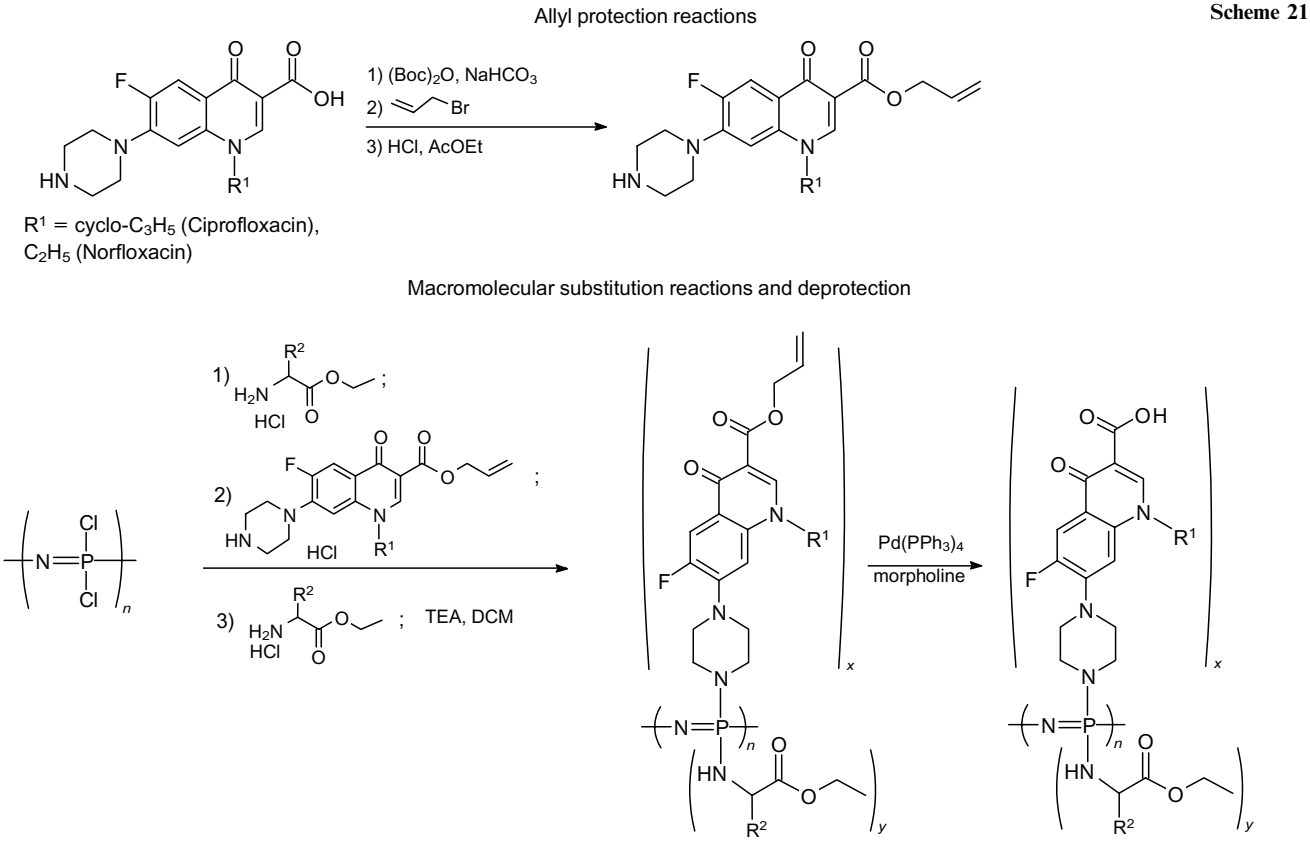


Figure 22. Gold nanoparticles in POP-based hybrid polymersomes; mice were injected with equivalent doses of 5 mg kg^{-1} DOX·HCl in DOX·HCl, PNP-3D, EAuPNP-3D and IAuPNP-3D. Saline treatment was used as a control; changes in the tumour volume over 18 days (n = 5) (*a*) and therapeutic effects on mice that were treated with different drug systems (*b*). PNP-3 is polyphosphazene; EAuPNP-3 and IAuPNP-3 are polymersomes loaded with Au nanoparticles prepared beforehand (E) or *in situ* (I); D stands for doxorubicin loading.[158]

Scheme 21



Symbols represent substitution patterns

	Ciprofloxacin (C)	Norfloxacin (N)
Alanine ethyl ester (A)	AC′ (12%) : AC	AN
Glycine ethyl ester (G)	GC	GN
Phenylalanine ethyl ester (P)	PC	PN

Ciprofloxacin (R^1 = cyclo-C_3H_5)
AC: R^2 = CH_3, x = 0.5, y = 1.5;
AC′: R^2 = CH_3, x = 0.24, y = 1.76;
GC: R^2 = H, x = 0.48, y = 1.52;
PC: R^2 = Bn, x = 0.42, y = 1.58

Norfloxacin (R^1 = C_2H_5)
AN: R^2 = CH_3, x = 0.44, y = 1.56;
GN: R^2 = H, x = 0.44, y = 1.56;
PN: R^2 = Bn, x = 0.48, y = 1.52

these POP-based composite polymersomes were found to be hemocompatible and highly stable to ribonuclease. These results show that the use of POP-based composite polymersomes for therapeutic delivery provides an efficient way for human lung cancer therapy.[226] Tian *et al.* synthesized POPs containing antibiotics, ciprofloxacin or norfloxacin, as side groups together with ethyl esters of various amino acids such as glycine, alanine and phenylalanine. Scheme 21 shows the synthetic route to the POPs comprising 1) allyl protection reactions, 2) macromolecular substitution reactions and 3) deprotection.[227]

