Poly(alkylene oxide) Copolymers for Nucleic Acid Delivery
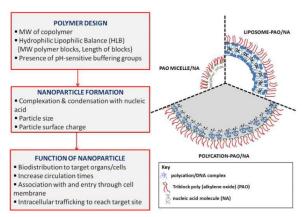
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CONSPECTUS

The advancement of gene-based therapeutics to the clinic is limited by the ability to deliver physiologically relevant doses of nucleic acids to target tissues safely and effectively. Polymer and lipid based nano-assemblies have been successfully employed over the last couple of decades for the delivery of nucleic acids to treat a variety of disease states. Results of phase I/II clinical studies to evaluate the efficacy and biosafety of these gene delivery vehicles have been encouraging, thus promoting the design of more efficient and biocompatible systems. Research has focused on designing carriers to achieve biocompatibility, stability in the circulatory system, biodistribution to target the disease site, and intracellular delivery, all of which enhance the resulting therapeutic effect.

The family of poly(alkylene oxide) (PAO) includes random, block and branched polymers, among which the ABA type triblocks copolymers of ethylene oxide (EO) and propylene oxide (PO) (commercially known as Pluronic®) have received the greatest consideration. In this Account, we highlight examples of polycation-PAO conjugates, liposome-PAO formulations, and PAO micelles for nucleic acid delivery. Among the various polymer design consideration, which include molecular weight of polymer, molecular weight of blocks, and length of blocks, it has been found that the overall hydrophobic-lipophilic balance (HLB) is a critical parameter in defining the behavior of the polymer conjugates for gene delivery. The effects of varying this

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parameter are discussed in the context of improving gene delivery processes, such as serum-stability and association with cell membranes. Other innovative macromolecular modifications discussed in this category include the work done by our group to enhance the serum stability and efficiency of lipoplexes using PAO graft copolymers, development of a PAO gel-based carrier for sustained and stimuli responsive delivery, and biodegradable PAO-based amphiphilic block copolymers.

INTRODUCTION

Progress in the field of non-viral gene-based therapies requires improvements in the systemic and cellular delivery of nucleic acids in the form of plasmid DNA or synthetic oligonucleotides.[1,2] Non-viral carriers consisting of synthetic and natural cationic polymers, liposomes, and non-ionic copolymer micelles have demonstrated moderate success in overcoming some of the delivery challenges.[3] While non-viral carriers still exhibit considerably lower gene transfection efficiency than viruses, they remain a favorable alternative to viral vectors based on their superior safety profile and potential for further development.[4] At the cellular level, the carrier serves to promote association with the target cell surface, penetration/fusion with the cell membrane, escape from the endolysosomal pathway, and dissociation from the therapeutic cargo for release to the target site of action (cytoplasm/nucleus). To overcome the issues of cytotoxicity and poor pharmacological distribution that exist at the systemic level, the most widely accepted modification has been to incorporate hydrophilic polymers, such as poly (ethylene oxide) (PEO), into carrier chemistry.[5] PEO has been used widely in drug and nucleic acid delivery systems to increase the colloidal stability of the carrier and to restrict its interactions with serum proteins and immune system components. However, depending on the amount, molecular weight and organization of PEO on a carrier, its hydrophilic nature can hinder association with cell membranes, thus drastically reducing the delivery of therapeutic cargo into cells. For this reason, the use of PEO has been broadened to amphiphilic poly(alkylene oxide) (PAO) copolymers containing both a hydrophilic component and a hydrophobic component. Most of the amphiphilic block copolymers in this category consist of the hydrophilic segment, PEO, and a hydrophobic component, such as poly(propylene oxide) (PPO), poly(butylene oxide) (PBO), poly(methyl methacrylate) (PMMA), polystyrene, or polyesters such as polylactides, polylactones, polyglycolides. These hydrophobic components serve to increase association with the hydrophobic portions of the cell membrane, and thereby cell entry. The composition and architecture of the subunits, and parameters such as molecular weight and length of the polymer blocks, have been tuned to enhance the delivery of the therapeutic cargo to the target cells of interest. Typically, nucleic acids are formulated as nanoparticles, where characteristics such as the particle size, surface charge, and biological functionalities may be manipulated to promote longer blood circulation half-lives, association with cells for entry, and intracellular trafficking of the therapeutic cargo to the target site of action.

