Loczenski Rose, Vanessa and Mastrotto, Francesca and Mantovani, Giuseppe (2016) Phosphonium polymers for gene delivery. Polymer Chemistry, 8 . pp. 353-360. ISSN 1759-9962

Phosphonium polymers for gene delivery

Vanessa Loczenski Rose,[a,b] Francesca Mastrotto,[a] Giuseppe Mantovani[a]

As published in Polymer Chemistry (RSC) as accepted manuscript - Polymer Chemistry, 2016, DOI: 10.1039/C6PY01855F

Phosphonium salt-containing polymers have very recently started to emerge as attractive materials for the engineering non-viral gene delivery systems. Compared to more frequently utilised ammonium-based polymers, some of these materials can enhance binding of nucleic acid at lower polymer concentration, and mediate good transfections efficiency, with low cytotoxicity. However, for years one of the main hurdles for their widespread application has been the lack of general routes for their synthesis. To date a range of polymerisation techniques have been explored, with the majority of them focussing on radical polymerisation, especially controlled radical polymerisation (CRP) techniques – ATRP, NMP and RAFT polymerisation - both by polymerisation of phosphonium monomers or by post-polymerisation modification of polymer intermediates. This review article aims at discussing key differences and similarities between phosphonium-and other analogous cations, how these affect binding to polynucleotides, and will provide an overview of the phosphonium polymer systems that have been utilised for gene delivery.

1. Introduction

The recent years have witnessed a growing interest in the development of polymer-based non-viral systems for the delivery of polynucleotides such as siRNA and pDNA, both *in vitro* and *in vivo*.[1-5] To this aim, synthetic polymers are particularly attractive, due to increasingly efficient chemical routes for their synthesis, and the possibility to further modify the delivery systems through post polymerisation strategies.[6] Within this context, controlled radical polymerisation (CRP, also known as reversible-deactivation radical polymerisation, RDRP) techniques have been extensively investigated, as they enable control over the polymer composition, architecture as well as molecular weight and dispersity.[7, 8] CRP techniques relevant for the synthesis of gene delivery vehicles[9] are copper-mediated living radical polymerisation,[10] especially atom transfer radical polymerisation (ATRP),[11, 12] reversible addition fragmentation chain transfer (RAFT)[13, 14] polymerisation, and nitroxide-mediated polymerisation (NMP).[15]

One of the most common strategies to deliver oligo/polynucleotides *in vitro* and *in vivo* is to assemble them into polyplexes, where they are bound to suitable polymer carriers through non-covalent interactions between multiple copies of negatively charged nucleotide phosphate groups and polycationic polymers[16] (Figure 1). When the polymer cationic units are nitrogen-based (ammonium),

the relative proportion of positively charged groups within the polymer and phosphate anions in oligo/polynucleotides is referred to as the N^+/P^- ratio. This parameter is critical in the formulation of polyplexes, as it can affect their overall size, transfection efficiency (non-viral vectors have typically subviral performances[17]), and most importantly cell cytotoxicity.[12]

Figure 1. Assembly of non-covalent polymer-polynucleotide polyplexes.

Conversely, the potential of phosphonium-containing polymers for nucleic acid delivery has not yet been fully recognised, most likely due to the few synthetic routes for their synthesis in satisfactory yield and purity, and the cost, toxicity and pyrophoric nature of some of the organic phosphine precursors necessary for their preparation.[18, 19] To date only a relatively small number of studies successfully implemented phosphonium-based polymers for gene delivery.[20] This review will provide a comprehensive overview of the state of the art of the field.

2. Phosphonium versus ammonium cations: similarities, differences and potential advantages for gene delivery

Phosphonium-containing polymers have been investigated as potential alternatives to their corresponding ammonium analogues. Being generally less prone to undergo Hofmann elimination and Menschutkin degradation, macromolecules containing phosphonium cations have often a superior thermal stability.[20] Enhanced stability of phosphonium monomers can potentially provide additional advantages – e.g. their use under more challenging experimental conditions, thus allowing to expand polymerisation techniques for the synthesis of phosphonium-based macromolecules. In gene delivery, studies reported improved abilities of phosphonium polymers due to bind nucleic acids, enhance transfection efficiency, and decrease cell cytotoxicity.[21, 22] Moreover, these materials were found to bind polynucleotides at lower P^+/P^- ratios (in analogy to N^+/P^- ratio for ammonium-containing polymers, P^+/P^- ratio is the molar ratio between the positively charged phosphonium groups (P^+) in the polymer, and the negatively charged phosphate group (P^-) of the polynucleotide) than their corresponding ammonium analogues.[21-23] Although specific mechanistic studies have not been carried out as yet, these results could be explained, at least in part, with the intrinsic differences between nitrogen- and phosphorous-based cations (Figure 2).

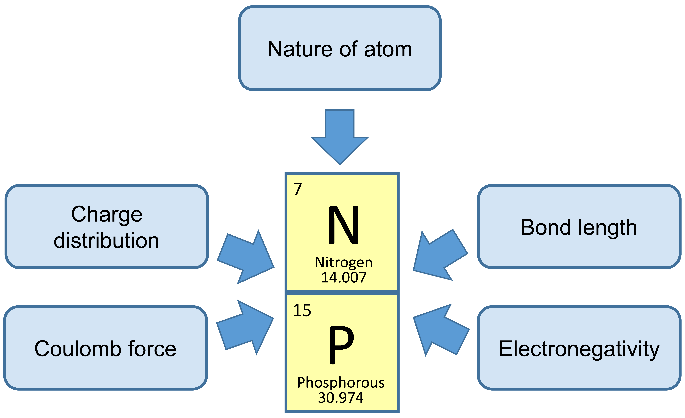

Figure 2. Factors influencing the interaction between positively charged ammonium and phosphonium centres, and nucleic acids: Nitrogen *vs.* Phosphorus cations.