The hydrolysis of all of these POPs resulted in nearly neutral hydrolytic end products, with the pH being in the range of 5.9 to 6.8. About 5% to 23% mass loss with 4% to 30% antibiotic release was found for POP films. Meanwhile, 40% to 45% mass loss with 45% to 50% antibiotic release was observed for POPs nano/microfibres. Fast degradation rate and antibiotic release were observed for POPs containing alanine or glycine ethyl ester as compared with POPs co-substituted with phenylalanine ethyl ester with antibacterial activity throughout degradation period (six months), thus indicating the release of ciprofloxacin or norfloxacin from POPs (Fig. 23). Hence, the above results showed that POPs based on various therapeutic agents can be designed with required parameters and applied as biomaterials for tissue engineering. Moreover, these POPs can be used as coating materials and other implantable devices for post-surgery applications, or as pro-drugs for the treatment of long-lasting local infection diseases.[227]

Teasdale *et al.* synthesized a series of hydrophilic, multifunctional POPs using Boc-protected hydrazide linker and co-substitution with hydrophilic polyalkylene oxide side groups *via* living cationic polymerization for targeted delivery of anticancer drugs (epiubicin and folic acid), as shown in Scheme 22.[228] These drug-loaded POPs showed pH-dependent drug release over periods of a few days. Their degradation rate was found to depend on the type of substituents. These multi-functional POPs showed that the incorporation of amino acid ester and other biodegradable side groups provides opportunities for biomedical applications, especially for targeted delivery of anticancer drugs.[228]

A number of attempts have been made to develop several antimalarial drugs in order to find out more effective and less toxic compounds. POP-based drug conjugates have been used for treatment of malaria. Currently, resistance to antimalarial drugs has developed. The simultaneous loading of two antimalarial drugs having different modes of action provides opportunities to inhibit the growth of resistance to either of the components. The combination therapy showed significant antimalarial ability with minimal drug contents as compared to the standard drug combination. Kumar *et al.* designed a series of POP drug conjugates using 2-propoxy, 4-acetamidophenoxy, 4-formyl and 4-aminoethylbenzoate as side groups co-substituted with primaquine (PQ) and dihydroartemisinin (DHA) drugs (Scheme 23).[229]

These synthesized POP drug conjugates were fabricated to nanoparticles to achieve novel controlled combined drug delivery approach for radical cure of malaria. These nanoparticles demonstrated a two-step drug release in *in vitro* studies, in particular, the initial burst release was followed by a sustained release due to non-Fickian diffusion and Fickian diffusion patterns, respectively. Their antimalarial efficacy was investigated through *in vivo* experiments by using plasmodium berghei (NK65 resistant strain) infected mice at different doses (Fig. 24). These nanoparticles were found to be effective against drug resistant malaria owing to their longer protection times (about 35 days) without any risk of recurrence.[229]

Chen *et al.* synthesized a series of amphiphilic random poly(β-D-glucose-co-1-octyl)phosphazene (PGOP) copolymers with glycosyl functionalization degrees of 58.1% (PGOP-1), 74.1% (PGOP-2) and 87.0% (PGOP-3) using a two-step thiol – ene coupling reaction (Scheme 24).[230] These amphiphilic POPs were found to self-assemble into various morphological shapes, including spheres, rods and vesicles both in water and in water – DMF mixtures; particular structures were obtained *via* adjusting the ratio of water and DMF in the mixture as well as hydrophilic or hydrophobic content of POPs. At first, these POPs were dissolved in DMF and then slowly transferred to water. In the 2% to 0% range of water content, micelles were observed. When more water (23% – 28%) was added, then rod-like structures were found and ultimately these rod-like structures were converted into vesicles upon addition of more water (> 50%), as shown in Fig. 25. The self-assembly is due to hydrophilic and hydrophobic interactions between the polymer and the solvents. These POPs can be used for controlled release of both hydrophilic and hydrophobic drugs.[230]

Recently, Akram *et al.* synthesized three new co-substituted POPs using three different side groups with different hydrophobicity and formed their blends with PMMA (75 : 25).[129] These blends were used to fabricate microspheres for drug delivery applications. The microspheres were found to exhibit good encapsulation behaviour and sustained release of the aspirin drug (Fig. 26).[129]

The reductive responsive POP nanovehicles have been tested owing to their ability to deliver anticancer drugs to intracellular parts. Amphiphilic POPs were synthesized using hydrophilic reductive sensitive amino-terminated PEG (PEG-SS-NH_2) and D-phenylalanine methyl ester (DPME) hydrophobic groups with different mole fractions (Scheme 25).[159]

Solvent-dependent self-aggregation phenomenon was found in these POPs due to their amphiphilic nature, and spherical micelles (~50 nm size) were formed. Various organic co-solvents, THF, DMF and DMSO, were used to fabricate micelles *via* dialysis procedure. Micelles loaded with a number of hydrophobic drugs such as camptothecin (CPT) and indomethacin were cleaved in a micro-reductive environment using dithiothreitol (DTT) as a reducing agent. About 70% – 80% of CPT release was found within 15 h. The encapsulation efficiency of CPT was dependent on the nature of solvent and the mole ratios of side groups in the POP.[159]

Henke *et al.* prepared two POPs with controlled molecular weight and relatively narrow PDI by living cationic polymerization using a Boc-protected amine linker and jeffamine as substituents (Scheme 26).[231]