Traditionally, nanoparticle carrier chemistries have employed polycations and cationic liposomes that associate with and deliver the therapeutic nucleic acid to the target cells of interest. However, their serum instability, intrinsic cytotoxicity of polycations and colloidal instability of liposomes limited their application. Amphiphilic PAO block copolymers have been incorporated as conjugates with polyplexes (polycation-nucleic acid complexes), or as supramolecular assemblies with lipoplexes (liposome-nucleic acid complexes), or directly as a polymeric carrier to overcome cytotoxicity and stability issues. Some of the other advantages of using PAO block copolymers include their non-ionic chemistry, which reduces or shields the charge on polyelectrolytes; easy tunable amphiphilicity; high colloidal stability, which makes them easily injectable; and very low critical micelle concentration (CMC), which makes them efficient at lower dilutions. Here in this Account, we will

highlight several classes of PAO formulations for nucleic acid delivery applications and current understanding of how the properties of the PAO impact on the biological steps of nucleic acid delivery.

PHYSICAL PROPERTIES OF AMPHIPHILIC PAO BLOCK COPOLYMERS

PAO amphiphilic block copolymers spontaneously self-assemble into a variety of nanoparticle morphologies including spheres and rods that typically have inner hydrophobic core regions and outer hydrophilic shell regions, and polymersome vesicles that have bilayers surrounding an aqueous core. The thermodynamics underlying PAO self-assembly have been extensively reviewed elsewhere.[6,7] PAOs used in drug delivery typically have either A-B diblock or A-B-A triblock architectures, where the A blocks are hydrophilic poly(ethylene oxide) (PEO) that form the shell and the B blocks are hydrophobic poly(propylene oxide) (PPO) or poly(butylene oxide) (PBO) that form the core.[8] PAO self-assembly is an entropy-driven process dominated by the hydrophobic block's interactions with water as shown by applications of Flory-Huggins theory[9,10] and characterized by a critical micellization temperature (CMT) and critical micelle concentration (CMC) that are dependent upon the molecular composition of the PAO, including the copolymer molecular weight, the monomer chemistry, the lengths of the hydrophilic and hydrophobic segments, and the linear or branched architecture of the macromolecule (Table 1).[11] Increasing the PEO length increases the copolymer solubility and hence increases the CMC, whereas increasing the PPO or PBO length decreases the solubility and hence decreases the CMC.[12] The CMC decreases and the micelle hydrodynamic radius increases with increasing temperature for PEO-PPO-PEO triblocks, with typical micellar radii between 2 and 8 nm at room temperature.[13] In a concentrated aqueous solution of PAO block copolymers, a critical gelation temperature (CGT) is observed above which the micellar liquid transitions into the lyotropic liquid crystalline phase characterized by a semisolid gel-like structure. For PAOs, the hydrophilic-lipophilic balance (HLB) is indicative of the relative solubility of the copolymers in aqueous and organic (e.g., membrane lipid) media and can be empirically determined by a linear combination of EO and PO group contribution numbers.[14]

The PAO copolymers form stable complexes in aqueous solutions with nucleic acid molecules by condensing plasmid DNA or antisense oligonucleotides to form stable and compact nanostructures (Figure 1).[15–18] To date, several groups have extensively explored amphiphilic triblock, poloxamer (Pluronic®), diblock polyetheramines (Jeffamines®), and star-shaped block copolymers (Tetronic®) as intracellular gene delivery carriers or as conjugates to gene delivery carriers.[16] Pluronic molecules consist of alternating blocks PEO and PPO of varying chain lengths and repeat units, Tetronic macromers consist of four PEO/PPO block chains bonded to a protonatable ethylene diamine central group, and Jeffamine copolymers consist of a primary amino group attached to the end of a polyether backbone (of either PO, EO, or mixed PO/EO) (Figure 2).