P *vs.* N: chemico-physical considerations. Both nitrogen and phosphorus elements belong to Group 15 of the periodic table, and possess 5 valence electrons. Being below nitrogen within the same group, phosphorus (atomic number 15, electronegativity 2.19) has larger atomic radius and is less electronegative than both nitrogen (atomic number 7, electronegativity 3.04) and carbon (electronegativity 2.55) (Figure 2). Ammonium salts are therefore intrinsically smaller than their phosphonium analogues (C-N: ~1.53 Å; C-P: ~1.81 Å), which also allow them to be closer to their anionic counterions.[24] Importantly, different partial charges and charge distributions have been suggested, where for phosphonium ions the positive charge is centered at the P atom, whereas in the ammonium group is comparatively more distributed through the adjacent carbons, resulting in a weaker cationic charge in the ammonium groups.[21, 25] Consequently, in quaternary ammonium moieties the adjacent hydrogen atoms (α- and β- methylene hydrogens) are also more positively charged, which results in stronger hydrogen bonding, further shortening contact distances with their anionic counterions.[24]

Ab initio calculations performed by Colby and co-workers with tetrabutylphosphonium/ammonium salts further supported the differences in charge distributions of ammonium/phosphonium cations and their adjacent groups. Being less electronegative than the adjacent carbons, the phosphorus atom of n-Bu_4P^+ carries a positive charge (+1.1e) leaving the outer atoms to share (-0.1e).[25] This partially negative shell surrounding the cationic P atom in turn provides a partial shielding which weakens the coulombic interaction with anionic counterions. Conversely, being more electronegative that the bound carbon atoms, in *n*-Bu_4N^+ nitrogen carries a negative charge (-0.5e) leaving the outer atoms to share (+1.5 e), which results in higher attraction towards anions. Coutinho and co-workers came to similar conclusion when analysing charge distribution of tetra-alkyl-phosphonium and –ammonium ionic liquids, again suggesting that the P atom possessed higher positive charge, whilst the N centre delocalised more the positive charge towards adjacent alkyl groups.[26]

Within a gene delivery context, evidence suggesting phosphonium's lowered attraction towards anions would provide enhanced DNA release, thus partially explaining the increased transfection, although Frechet and co-workers have also suggested that the more localised charge on the phosphorus atom, might potentially favour nucleic acid binding with phosphonium-based polymers.[21]

P *vs.* N: toxicity profiles *in vitro* and *in vivo*. A potential benefit of some phosphonium-containing materials is a lower cytotoxicity compared to their ammonium analogues. This was first described in a study by Stekar *et al.* which focussed at identifying analogues of anti-neoplastic synthetic phospholipids edelfosine and miltefosine, with better tolerability and higher cytostatic activity. Novel analogues were synthesised by replacing the nitrogen atom (N) of 2-O-methyl-1-O-octadecyl-*rac*-glyceryl-3-phosphocholine and octadecyl phosphosphocholine with either arsenic (As) or phosphorus (P) (Figure 3), and the resulting phosphonium and arsonium phospholipids were found to have comparatively lower acute toxicity in a mouse model, when compared to their parent choline phospholipids.[27] However, the new analogues retained a similar antineoplastic activity of their parent phospholipids, as assessed *in vitro* in various cell lines (lymphocytic leukemia cells (L1210), KB cells (a subline of HeLa cells) and DS cells (B lymphocyte)) and *in vivo* using rat bearing 7,12-dimethylbenz(a)anthracene induced carcinomas. Although the exact mechanism(s) behind the observed differences was not investigated at this stage, the reduced acute toxicity resulting in weaker parasympathomimetic activity could be potentially related to the greater covalent radii of phosphonium and arsonium ions, which resulted in larger complexes.[27]

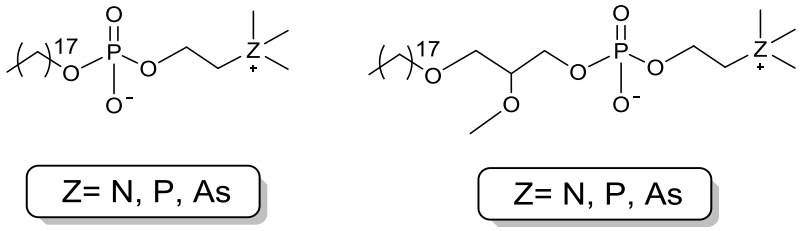

Figure 3. Antineoplastic active phospholipids octadecyl phosphosphocholine (left) and 2-O-methyl-1-O-octadecyl-rac-glyceryl-3-phosphocholine (right) analogues with different Group 15 quaternary centres (N, P, and As) investigated by Stekar *et al*.[27]

A study by Clément and co-coworkers on cationic phosphonolipids demonstrated that changing the nature of the cationic polar head from ammonium to phosphonium or arsonium, resulted in more efficient DNA transfection of β-galactosidase in airway epithelial cells (CFT1 cells) and HeLa cells. Furthermore, reduced cytotoxicity was observed in myelogenous leukemia cells (K562) transfected with a phosphonolipids containing a phosphorus or arsenic-based quaternary groups compared to the corresponding ammonium lipids.[28] In a subsequent work the same authors investigated a large library of cationic phosphonolipids with variable structural parameters, including the nature of cationic quaternary moieties - ammonium, phosphonium or arsonium, as part of gene delivery vectors, both *in vitro* and *in vivo*.[29] Using a luciferase assay, it was found that the replacement of ammonium groups

with analogue phosphonium or arsenium moieties improved cell transfection and reduced cytotoxicity in a range of cell lines (HeLa, CFT1, K562).

In terms of phosphonium-containing polymers, a seminal work by Frechet and co-workers showed that structurally analogous polyacrylates bearing triethyl-phosphonium repeating units had lower cytotoxicity compared to the corresponding triethyl-ammonium analogues.[21] Cell viability was assessed using a metabolic WST-1 proliferation assay employing a range of polymer concentrations (50-500 μg mL^{-1}), and was measured 48 hours after polymer exposure. Furthermore, better cell viability was observed for the triethyl-phosphonium based polymer after transfection with siRNA polyplexes in comparison to its ammonium analogues. In a subsequent work using similar polymers in 3T3 mouse cell line, we found no significant difference in cytotoxicity between phosponium and ammonium-containing polymers.[30]

Using tributyl- and triethyl-ammonium and phosphonium polystyrenes, no differences in cytotoxicity profiles were found for both polymers and corresponding polynucleotide polyplexes in HeLa cells, as estimated by a MTT cell viability assay.[22]

Overall, current evidence suggests that phosphonium polymers generally possess a cytotoxicity profile equivalent or more favourable than their ammonium-based analogues, which could open the way for a more widespread application of these materials in gene delivery.