After deprotection, the free NH_2 linker provided binding sites for the subsequent conjugation with monocarboxylated Pt^{IV} complexes (A and B). These water-soluble conjugates simultaneously released the Pt^{II} active species, oxaliplatin, and a cisplatin derivative upon intracellular reduction. These POP – platinum(II) drug conjugates were found to be highly soluble in water, but with a long hydrolytic degradation time. Significantly enhanced (30-fold) cellular uptake and improved tumour shrinkage ability were observed with less resistance to drug-resistant sublines compared with free Pt^{IV} complexes. However, their cytotoxicity was still lower than that of clinically established Pt^{II}

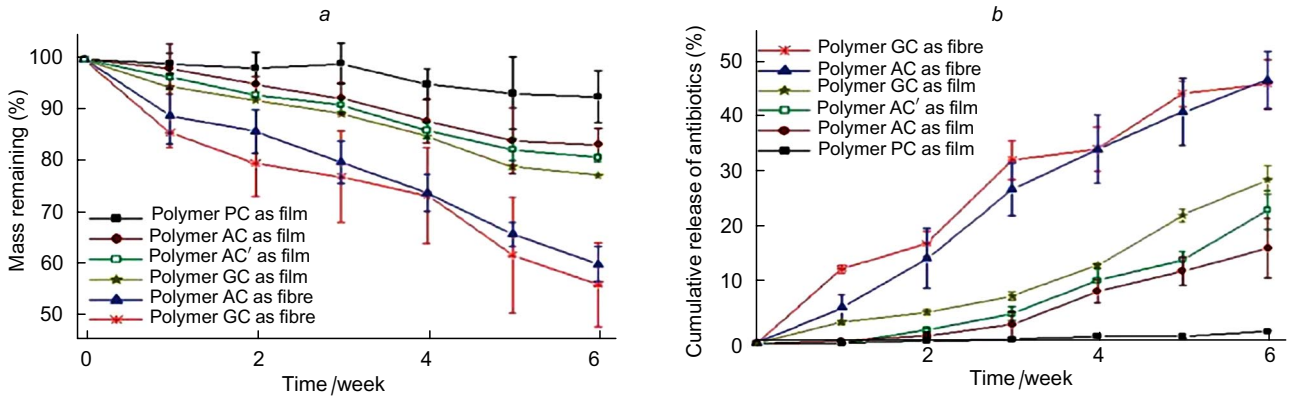
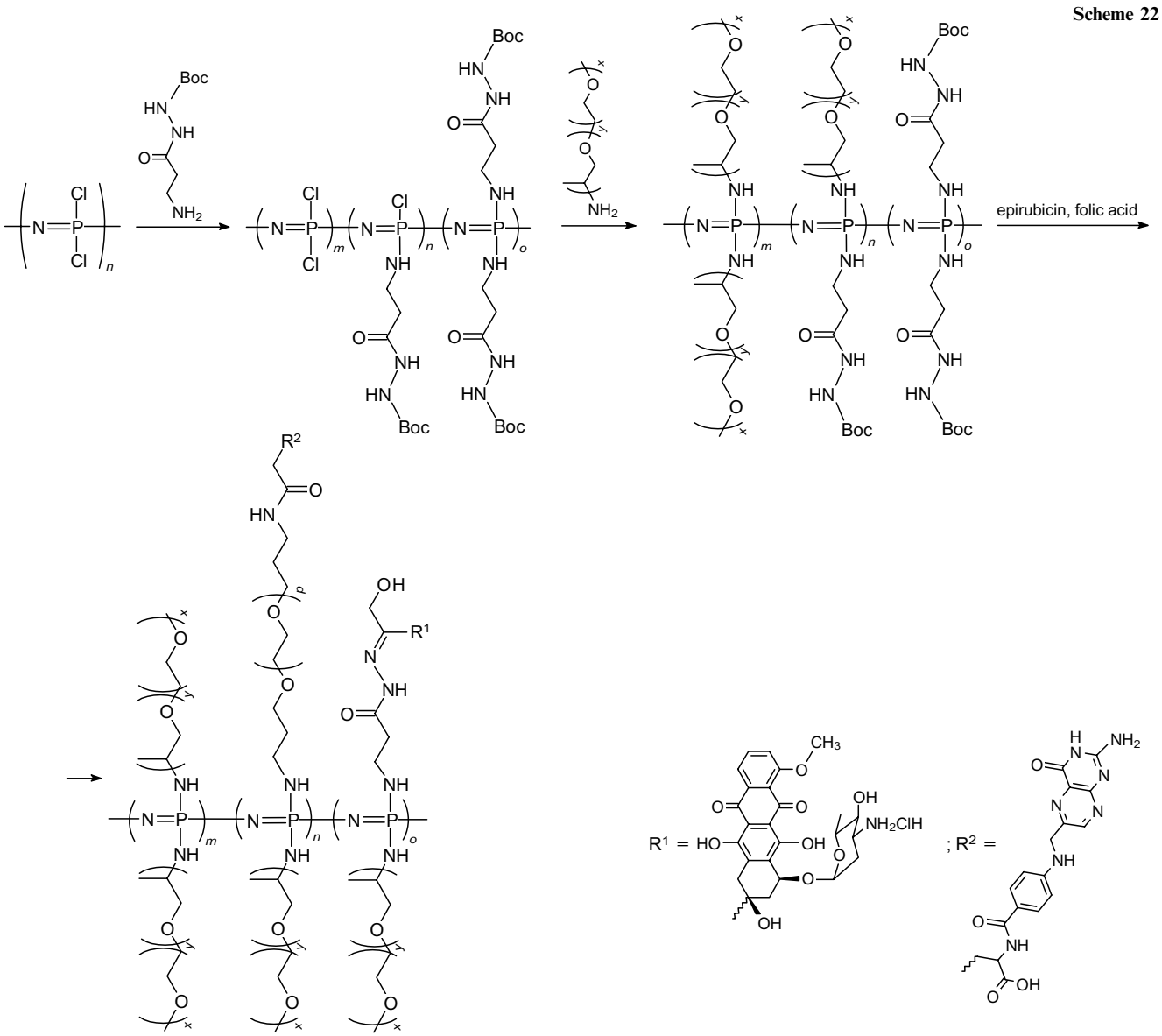
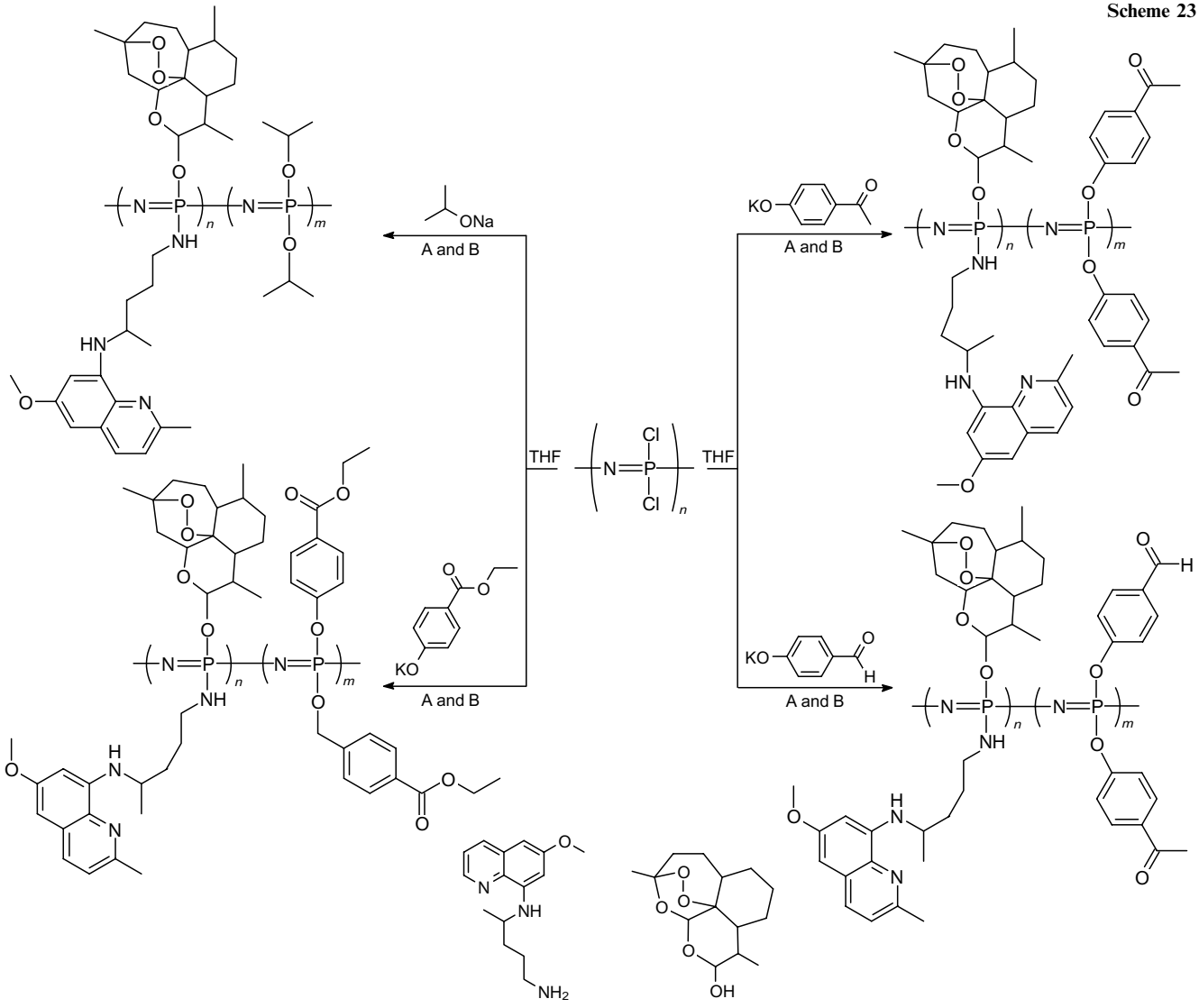