Due to their amphiphilic structures, PAOs are highly surface active, producing aqueous surface tensions of about 36 – 40 mN/m, depending on the ratio of hydrophilic to hydrophobic blocks.[19] The surfactant properties of PAOs are essential to their interaction with biological membranes, enabling either membrane disruption or the sealing and repair of damaged membranes, depending upon the structure of the PAO and the state of the biological membranes.[20,21]

POLYALKYLENE OXIDE COPOLYMER – POLYCATION COMPLEXES

Cationic polymers such as polyethyleneimine (PEI) are commonly employed as carriers for nucleic acids because of their ability to bind with negatively charged DNA or RNA by electrostatic interactions. The resulting net charge of the complex of carrier/nucleic acid

dictates its interactions with serum proteins and associations with cell membrane surfaces. The positive charge of many carriers promotes interaction with negatively charged components on the cell surface such as proteoglycans; however, it also tends to promote undesired interaction with serum proteins. The specific chemistry of PAO-polycations affects DNA condensation, serum-stability of the nanoparticle and cell surface activity.

The serum stability of PEI/DNA and Pluronic-PEI/DNA conjugates, the latter varying in their hydrophilic–lipophilic balance (HLB), and their delivery of DNA to NIH/3T3 cells were compared.[24] The conjugates incorporating Pluronic with higher HLB values (e.g., F68) displayed higher stability in up to 50% serum concentration in media, and in turn produced significantly increased gene expression. The concentration of Pluronics was found to be critical for serum stability, as the deposition of serum components onto PAO-polycation conjugates was inhibited only when the concentration of Pluronics of higher HLB was in the range of 1 to 3%. It was also seen that efficient transfection by PEI-DNA complex required Pluronics to be first mixed with DNA, followed by complex formation with PEI.[24] Similarly, the order of addition was found to affect the size of the nanoparticle, with a diameter of 166 nm when Pluronic P123 was mixed with DNA prior to addition of P123-g-PEI conjugate, as compared to 650 nm when Pluronic is added to the complex of P123-g-PEI/ DNA[25].

A unique set of copolymers was created by grafting Jeffamine M-2070 (PO/ EO mol ratio 10/31, MW 2000 Da) chains onto guanidinylated linear and branched PEI[26]. For low molecular weight PEI, which generally has reduced cytotoxicity but alone does not form polyplexes or transfect cells efficiently, guanidinylation and Jeffamine conjugation promoted complex formation with nucleic acids, low cytoxicity, enhanced stability in medium with 10% serum, and which resulted in several-fold higher transfection in NIH/3T3 and Cos-7 cell lines, compared to that of their parent PEI species and PEI-DNA polyplexes.

A similar approach involved the plasmid transfection of CHO cells using PEO or L92 conjugated with poly [2-(dimethylamino) ethyl methacrylate] (pDMAEMA). Both these conjugates formed nanocomplexes with DNA in the size range of 100–250 nm. The L92-pDMAEMA conjugate displayed higher surface activity (determined by measuring surface tension) compared to PEO-pDMAEMA at pH 5 and 7, and correspondingly higher transfection. Further, addition of free Pluronic P123 to L92-pDMAEMA conjugates increased further the transfection efficiency, presumably by a mechanism involving the hydrophobic stabilization of the L92-pDMAEMA conjugate.[27] The concept of adding free Pluronic to stabilize the carrier system has been applied to other formulations of Pluronic-polycation conjugates, as well as to non-PAO based delivery systems.[28,29] However, this effect is not universal, as the addition of free Pluronic P85 to a conjugate of P85-poly({*N*-[*N*-(2-aminoethyl)-2-aminoethyl] aspartamide} (P85-P[Asp(DET)]) did not improve transfection.[30]

PAO-polycation conjugates have also proven effective *in vivo*. The P85-g-PEI-based transfection system proved to be efficient for Ku86 antisense oligonucleotide (AON) delivery *in vivo* to human colon adenocarcinoma xenografts. In comparison to PEI-AON complexes, P85-g-PEI-Ku86 AON complexes demonstrated high stability in serum-containing solutions by reducing aggregation in the bloodstream, and also reduced toxicity effects.[31] This delivery system displayed significant inhibition of tumor growth when used in conjunction with ionizing radiation (IR), showing therapeutic promise in a clinical setting.