3. Phosphonium-based polymers for gene delivery.

Phosphonium salts and their corresponding polymers have been investigated in a range of applications which span from ion exchange membranes[18, 31] and selectively permeable membranes,[32] treatment of drinking water,[33] to cationic biocides,[34-37] cell-penetrating agents,[38] and ionomers.[39, 40] Various polymerisation and post-polymerisation approaches have been utilised to generate phosphonium-containing polymers, however only relatively recently phosphonium polymers have been exploited as biological active polymers with antimicrobial properties[34-37, 41-44] and for the delivery of nucleic acid[21, 23, 30]. Examples of phosphonium-based polymers investigated for gene delivery are shown in Figure 4.

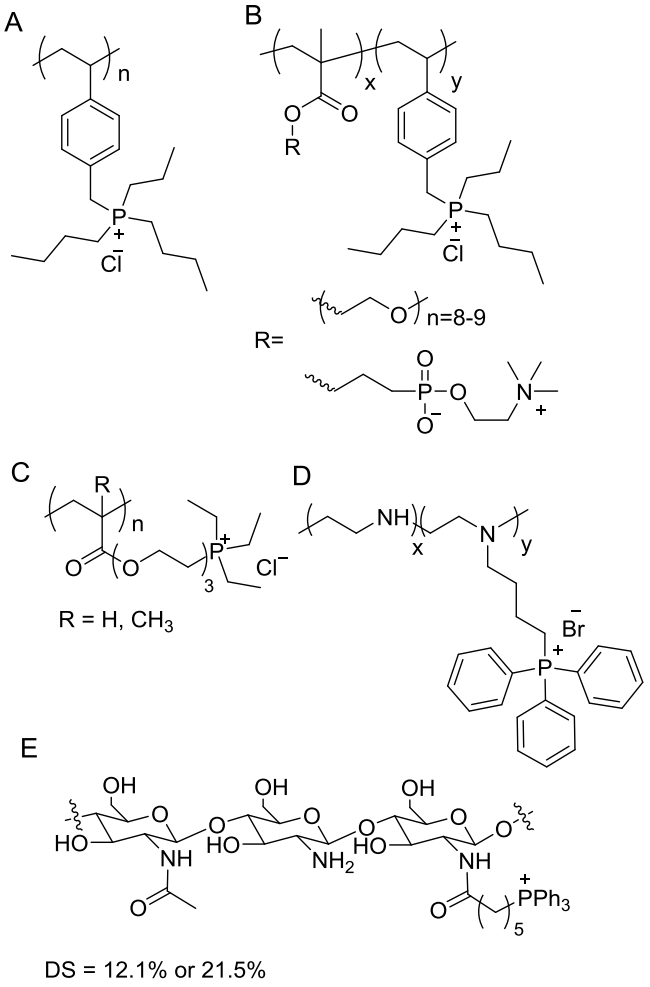

Figure 4. Examples of of phosphonium-containing polymers investigated for delivery of DNA and siRNA. A) (4-vinylbenzyl)tributylphosphonium chloride[22]; B) (4-vinylbenzyl)tributylphosphonium chloride-containing block polymers[23]; C) triethylphosphonium poly(meth)acrylates[21, 30]; D) triphenylphosphonium bromide-substitited PEI[45]; E) *N*-phosphonium-containing chitosan[41].

Controlled radical polymersation (CRP): synthesis of phosphonium-based polymers and their use for gene delivery. This section will first illustrate several approaches to synthesise phosphonium-containing polymers by CRP, then will discuss examples where these polymers were utilised for gene delivery.

The first examples of synthesis of phosphonium polymers by CRP were reported by Wang and Lowe.[46, 47] RAFT polymerisation of 4-vinylbenzyl (trimethylphosphonium) chloride and 4-vinylbenzyl (triphenylphosphonium) chloride styrenic monomers was carried out under aqueous conditions at 80°C employing 2-(2-carboxy-ethylsulfanylthiocarbonylsulfanyl) propionic acid trithiocarbonate RAFT CTA. Polymerisations followed linear first-order kinetics and were well-controlled, with homopolymers featuring $Đ \leq 1.07$. The resulting macro-CTAs were further chain extended with 4-vinylbenzoic acid to give diblock copolymers polyampholytes - polyzwitterions that contain, or potentially contain, both cationic and anionic residues located on different repeat units – which were found to self-assemble in supramolecular structures at pH 2.0, below the pKa of its benzoic acid repeating units (Figure 5).

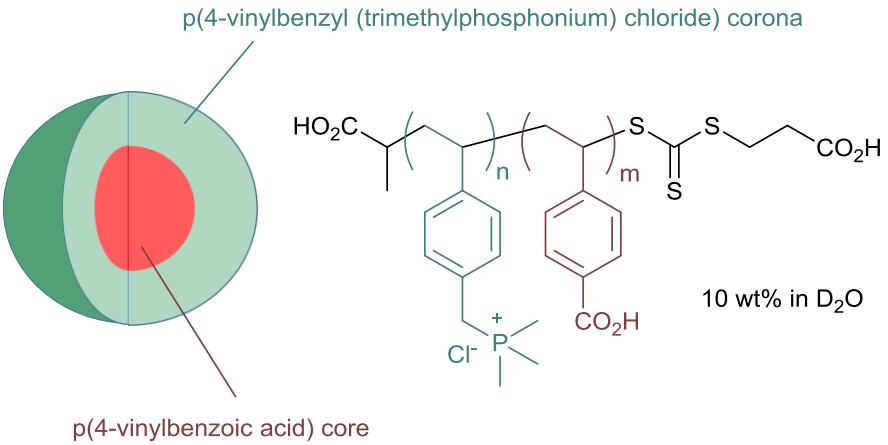

Figure 5. poly(4-vinylbenzyl (trimethylphosphonium) chloride)-*b*-(4-vinylbenzoic acid) polyampholytes synthesised by Wang and Lowe by aqueous RAFT polymerisation.[46]

RAFT polymerisation was also employed in a subsequent work by Stokes, Beyer and co-workers to synthesise a library of well-defined poly((trimethyl(4-vinylbenzyl)phosphonium chloride)-*r*-styrene) random copolymers with a range (15 - 98 mol %) of cationic repeating units.[48] Copolymers were designed to be potentially incorporated within anion exchange membranes, and their thermal properties were evaluated by differential scanning calorimetry and thermogravimetric analysis. Aiming at analogous applications, Balsara's group synthesised a range of poly[styrene-*b*-((2-acryloxy)ethyltributyl-phosphonium bromide)] diblock copolymers by RAFT polymerisation.[49] A pSty macro CTA was first prepared, followed by chain extension with bromoethyl acrylate, and the resulting reactive copolymer treated with tributhylphosphine to afford the desired phosphonium-containing diblock copolymers (Figure 6).