Figure 23. Hydrolytic degradation of POPs (*a*) and cumulative release profile of POPs containing ciprofloxacin as films and as fibre mats (*b*).[227]

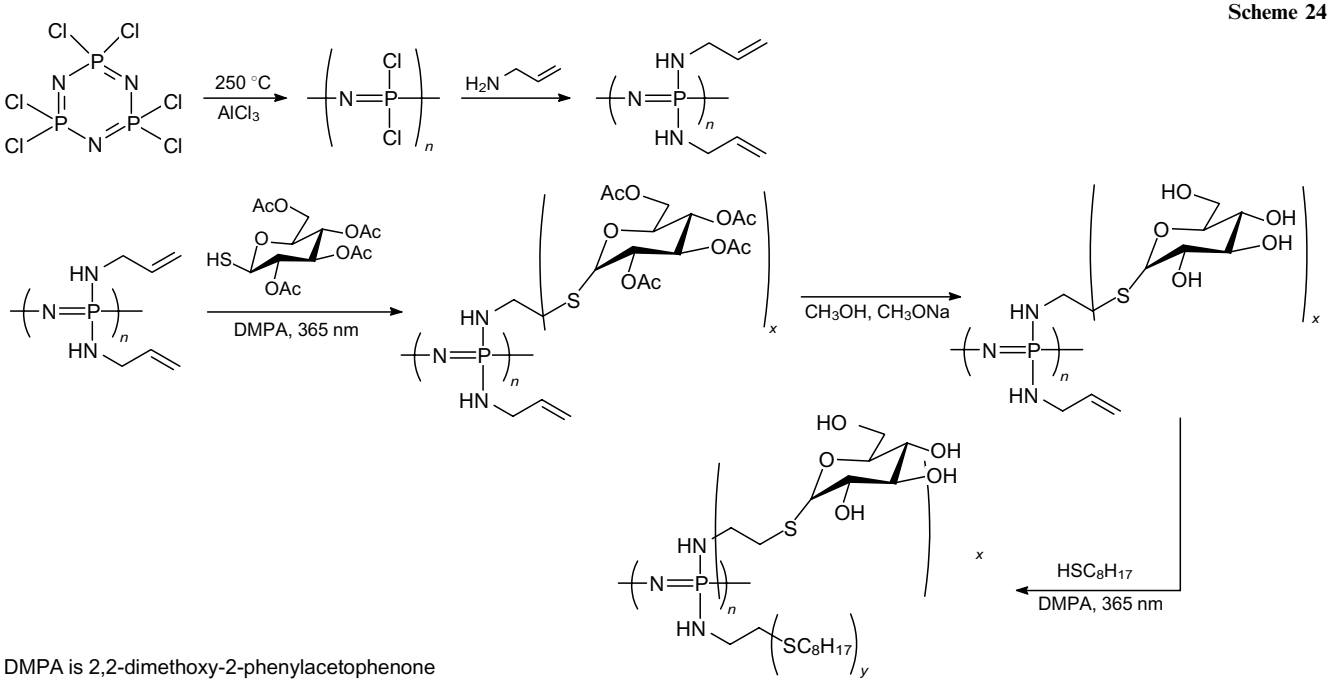
Scheme 22



Scheme 23



Scheme 24



DMPA is 2,2-dimethoxy-2-phenylacetophenone

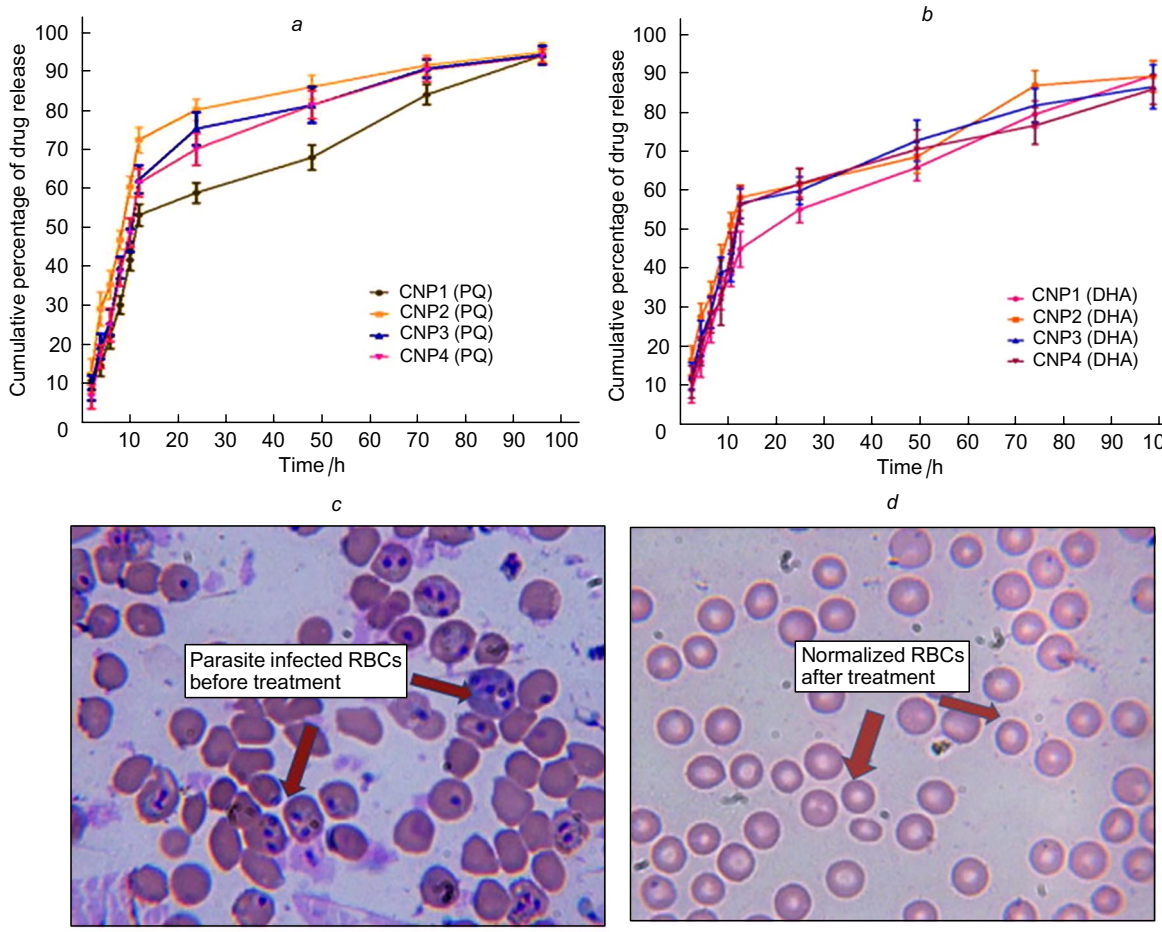


Figure 24. Cumulative percentage of drug release at pH 7.4 for primaquine (*a*), dihydroartemisinin (*b*) and infected red blood cells (before treatment) (*c*) and normalized red blood cells (RBCs) (after treatment) with