POLYALKYLENE OXIDE COPOLYMER –LIPID COMPLEXES

Cationic lipids have been employed widely for the delivery of short interfering RNA (siRNA), antisense oligonucleotides (AONs) and plasmid DNA in several *in vitro* and *in*

vivo transfection studies over the past decade.[32–35] The chemistry of liposomes is appealing because they are naturally amphiphilic and similar to biological membranes in terms of phospholipid structure. Furthermore, PAO copolymers can be incorporated into liposome/ nucleic acid complexes (lipoplexes) with the goal of increasing half-life in circulation and reducing toxicity by shielding the positive charge and modulating the surface activity of the carrier.

Liposomes based on sphingosine and dioleoylphosphatidylethanolamine (DOPE) were modified by the addition of Pluronic 188 to act as a non-ionic surface-active agent that stabilizes the DNA/liposome complex.[36] This formulation achieved enhanced transfection in SKOV-3, NPC076, Huh7, HeLa, AGS, NIH/3T3 and A431 cell lines, whereas formulations without Pluronic 188 failed to display transfection activity over a range of DNA/lipid ratios. This formulation was investigated *in vivo* on male BALB/c mice, in which SP-DOPE/DNA and Pluronic 188 mediated higher levels of luciferase expression in all the major organs, while also maintaining prolonged gene expression in lung and spleen.

Liposomes based on egg phosphatidylcholine (PC), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), dipalmitoyl rhodamine phosphatidyl- ethanolamine (DPRhPE) and PEG-distearoyl phosphatidylethanolamine (PEG5000- DSPE) were modified using a temperature-sensitive PAO block copolymer, Pluronic F-127, in order to develop a general mechanism to de-stealth liposomes at the target site and in turn achieve better liposome-cell adhesion. Pluronics F127 was found to be the most suitable candidate in the Pluronics family owing to its large PEO segments that coat the liposome surface, PPO segments that provide anchorage to the membrane, and for its CMT (critical micelle temperature), which lies in the physiological range (i.e., 37°C). Experiments demonstrated that adhesion of the Pluronic modified liposomes to CHO cells was inhibited at temperatures above the CMT where the Pluronics form micelles, but not at temperatures below CMT where they exist as unimers.[37]

Our group's approach to liposome-mediated AON delivery involves the addition of amphiphilic graft copolymers to a cationic liposome, such as DOTAP (schematic represented in Figure 3). The polymer backbone consists of poly(propylacrylic acid) (PPAA), a pH-sensitive hydrophobic polymer that induces endosomal membrane destabilization and release of therapeutic cargo. PPAA was modified by grafting on to its backbone either PEO or Jeffamine (EO: PO = 31:10). The properties of the resulting graft copolymer conjugates, PPAA-g-PEO and PPAA-g-Jeffamine, can be tuned by the molecular weight of the backbone polymer, the density of grafting, and by the proportion of EO to PO groups. Enhancement of AON delivery in CHO and A2780 cells has been observed with PPAA-g-PAO conjugates vs. PPAA without any modification.[38] The DOTAP/AON/PPAA-g-PAO graft copolymer delivery system incorporates PPAA and PAO to maintain a sufficient degree of hydrophobic character required for membrane (plasma and endosomal) association, along with hydrophilic character required for serum stability.

To study the effects of varying copolymer HLB on AON delivery, we varied the graft density of PEO or Jeffamine M-2070 onto the PPAA backbone to create copolymers ranging from 1–10 mol% grafting. The degree of membrane penetration, as measured by a calcein dye leakage assay, decreases with increasing graft density, presumably due to increased steric hindrance between the copolymer and membrane (Figure 4). This result correlates directly with the degree of gene silencing (in complex with DOTAP/AON) within the set of graft copolymers. Native PPAA (0% grafting) added to DOTAP/AON is not as effective for gene silencing despite having strong membrane interactions due to its serum instability. The 1 mol% grafting of PEO/Jeffamine onto PPAA strikes an optimum balance of steric stabilization and hydrophobicity, allowing simultaneously for minimal interactions with serum components (data not shown) while maintaining favorable association with

membranes, leading to efficient delivery of antisense cargo into cells and subsequent gene silencing.