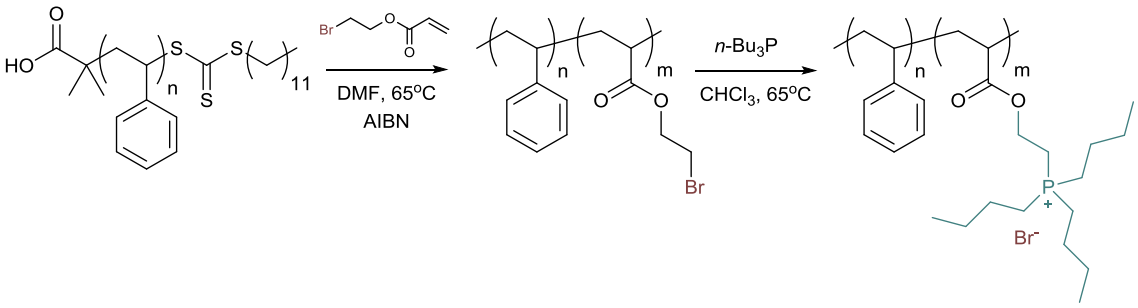

Figure 6. Poly[styrene-*b*-((2-acryloxy)ethyltributyl-phosphonium bromide) prepared by RAFT polymerisation by Balsara and co-workers.

Nitroxide-mediated polymerisation (NMP) was successfully utilised by Long and coworkers to synthesise a family of phosphonium-containing ABA triblock copolymers, where the number of ionic repeating units as well as the length of the hydrophobic alkyl substituents on the phosphonium cation were systematically varied.[50] Polymerisation from a (DEPN-terminated poly(*n*-butyl acrylate) di-functional nitroxide initiator of (tributyl-4-vinylbenzyl phosphonium chloride or trioctyl-4-vinylbenzyl phosphonium chloride monomers at 125°C in DMF afforded the desired ABA block copolymers

(Figure 7). These materials were assessed for their potential to be incorporated in alkaline fuel cell membranes and for melt processing, by analysing their thermal and thermo-mechanical properties as well as self-assembly properties, by employing differential scanning calorimetry, glass transition measurements, dynamic mechanical analysis and TEM.

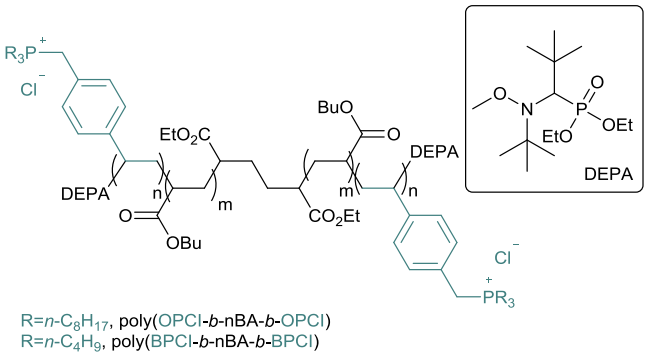

Figure 7. Synthesis of phosphonium containing triblock polymers by NMP. Adapted from Ye *et al.*[31]

The first example of phosphonium polymers prepared by ATRP was reported by Borguet and Tsarevsky, who employed initiators for continuous activator regeneration atom transfer radical polymerisation (ICAR ATRP) to synthesise well-defined poly(4-vinylbenzyltriphenylphosphonium) materials (Figure 8).[51] Interestingly, initial attempts to polymerise vinylbenzyltriphenylphosphonium chloride via ICAR ATRP conditions were unsuccessful, likely due to displacement of the tris(2-pyridylmethyl)amine (TPMA) ligand from Cu(I) and Cu(II) ATRP catalytic species by monomer chloride counter ions. Pleasingly, the replacement of chloride- with less coordinating tetrafluoroborate counterions in the phosphonium monomers resulted in successful polymerisation, using ethyl-2-bromoisobutyrate as the initiator, TPMA as the ligand, and AIBN as the radical initiator, at 70°C in DMF (Figure 8). To confirm the end-group fidelity of the process, chain extension was carried out again by ICAR ATRP, using either styrene to give block-copolymers, or 4VBTPPBF4 to afford a higher molecular weight homopolymer. The final polymers had M_n 15.0-28.4 kDa and Đ = 1.31-1.51.

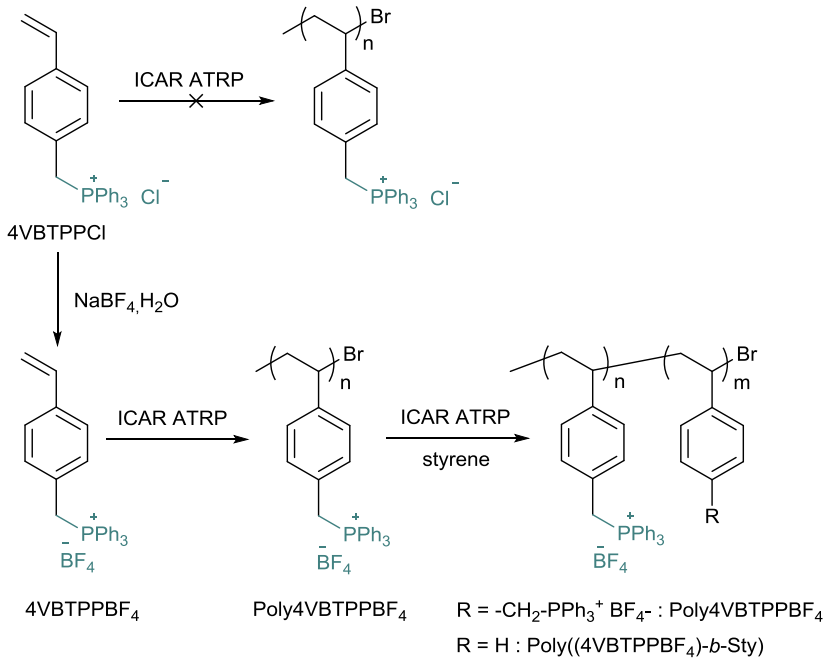

Figure 8. Synthesis of phosphonium-containing polystyrenes by ICAR ATRP. 4-vinylbenzyltriphenylphosphonium chloride monomer was first converted into its corresponding tetrafluoborate salt to allow polymerisation under ICAR ATRP conditions. Adapted from Borguet and Tsarevsky.[51]