POP – drug conjugate nanoparticles (*d*). CNP1, CNP2, CNP3 and CNP4 are nanoparticles fabricated from different polymer – drug conjugates with the OPr^i, OC_6H_4NHAc-4, OC_6H_4CHO-4, and $NHC_6H_4CO_2Et$-4, respectively.[229]

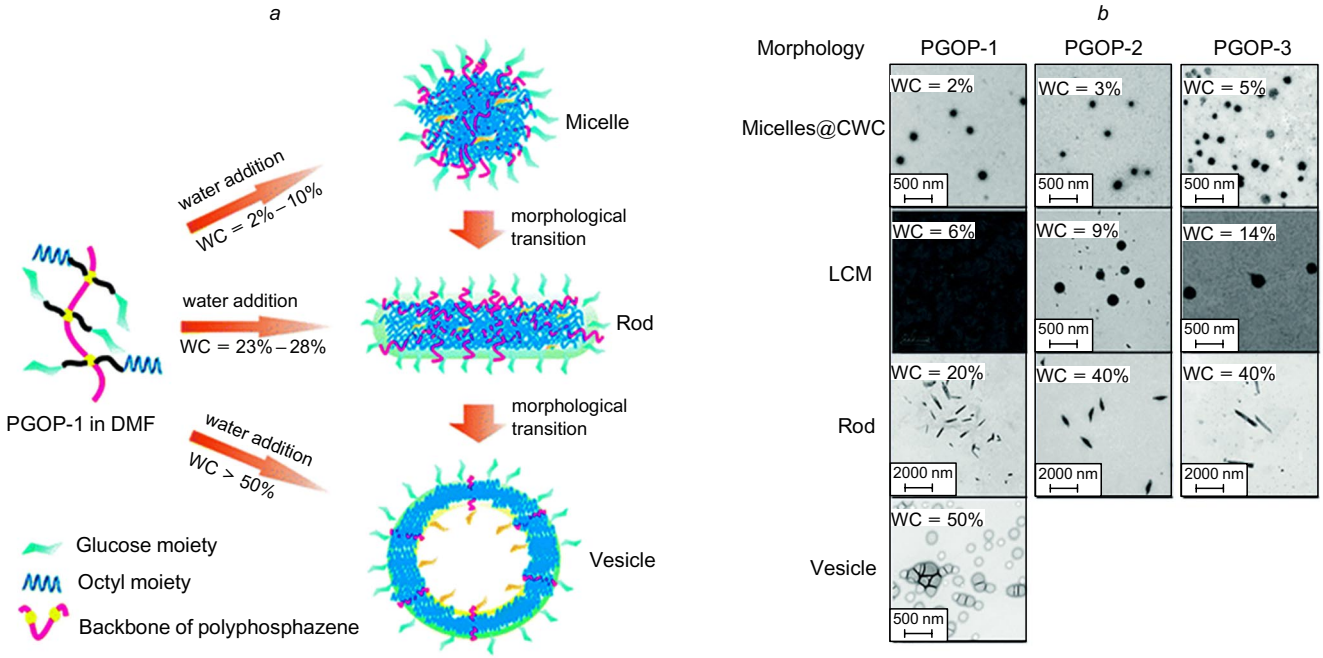


Figure 25. Morphological change of poly(β-D-glucose-co-1-octyl)-phosphazenes in a DMF – H_2O mixture (*a*) and TEM images of self-assembled morphologies of POPs with different water contents (WC) (*b*).[230] LCM are large compound micelles, CWC is critical water content.