We have also characterized the pH-dependent membrane destabilization of the graft copolymers of PPAA with PEO and Jeffamine using a hemolysis assay. Modification of PPAA with high degrees of PEO and Jeffamine grafting (20 mol%) eliminates the pH-sensitive lysis effect of the parent polymer, PPAA.[38] In contrast, lower degrees of PEO and Jeffamine grafted at 1 mol%, onto PPAA retains the effect lysis effect at acidic pH of 5 and at the absence of lysis at neutral pH of 7 (data not shown). Furthermore, conjugation of Jeffamine M-2005 (PO/EO=29/6) with PPAA produces a very hydrophobic copolymer that displays high degrees of hemolysis throughout the pH range 5 to 7, thus proving to be an unfavorable graft polymer chemistry because of this cytotoxic effect.

POLY (ALKYLENE OXIDE) COPOLYMERS AS ADJUVANT AGENTS TO NAKED DNA

PAO block copolymers have been used as adjuvants to DNA vaccines to promote an immune response.[39] Intramuscular delivery of adjuvant-plasmid DNA significantly increased gene expression in myocytes and keratinocytes at the site of administration in a mouse model of skeletal muscle. Moreover dendritic cells (DCs) and macrophages in the distal organs, including draining lymph nodes and spleen, also showed increased expression of reporter gene. The efficient cellular distribution of transgene elicits systemic and local expansion of antigen presenting cells (DCs and macrophages), an effect suggested to be attributable to Pluronics. It has also been suggested that the surface activities of PAO block copolymers affect positively the duration and intensity of a particular immune response.[40] A Phase I clinical trial demonstrated the tolerance of a DNA vaccine that is formulated with poloxamer CRL1005 (PEO_{207}-PPO_7-PEO_{207}) to enhance cellular and humoral immune responses.[41]

Pluronics can influence transgene activity even when used in "free" form, without conjugation to DNA.[42] For example, enhanced expression of reporter and therapeutic genes was observed *in vivo* when the mixture of Pluronic L61 and Pluronic F127 (known as SP1017) was administered intramuscularly. Although this formulation did not condense DNA due to the lack of positive charges, the SP1017-plasmid DNA complex enhanced gene expression by 5–20 fold, compared to that obtained by naked DNA transfection. It should be noted that the enhanced gene expression *in vivo* opposes cell culture results with transfection of myoblasts and the murine muscle cell line, C2C12. This discrepancy between *in vitro* and *in vivo* results has been observed by others.[43,44] Similarly, several other studies reported enhanced transgene expression levels *in vivo* when Pluronic and Tetronic block copolymers were used as adjuvants.[45,46,47] Early studies demonstrated ocular delivery of plasmid DNA using the non-ionic PEO-PPO-PEO copolymer.[48] Several examples have demonstrated the use of Pluronic block copolymers for plasmid DNA delivery to target muscle dystrophy. For example, poloxamine 304, commercially known as Lutrol® (PEO_{75}-PPO_{30}-PEO_{75}) was utilized for plasmid DNA delivery to mouse muscle tissue.[49] Ocular and muscular applications share the common feature of involving delivery directly into the target tissues, avoiding systemic circulation issues. A phase II clinical study for the treatment of patients suffering from claudication as a result of moderate to severe peripheral arterial disease was conducted using VLTS-589 (plasmid encoding the endothelial secreted protein Del-1 in conjunction with poloxamer 188).[50] Similar results were observed between treatments of plasmid with poloxamer and poloxamer alone, with significant improvement in exercise capability, suggesting positive direct effects of poloxamer 188 in this application. A potentially exciting application is the use of nonionic PEO-PPO-PEO micelles for oral gene delivery.[51]