Long and co-workers synthesised phosphonium-containing AB-diblock polymers for DNA delivery by RAFT polymerisation.[23] The polymers consisted of a substituted polystyrene cationic phosphonium block for DNA complexation, and a second block synthesised from oligo-(ethylene glycol)$_9$ methyl ether methacrylate or 2-methacryloyoxyethyl phosphorylcholine for improved colloidal stability of the resulting delivery system. The degree of polymerisation of the cationic DNA-binding block was systematically varied, to give a library of AB copolymers with M_n: 33.8-54.4 kDa, with low dispersities ($Đ \leq 1.09$). Efficient DNA binding was observed at low (+/-) ratios $–P^+/P^-$ - in a gel shift assay. Low transfection efficiencies were observed in monkey kidney fibroblast (COS-7) and HeLa (cervical cancer cell line) cells. However, using a Luciferase assay good transfection efficiencies, comparable to commercially available transfection agent jet-PEI (a linear poly(ethylene imine)), were demonstrated in hepatoma-derived cells (HepaRG) with good cell viabilities (≥80%) at low polymer-DNA ratio (+/-) ratio (2), whilst EGFP-C1 plasmid was used for confocal microscopy experiments.

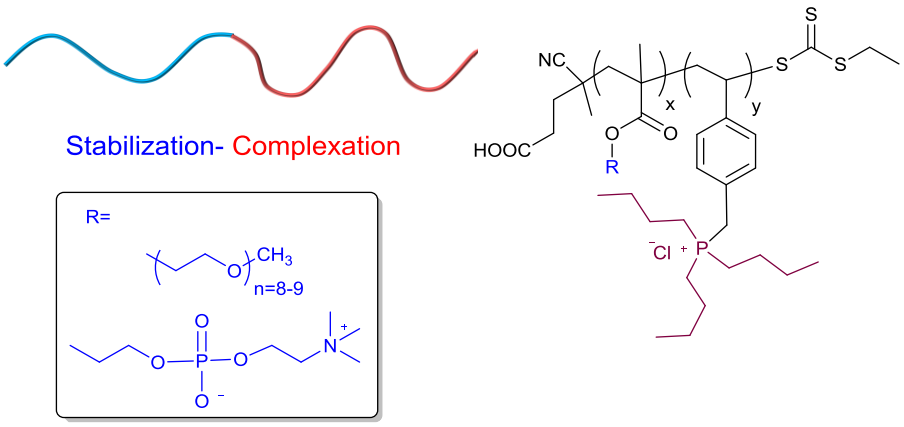

Figure 9. Phosphonium-based diblock polymers for application in gene delivery, synthesised *via* RAFT polymerisation by Long and co-workers.[23]

Following a key study by Frechet and co-workers[21] (*vide infra*), we recently synthetised a library of quaternary ammonium and phosphonium polymethacrylates for short interfering RNA delivery.[30] The nature of the charged heteroatom (N vs P) as well as the length of the spacer separating the cationic units from the polymer backbone (oxyethylene *vs.* trioxyethylene) were systematically varied to identify structure-function relationships for these materials (Figure 10). Results showed that both longer and more flexible trioxyethylene spacers, and phosphonium cations resulted in RNA polyplexes that were more stable in the presence of heparin competitive polyanions. Interestingly, whilst all RNA polyplexes were efficiently internalized by GFP-expressing 3T3 cells, no appreciable siRNA-mediated GFP knockdown was observed, possibly due to inefficient polyplex endosomal escape. However, Survivin gene knockdown was achieved in HeLa cells by replacing siRNA with multimerized liRNA, showing that the macromolecular structure of RNA can be key for RNA interference.

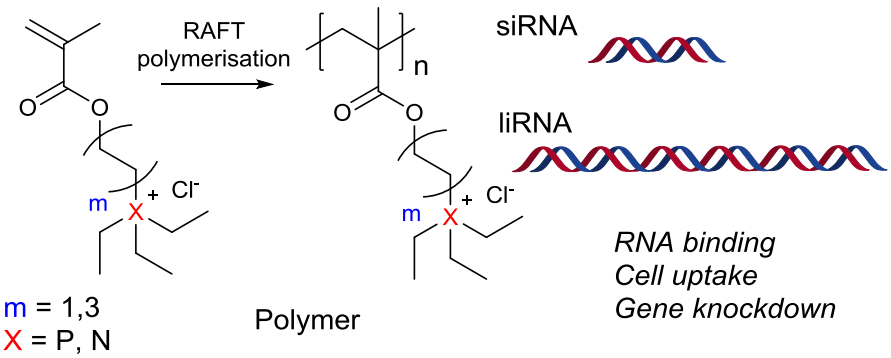

Figure 10. Quaternary ammonium and phosphonium polymethacrylates for RNA delivery synthesised *via* RAFT polymerisation by Mantovani and co-workers.[30]

Phosphonium polymers for gene delivery prepared with other techniques. Closely related to CRP, 'conventional' free radical polymerisation has also been utilised to synthesise polycationic polymers for gene delivery. Long and co-workers polymerised styrenic ammonium and phosphonium monomers to generate a small library of poly(triethyl-(4-vinylbenzyl)ammonium chloride) (PTEA), poly(tributyl-

(4-vinylbenzyl)ammonium chloride) (PTBA), poly(triethyl-(4-vinylbenzyl)phosphonium chloride) (PTEP) and poly- (tributyl-(4-vinylbenzyl)phosphonium chloride) (PTBP)[22]. A key aim of this study was to study structure activity relationships for these materials, by varying the length of the polymer *N*- and *P*-alkyl substituents. DNA binding assays with a gWiz-Luc plasmid showed more efficient DNA binding for phosphonium polymers over their ammonium analogues. Furthermore, tributylphosphonium-based polymers (PTBP) showed significant higher transfection activity of luciferase DNA ($p>0.05$) over the corresponding triethylphosphonium- and tributylammonium (PTBA)-polymers and commercially available Superfect in serum free conditions, again underlying the importance of the macromolecular features of the polycationic complexing polymer in transfection efficiency.