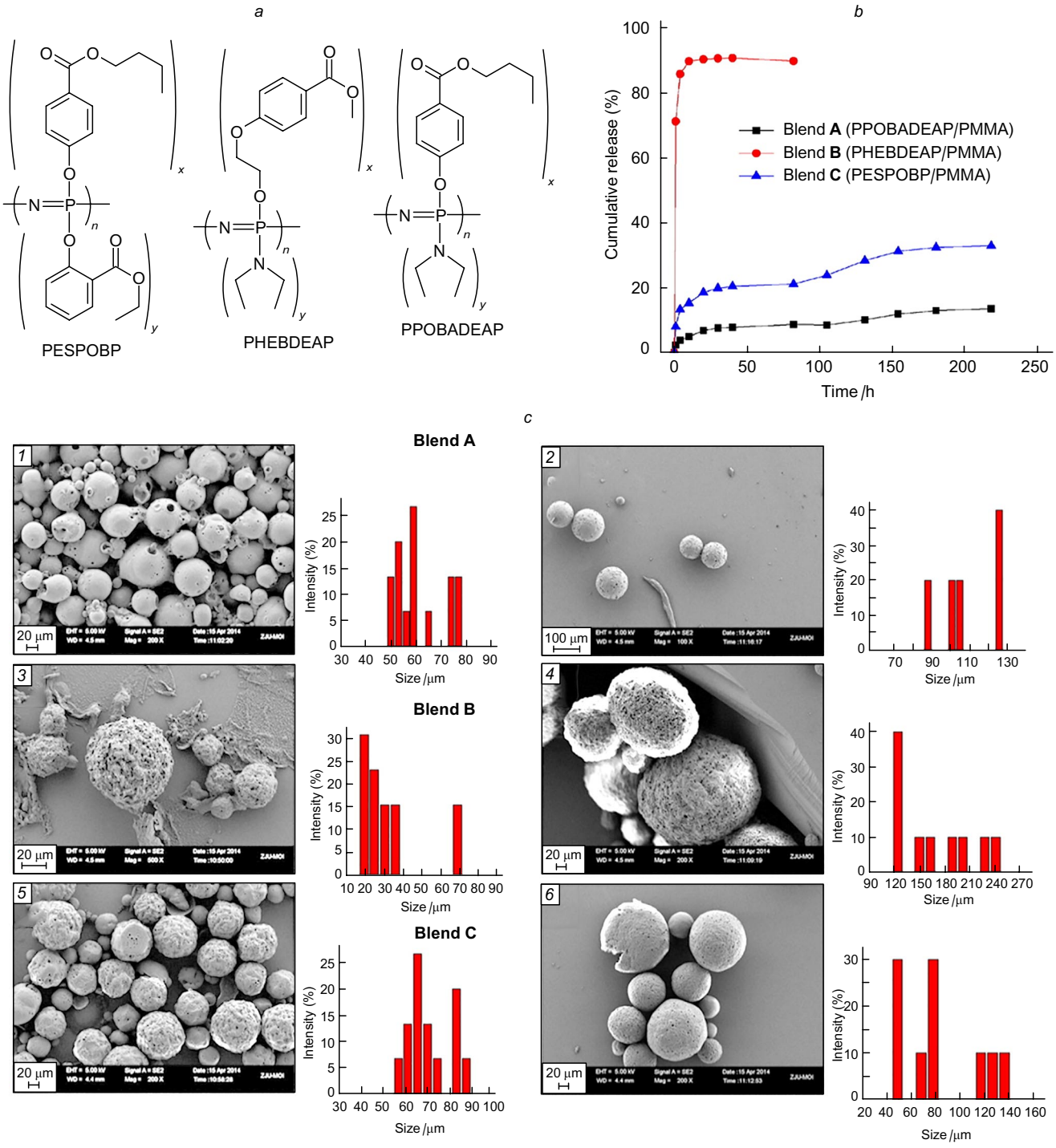
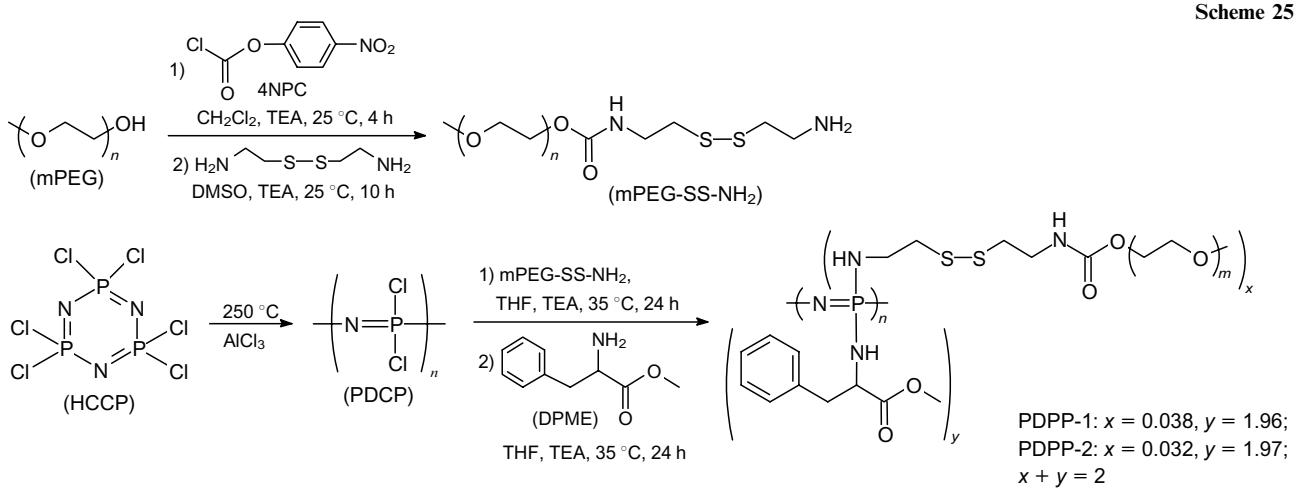


Figure 26. The chemical structures of POPs (*a*), percentage of cumulative release *in vitro* of aspirin at 37 °C and pH 7.4 (*b*) and SEM images of blank (*1*, *3*, *5*) and drug-loaded microspheres (*2*, *4*, *6*) of blends **A** (PPOBADEAP/PMMA), **B** (PHEBDEAP/PMMA) and **C** (PESPOBP/PMMA) with their mean diameters (*c*).[129]