The pathway of cellular entry can be influenced by the HLB of the amphiphilic block copolymer. To this end, Chevre et al. have reported that Lutrol®, with 80% PEO, enhances gene delivery by promoting plasma membrane transport via direct fusion, and amphiphilic polymer P85 with 50% PEO promotes DNA transfection in a promoter-dependent manner and activates the NF-kB signaling pathway.[52] Furthermore, the state of aggregation of Pluronic chains in aqueous solution (unimer or micelle) affects the route of endocytosis. For example, P85 unimers were shown to enter cells by caveolae-mediated of endocytosis, while P85 micelles enter by clathrin-mediated endocytosis in the model MDCK (Madin-Darby canine kidney) cells.[53] A possible explanation for this behavior is that PPO blocks in Pluronics unimers recognize cholesterol-rich domains in the cell membranes, mediate association and allow for entry by caveolae-mediated endocytosis. In contrast, the micelle structure is such that the PPO blocks are buried, thus making them less likely to interact with lipid portions of the cell membrane. The ability to control the route of endocytosis via the unimer-micelle equilibrium motivates the employment of amphiphilic copolymers in drug and nucleic acid delivery systems.

BIODEGRADABLE AND GEL FORMULATIONS OF PAO

For applications such as wound healing, topical or depot delivery with hydrogels can be employed. Reversible gels are attractive for nucleic acid delivery due to the feature that their network is not held together by covalent bonds, but instead by physical crosslinks that are dictated by the self-ordered micellar morphology of the PAO.[57] On the other hand, without modification these gels are likely to result in burst rather than controlled release of therapeutic cargo.[58] To regulate the diffusion of entrapped therapeutics through these gels and to maintain their integrity, several modifications have been practiced to date, such as incorporation of labile groups in the polymer chain, resulting in a self-degradable polymer.[59] For example, the biodegradable poly(lactic-co-glycolic acid) (PLGA) was copolymerized with PEG to form the triblock, PEG-PLGA-PEG.[43] The critical gelation temperature can be tuned by altering molecular characteristics of the blocks. The degradability of these polymers makes them an appropriate therapeutic carrier especially in treatments where localized and controlled release is advantageous.

A variety of block copolymers combine biodegradable segments with PAO segments. For example, multi-block copolymers based on poly (L-lactic acid) and PEO were observed to degrade to 20% of the initial molecular weight within 72 hr under physiological conditions. Furthermore, the shielding effect of PEO stabilized the copolymer/pDNA conjugates, allowing transfection in the presence of serum proteins.[55] Polycaprolactone has also been copolymerized with PEO and a variety of cationic groups to create effective vehicles for plasmid DNA and siRNA delivery.[60,61]

CONCLUSIONS

Nucleic acids have tremendous potential for impact in a range of therapeutic applications involving either gene transfer with plasmid DNA or gene silencing with synthetic oligonucleotides. Research in the field of PAO-based carriers for nucleic acid delivery has explored PAO micelles and PAO conjugates with polycations and liposomes. The advantage of PAO chemistry, compared to that of liposomes, is that the former is more flexible, with respect to allowing incorporation of two or more chemical moieties in graft or block form to meet design criteria such as nucleic-acid binding, membrane-lysis, or improved systemic circulation. The PAO chemistry needs to be optimized depending on the polycation that is being used for nucleic acid complexation, but generally PAOs of higher molecular weight (5 kDa), of triblock configuration for micelle formation, and higher HLB (values 20 of higher) significantly improve activity.

Particular attention has thus far been focused on the effects of PAO HLB on nucleic acid delivery. From a carrier formulation standpoint, development work has utilized free PAOs and PAO-conjugates that result in greater nucleic acid delivery and transfection efficiency. Further research is required to better establish the relationship between the physicochemical properties of PAO copolymers and their functions in the gene delivery process, specifically their effects on serum stability, membrane penetration, endosomal escape and intracellular trafficking, and how these correlate with gene expression or gene silencing. The correlation between copolymer HLB and membrane penetration, and further between membrane penetration and gene silencing activity, has been an important step in a path that can be followed to better define and control how the chemistry of PAO-based carriers affects gene delivery mechanisms and efficiency.

Acknowledgments

Biographies

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Charles M. Roth is an Associate Professor in the Department of Chemical and Biochemical Engineering and Department of Biomedical Engineering at Rutgers, The State University of New Jersey. He received his Ph.D. in chemical engineering from University of Delaware and was a postdoctoral fellow and subsequently Instructor in Bioengineering at Harvard Medical School and Massachusetts General Hospital. Dr. Roth's research interests are broadly in the areas of molecular and nanobioengineering with an emphasis on gene silencing technology and engineering approaches to cancer.