Following the application of polyphosphonium polymers for pDNA delivery, Frechet and co-workers demonstrated for the first time the application of phosphonium-containing polymers for siRNA delivery (Figure 11)[21] A library of polyphosphonium polymers was generated in two steps from commercially available polyacrylic acid (PAA) which was first esterified with 2-[2-(2-chloroethoxy)ethoxy]ethanol and the resulting intermediate was then reacted with different organic phosphines - triethylphosphine, tri(*tert*-butylphosphine), tris(3-hydroxy propyl) phosphine, triphenylphospine - and triethylamine. The triphenylphosphine-derived polymer was insoluble in water, whilst the tri(*tert*-butylphosphine) derivative was found to be extremely cytotoxic even at low polymer concentrations. Both polymers were therefore not utilised to generate siRNA delivery vehicles. However, the triethylphosphonium acrylate based polymer gave transfection efficiencies of 65%, while maintaining extremely good cell viability (100%) in HeLa-Luc (luciferase expressing) cells. In comparison its polymeric ammonium analogue gave only 25% transfection efficiency and 85% cell viability. This study suggested that these phosphonium-containing polymers were less cytotoxic and gave higher transfection efficiency than their corresponding ammonium polycations.

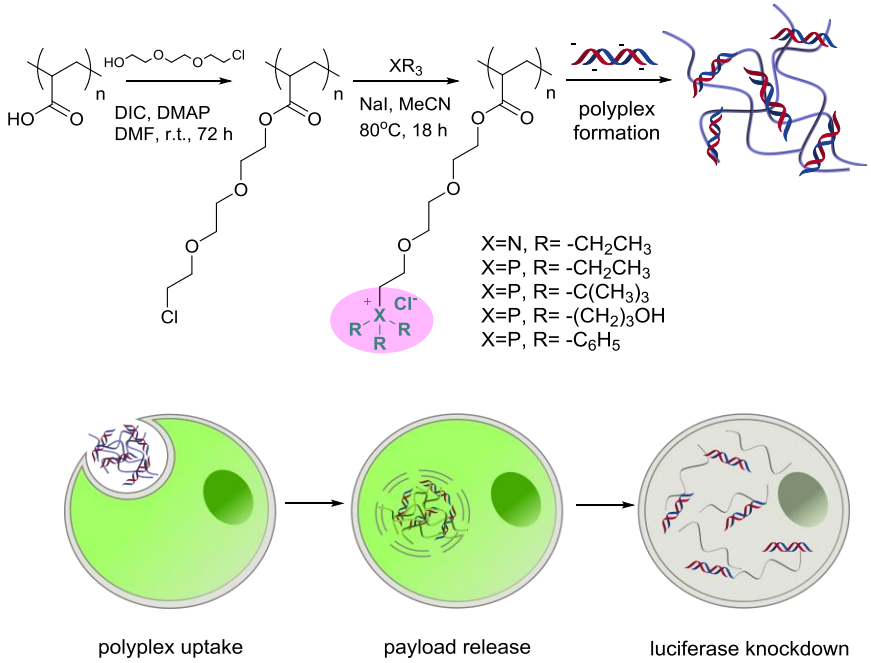

Figure 11. Phosphonium- and ammonium-based polyacrylates synthetised by Frechet and co-workers for siRNA delivery. Adapted from Ornelas-Megiatto *et al*.[21]

The preparation of novel ammonium-phosphonium (N-P) hybrid polymers containing secondary and tertiary amines along with phosphonium moieties in a single polymeric chain has been described by Kumar and co-workers (Figure 12).[45] A set of *N*-butyl(triphenylphosphonium bromide) (BTP)-grafted-linear polyethylenimine (lPEI) polymers (BTP-*g*-lP), was prepared by reaction of (4-bromobutyl)triphenyl-phosphonium bromide with lPEI to introduce butyl(triphenylphosphonium bromide) pendant groups onto the lPEI polymeric backbone. Different lPEI:BTP molar ratios were used, resulting in a library of grafted polymers with different BTP content (10, 20, 30, 40 and 50%). Polyplexes of polymers and DNA had hydrodynamic diameters in the 249–307 nm range and a positive surface charge (+31-34 mV), as measured by DLS and zeta potential respectively. The GFP fluorescence and hence ability to transfect DNA was measured spectrofluorometrically in cell lysates after 36 hours of incubation with polyplexes. The polymer containing 30% BTP (BTP-*g*-lP3/pDNA) showed the best results with ~3.6 and 7.1-fold higher transfection efficiency in lung cancer (A549) and breast cancer (MCF-7) cells, respectively, compared to native lPEI. The same complex displayed ~1.8–8.4 fold higher transfection compared to commercially available Lipofectamine, Superfect and GenePORTER 2 in both cell lines investigated. In addition, the potential of BTP-*g*-IP3 for siRNA delivery was evaluated in a GFP expressing MCF-7 cell line, and improved gene knockdown was shown over commercial available Lipofectamine. All formulations tested did not show significant cytotoxicity at the concentrations employed for the transfection experiments.

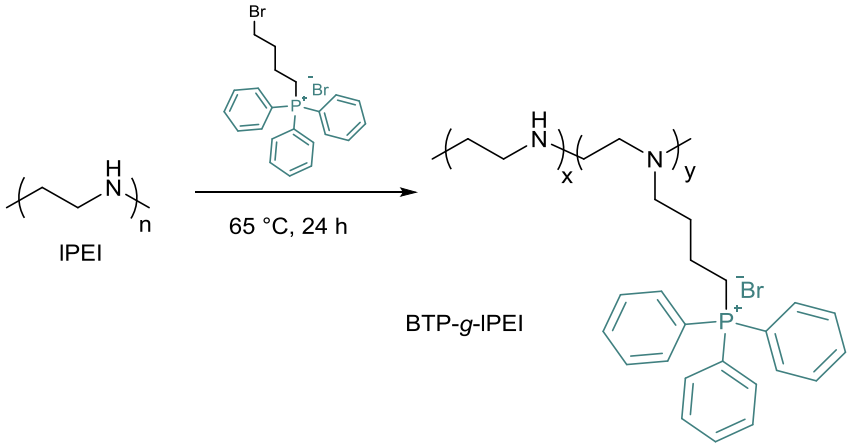

Figure 12. Synthesis of BTP-*g*-lPEI polymers with 10-50% substitution of grafted BTP on lPEI, adapted from Kumar and co-workers.[45]