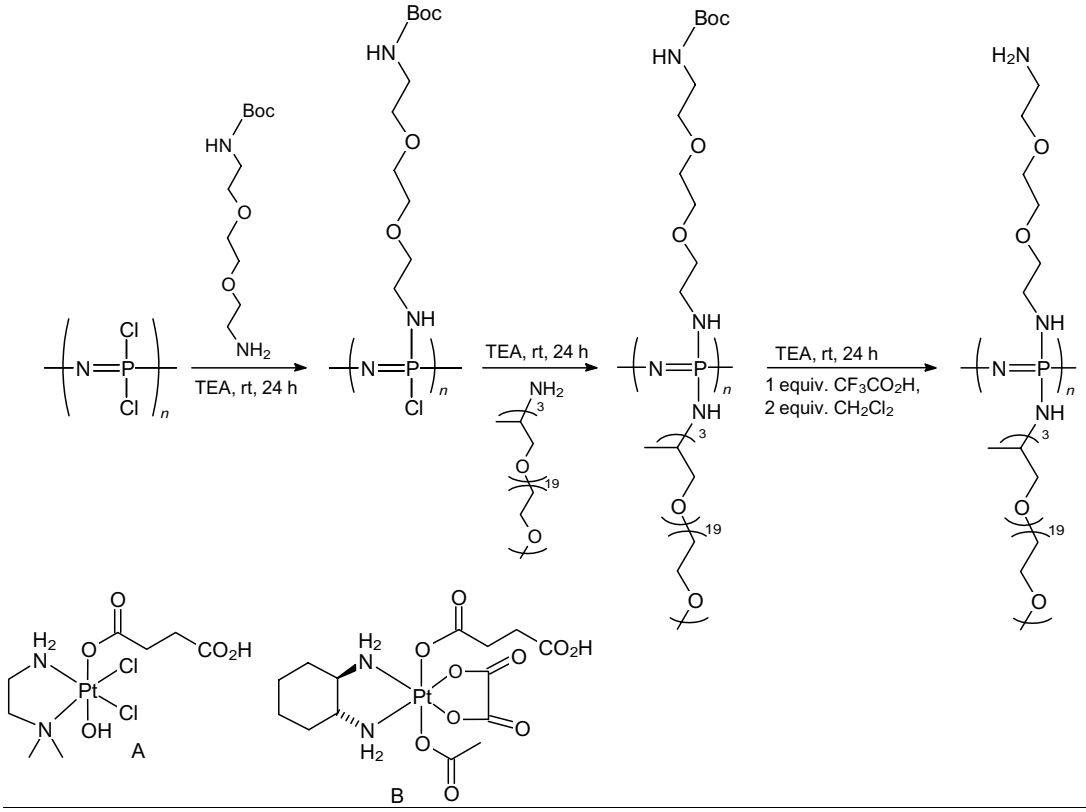
drugs, perhaps because of slow reduction. A considerably high cytotoxicity was observed over long period of times. Hence, these studies showed a progress in the development of anticancer drug carriers. However, there is a need to improve their reduction kinetics and endosomal release of free platinum(II) drugs.[231]

Linhardt *et al.* synthesized a series of water-soluble and biodegradable POPs by grafting peptide sequences onto the PDCP backbone and subsequent co-substitution with hydrophilic polyalkylene oxide, jeffamine M-1000.[232] These POPs showed excellent opportunities as polymeric carries for therapeutic drug delivery through blood circulation.[232] In order to overcome the limitations [233] associated with conventional nanomedicine, cancer therapy is gradually changed into more advanced form of personal therapy, *i.e.*, tumour-targeting multi-functional theranostics therapy,[234, 235] which makes it possible to perform both the diagnostic and therapeutic functions at the same time.[232]

Scheme 25



Scheme 26



5. Conclusion

Poly(organophosphazenes) (POPs) are the emerging class of biocompatible and biodegradable materials that possess almost outstanding properties with increasing biomedical applications, particularly, for tissue engineering and drug delivery. Poly(organophosphazenes) have advantages over the natural polymers due to their biocompatibility, synthetic flexibility, structural diversity of wide range of available side groups and biodegradability into non-toxic end products. Selective and easy substitution of side groups offer opportunities for adjusting the degradation rate, mechanical properties as well as to enhance the biocompatibility. In addition, osteocompatibility and osteoinductivity have made these polymers promising candidates for tissue engineering. Their various polymeric forms, *i.e.*, microspheres, nano/microfibres, micelles, polymersomes, hydrogels and nanoconjugate linear polymers provide different carriers to efficiently deliver various hydrophilic/hydrophobic therapeutic agents both *in vitro* and *in vivo*. Moreover, blending is another option to get desired properties of POPs.

However, there are still many challenges in both the synthetic and application aspects in the field of POPs. It is desired to develop multifunctional POPs for dual drug loading,[222] combination therapy, drug resistance reversal,[223] tissue engineering[3,173,236] and theranostic applications.[181] The introduction of extremely bulky nucleophiles as side groups to the phosphazene backbone is another interesting area of POPs.[237–239] To synthesize POPs with minimum toxicity, containing high payload, long-term blood circulation, accumulation in the injured tissue and

then controlled or quantitative release (in the case of anticancer drug) of therapeutics in response to stimuli are apparently the desired level of functionalities in POPs. In PDCP, each repeating unit has two active chlorine atoms attached to the phosphorus atom, which can be replaced with a wide structural diversity of available substituents, *i.e.*, amino acids, antioxidants, vitamins, antibiotics and alkoxy groups *via* three well-known linkages, that is, the P – N linkage, the P – O linkage and the P – C linkage, to endow POPs with characteristics properties which determine their applications. Investigation of other linkages like P – B, P – Si, *etc.* and their properties for biomedical applications will be the future work.

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