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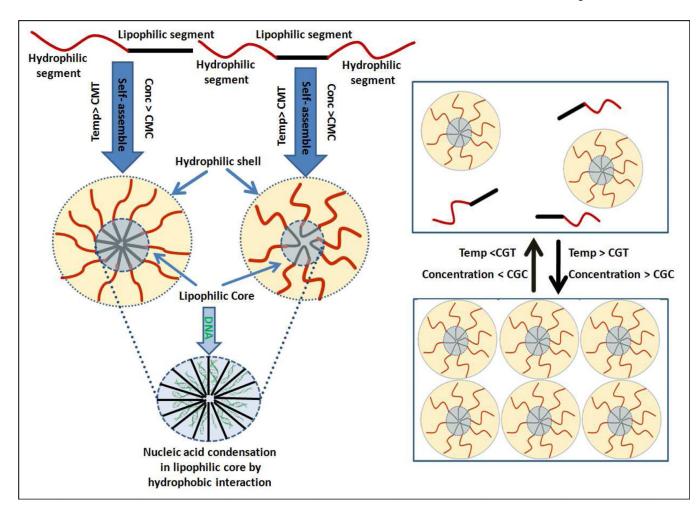
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Figure 1.
Formation of nucleic acid nanocomplexes with diblock and triblock PAO copolymers.
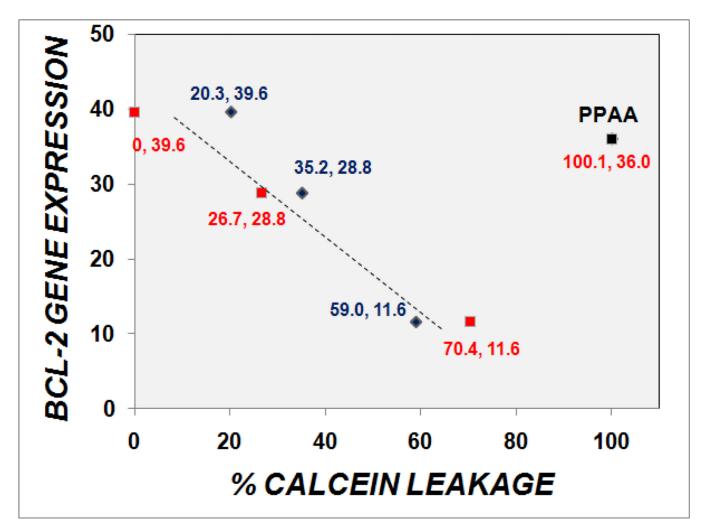
Figure 2.
Chemical architectures of polymers comprising ethylene oxide and propylene oxide groups.

FORMATION OF NANOCOMPLEXES	PARTICLE SIZES	COMPLEXATION WITH NUCLEIC ACIDS
PAO MICELLE/NA	170-620nm[39,43]	▪ Hydrogen bonding ▪ Hydrophobic interactions
POLYCATION-g-PAO/NA	85 -250nm[18-21]	▪ Electrostatic interactions
DOTAP/NA/PgPAO	150-400nm[29]	▪ Electrostatic interactions ▪ Hydrophobic interactions ▪ Hydrogen bonding

Figure 3.
Morphological structures of nanocomplexes formed between PAO/nucleic acid, PAO/
polyplexes and PAO/lipoplexes.

Figure 4.
Correlation between the membrane activity of PPAA-g-PAO polymers and gene silencing activity of DOTAP/PPAA-g-PAO/AON vector. Gene silencing was determined by inhibition of bcl-2 expression in A2780 cells (measured using real-time PCR) and membrane activity was measured by calcein dye leakage assay. Red square indicates graft density of PEO onto PPAA of 1, 5, 10 mol% (from right to left), and blue diamond indicates graft density of Jeffamine onto PPAA of 1, 5, 10 mol% (from right to left).