N-phosphonium-containing chitosans (NPCSs) with variable degrees of substitution (12.1 and 21.5 mol%) have been synthesised by Guo's group as DNA-complexing macromolecules (Figure).[52] Chitosan was reacted with (5-carboxypentyl) triphenylphosphonium bromide followed by purification by precipitation and dialysis. The size of NPCS/DNA complexes was found to be in the 110-160 nm range as measured by DLS. A MTT cell viability assay using human embryonic kidney (HEK 293) and HeLa cells revealed a comparable concentration-dependent decrease of cell viability for NPCSs and branched-PEI (25 kDa, utilised in this study as a reference transfecting polymer), using polymer concentrations ranging from 2 to 200 μg mL^{-1}. In both cell lines, NPCSs were found to be more cytotoxic than chitosan, but marginally less toxic than branched PEI. Transfection with EGFP-N1 plasmid showed increased DNA uptake using the NPCS/DNA complexes (N/P 16:1) over chitosan alone. The transfection efficiency with NPCS with a degree of substitution of 21.5% was comparable to the efficiency of branched PEI (bPEI) on HeLa cells at pH 6.5.

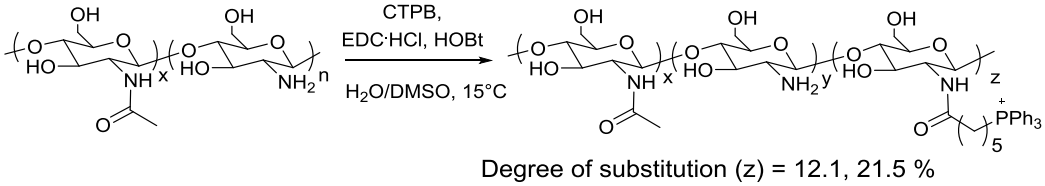

Figure 13. *N*-phosphonium chitosans (NPCSs) with variable degrees of substitution, investigated by Guo and co-workers as potential gene delivery vehicles.[52]

4. Conclusions

The application of phosphonium-containing polymers for biological purposes, such as nucleic acid delivery is still in its infancy, but has started to receive increasing interest. As discussed in this review, due to the peculiar properties of quaternised phosphonium centres, when appropriately designed phosphonium-based polymer systems can possess improved polymer properties over their corresponding ammonium-based materials, such as binding affinity to polynucleotides, transfection

efficiency and lower cytotoxicity. At present, more studies are required to further confirm those findings and understand the physico-chemical differences of ammonium- and phosphonium based gene delivery systems *in vitro* and *in vivo*.

Overall, the application of phosphonium-based polymers for nucleic acid delivery are still restricted, due to limited synthetic routes currently available. With few exceptions, the polymer systems discussed within this review were mostly prepared by conventional or controlled radical polymerisation (CRP) techniques, with the latter being particularly attractive due to the potential to produce polymeric materials with great control over their macromolecular features. This in turn may result in the development of classes of novel materials which could complement and expand the range of polynucleotide delivery systems currently available.

Acknowledgements

We would like to thank AstraZeneca and the Engineering and Physical Sciences Research Council (EPSRC) for funding our research through the EPSRC/ AstraZeneca Centre for Doctoral Training (CDT) in Targeted Therapeutics and Formulation Sciences (Grant code EP/D501849/1) and EP/J02158X/1.

Notes and references

[a] School of Pharmacy, Boots Science Building, University Park, University of Nottingham, Nottingham NG7 2RD, UK
[b] current address: Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY, UK

References

1. H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin and D. G. Anderson, *Nat. Rev. Genet.*, 2014, **15**, 541-555.
2. D. W. Pack, A. S. Hoffman, S. Pun and P. S. Stayton, *Nat. Rev. Drug Discov.*, 2005, **4**, 581-593.
3. Y. Yue and C. Wu, *Biomater. Sci.*, 2013, **1**, 152-170.
4. J. Chen, Z. Guo, H. Tian and X. Chen, *Mol. Ther.--Methods Clin. Dev.*, 2016, **3**, 16023.
5. A. Sizovs, P. M. McLendon, S. Srinivasachari and T. M. Reineke, in *Nucleic Acid Transfection*, eds. W. Bielke and C. Erbacher, Springer Berlin Heidelberg, Berlin, Heidelberg, 2010, DOI: 10.1007/128_2010_68, pp. 131-190.
6. D. Smith, A. C. Holley and C. L. McCormick, *Polym. Chem.*, 2011, **2**, 1428-1441.
7. F. J. Xu and W. T. Yang, *Prog. Polym. Sci.*, 2011, **36**, 1099-1131.
8. W. H. Heath, A. F. Senyurt, J. Layman and T. E. Long, *Macromol. Chem. Phys.*, 2007, **208**, 1243-1249.
9. D. S. H. Chu, J. G. Schellinger, J. Shi, A. J. Convertine, P. S. Stayton and S. H. Pun, *Acc. Chem. Res.*, 2012, **45**, 1089-1099.
10. C. Boyer, N. A. Corrigan, K. Jung, D. Nguyen, T.-K. Nguyen, N. N. M. Adnan, S. Oliver, S. Shanmugam and J. Yeow, *Chem. Rev.*, 2016, **116**, 1803-1949.
11. K. Matyjaszewski, *Macromolecules*, 2012, **45**, 4015-4039.
12. D. J. Siegwart, J. K. Oh and K. Matyjaszewski, *Prog. Polym. Sci.*, 2012, **37**, 18-37.
13. G. Moad, E. Rizzardo and S. H. Thang, *Chemistry – An Asian Journal*, 2013, **8**, 1634-1644.

14. M. Semsarilar and S. Perrier, *Nat. Chem.*, 2010, **2**, 811-820.
15. J. Nicolas, Y. Guillaneuf, C. Lefay, D. Bertin, D. Gigmes and B. Charleux, *Prog. Polym. Sci.*, 2013, **38**, 63-235.
16. V. Loczenski Rose, G. S. Winkler, S. Allen, S. Puri and G. Mantovani, *Eur. Polym. J.*, 2013, **49**, 2861-2883.
17. C. H. Jones, C.-K. Chen, A. Ravikrishnan, S. Rane and B. A. Pfeifer, *Mol. Pharm.*, 2013, **10**, 4082-4098.
18. C. Jangu, A. R. Schultz, C. E. Wall, A. R. Esker and T. E. Long, *Macromol. Rapid Commun.*, 2016, **37**, 1212-1217.
19. E. G. Tennyson, S. He, N. C. Osti, D. Perahia and R. C. Smith, *J. Mater. Chem.*, 2010, **20**, 7984-7989.
20. C. Jangu and T. E. Long, *Polymer*, 2014, **55**, 3298-3304.
21. C. Ornelas-Megiatto, P. R. Wich and J. M. J. Fréchet, *J. Am. Chem. Soc.*, 2012, **134**, 1902-1905.
22. S. T. Hemp, M. H. Allen, M. D. Green and T. E. Long, *Biomacromolecules*, 2012, **13**, 231-238.
23. S. T. Hemp, A. E. Smith, J. M. Bryson, M. H. Allen and T. E. Long, *Biomacromolecules*, 2012, **13**, 2439-2445.
24. D. J. Abdallah and R. G. Weiss, *Chem. Mater.*, 2000, **12**, 406-413.
25. S.-W. Wang, W. Liu and R. H. Colby, *Chem. Mater.*, 2011, **23**, 1862-1873.
26. P. J. Carvalho, S. P. M. Ventura, M. L. S. Batista, B. Schröder, F. Gonçalves, J. Esperança, F. Mutelet and J. A. P. Coutinho, *J. Chem. Phys.*, 2014, **140**, 064505.
27. J. Stekar, G. Nössner, B. Kutscher, J. Engel and P. Hilgard, *Angew. Chem. Int. Ed.*, 1995, **34**, 238-240.
28. E. Guénin, A.-C. Hervé, V. Floch, S. Loisel, J.-J. Yaouanc, J.-C. Clément, C. Férec and H. des Abbayes, *Angew. Chem. Int. Ed.*, 2000, **39**, 629-631.
29. V. Floch, S. Loisel, E. Guenin, A. C. Hervé, J. C. Clément, J. J. Yaouanc, H. des Abbayes and C. Férec, *J. Med. Chem.*, 2000, **43**, 4617-4628.
30. V. Loczenski Rose, S. Shubber, S. Sajeesh, S. G. Spain, S. Puri, S. Allen, D.-K. Lee, G. S. Winkler and G. Mantovani, *Biomacromolecules*, 2015, **16**, 3480-3490.
31. Y. Ye, K. K. Stokes, F. L. Beyer and Y. A. Elabd, *J. Membr. Sci.*, 2013, **443**, 93-99.
32. H.-S. Yim, C. E. Kibbey, S.-C. Ma, D. M. Kliza, D. Liu, S.-B. Park, C. E. Torre and M. E. Meyerhoff, *Biosens. Bioelectron.*, 1993, **8**, 1-38.
33. T. Zeng, J. J. Pignatello, R. J. Li and W. A. Mitch, *Environ. Sci. Technol.*, 2014, **48**, 13392-13401.
34. A. Kanazawa, T. Ikeda and T. Endo, *J. Appl. Polym. Sci.*, 1994, **53**, 1245-1249.
35. E.-R. Kenawy, F. I. Abdel-Hay, A. A. El-Magd and Y. Mahmoud, *React. Funct. Polym.*, 2006, **66**, 419-429.
36. Y. Xue, Y. Pan, H. Xiao and Y. Zhao, *RSC Advances*, 2014, **4**, 46887-46895.
37. A. Kanazawa, T. Ikeda and T. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 1993, **31**, 1441-1447.
38. Z. Song, N. Zheng, X. Ba, L. Yin, R. Zhang, L. Ma and J. Cheng, *Biomacromolecules*, 2014, **15**, 1491-1497.
39. H. Ghassemi, D. J. Riley, M. Curtis, E. Bonaplata and J. E. McGrath, *Appl. Organomet. Chem.*, 1998, **12**, 781-785.
40. S. Cheng, M. Zhang, T. Wu, S. T. Hemp, B. D. Mather, R. B. Moore and T. E. Long, *J. Polym. Sci., Part A: Polym. Chem.*, 2012, **50**, 166-173.
41. A. Kanazawa, T. Ikeda and T. Endo, *J. Appl. Polym. Sci.*, 1994, **53**, 1237-1244.
42. E.-R. Kenawy, F. I. Abdel-Hay, A. E.-R. R. El-Shanshoury and M. H. El-Newehy, *J. Controlled Release*, 1998, **50**, 145-152.
43. E.-R. Kenawy and Y. A. G. Mahmoud, *Macromol. Biosci.*, 2003, **3**, 107-116.
44. Y. Xue, H. Xiao and Y. Zhang, *Int. J. Mol. Sci.*, 2015, **16**.
45. R. Bansal, S. K. Tripathi, K. C. Gupta and P. Kumar, *J. Mater. Chem.*, 2012, **22**, 25427-25436.
46. R. Wang and A. B. Lowe, *J. Polym. Sci., Part A: Polym. Chem.*, 2007, **45**, 2468-2483.
47. A. B. Lowe, R. Wang, V. Tiriveedhi, P. Butko and C. L. McCormick, *Macromol. Chem. Phys.*, 2007, **208**, 2339-2347.

48. K. K. Stokes, J. A. Orlicki and F. L. Beyer, *Polym. Chem.*, 2011, **2**, 80-82.
49. P. Cotanda, G. Sudre, M. A. Modestino, X. C. Chen and N. P. Balsara, *Macromolecules*, 2014, **47**, 7540-7547.
50. S. Cheng, F. L. Beyer, B. D. Mather, R. B. Moore and T. E. Long, *Macromolecules*, 2011, **44**, 6509-6517.
51. Y. P. Borguet and N. V. Tsarevsky, *Polym. Chem.*, 2012, **3**, 2487-2494.
52. C. Qian, X. Xu, Y. Shen, Y. Li and S. Guo, *Carbohydr. Polym.*, 2013, **97**, 676-683.