Table 1

Physical characteristics of Pluronic block copolymers used in gene delivery applications

Copolymer	Molecular weight (Da)[a]	Composition [b]	N_{PO}/N_{EO}[c]	HLB [d]	CMC (M) [e]	ΔH [f] (kJ/mole)
L61	2000	EO_2-PO_{30}-EO_2	7.5	3	$1.1×10^{-4}$	183
L64	2900	EO_{13}-PO_{30}-EO_{13}	1.15	15	$4.8×10^{-4}$	185
L92	3650	EO_8-PO_{47}-EO_8	2.94	6	$8.8×10^{-5}$	278
L101	3800	EO_5-PO_{56}-EO_5	5.60	1	$2.1×10^{-6}$	380
L121	4400	EO_4-PO_{69}-EO_4	8.63	1	$1.0×10^{-6}$	401
F68	8400	EO_{76}-PO_{30}-EO_{76}	0.19	29	$4.8×10^{-4}$	91.6
F127	12,600	EO_{99}-PO_{69}-EO_{99}	0.35	22	$2.8×10^{-6}$	161
P85	4600	EO_{26}-PO_{39}-EO_{26}	0.75	16	$6.5×10^{-5}$	194
P105	6500	EO_{37}-PO_{56}-EO_{37}	0.76	15	$6.2×10^{-6}$	313
P123	5750	EO_{20}-PO_{69}-EO_{20}	1.73	8	$4.4×10^{-6}$	351

[a] Molecular weights are given by manufacturer (BASF Inc.)

[b] Composition is obtained from reference[22]

[c] Ratio of number of EO and PO blocks

[d] Hydrophilic/lipophilic balances (HLBs) of the copolymers were determined by manufacturer

[e] CMCs were determined using pyrene solubilization technique by Kabanov et al.[23].

[f] Enthalpy of micellization in dilute (0.5% w/v) solution: ΔH was measured by integrating the area under the peak in the DSC thermographs. Values are obtained from reference[22]

Table 2

Applications of poly(alkylene oxide) based block copolymers in nucleic acid delivery.

Carrier	Nucleic acid	Type of PAO block copolymer	Experimental model
Micelle	EPO, Luciferase reporter gene, and β-galactosidase	Mixture of Pluronics L61 and F127	Mice and rats with muscle dystrophy[42]
Micelle	pCAT, H minidys-GFP, SeAP, pTetO-mEPO	Poloxamine 304	Mice-skeletal muscle and heart muscle *in vivo*[54]
Micelle	Luciferase, GFP reporter gene	Pluronic P85	Mice-skeletal muscle, antigen presenting cells[39]
PEI-PAO	Anti-Ku86 AON	Pluronic P85	*In vivo*, Human colon adenocarcinoma[31]
P[Asp(DET)]-PAO	pCMV-luciferase reporter gene	Pluronic P85, PEO	MDA-MB-231 and A549 cells[30]
PEI	pSV- β galactosidase gene	F68, F127, P105, P94, L122, L61	*In vitro*/NIH/3T3[24]
Guanidinylated PEI-PAO, PEI-PAO	pCMV-β galactosidase gene	Jeffamine M-2070	CHO, NIH/3T3 and Cos-7[26]
SP/DOPE liposome	Luciferase reporter gene	Poloxamer 188	SKOV3, NPC076, Huh7, HeLa, AGS, NIH/3T3, A431, Mice[36]
DOTAP/PPAA liposome	Anti-GFP AON	Jeffamine M-2070	CHO, A2780[38]
DMAEMA-PAO	pCMV-β galactosidase gene	Pluronic L92, P123	CHO[27]
PLL-PAO polyplex	pSV-β galactosidase gene	PEO	293T[55]
PLGA-PAO-PLGA micelle	Luciferase reporter gene	PEO	293T[56]

Abbreviations used: CAT-chloramphenicol acetyltransferase, CMV-cytomegalovirus, GFP-green fluorescent protein, SV-simian virus, EPO-Erythropointin, H minidys- human minidystrophin, SeAP- secreted alkaline phosphatase, pTetO-mEPO-plasmid encoding the tetracycline inducible murine erythropoietin, PLL-poly(L-lysine), PLGA-poly(lactic-co-glycolic acid)