




Review

PEO-PPO-PEO Tri-Block Copolymers for Gene Delivery Applications in Human Regenerative Medicine—An Overview

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Abstract: Linear (poloxamers or Pluronic[®]) or X-shaped (poloxamines or Tetronic[®]) amphiphilic tri-block copolymers of poly(ethylene oxide) and poly(propylene oxide) (PEO-PPO-PEO) have been broadly explored for controlled drug delivery in different regenerative medicine approaches. The ability of these copolymers to self-assemble as micelles and to undergo sol-to-gel transitions upon heating has endowed the denomination of “smart” or “intelligent” systems. The use of PEO-PPO-PEO copolymers as gene delivery systems is a powerful emerging strategy to improve the performance of classical gene transfer vectors. This review summarizes the state of art of the application of PEO-PPO-PEO copolymers in both nonviral and viral gene transfer approaches and their potential as gene delivery systems in different regenerative medicine approaches.

Keywords: PEO-PPO-PEO copolymers; nonviral vectors; viral vectors; gene transfer

1. PEO-PPO-PEO Tri-Block Copolymers

Amphiphilic copolymers are particularly appealing materials due to their ability of simultaneously displaying the performance of hydrophilic and hydrophobic polymers. While the hydrophobic blocks are responsible for the water solubility from the individualized copolymer molecules and of the creation of a suitable interface with the aqueous surrounding, the hydrophobic blocks adsorb onto hydrophobic surfaces [1]. Among the different types of amphiphilic block copolymers, those based on tri-blocks of poly-ethylene oxide (PEO, hydrophilic blocks) and poly-propylene oxide (PPO, hydrophobic blocks) are among the most widely used in pharmaceutical formulations. PEO-PPO-PEO copolymers are normally classified in two families according to the structure of their main chain. They can be linear and bifunctional tri-blocks (PEO-PPO-PEO) known as poloxamers (Pluronic[®] or Lutrol[®]) or X-shaped (four arms PEO-PPO blocks) triblocks linked by a diamine central core known as poloxamines (or Tetronic[®]) [1] (Figure 1). The unique structure of poloxamines confers them with multi-stimuli responsiveness. In this context, the two tertiary amine central groups play an essential role, conferring thermodynamical stability and pH sensitivity while enabling further chemical modifications in order to acquire additional properties [2]. PEO-PPO-PEO copolymers are commercially available in a broad spectrum of molecular weights (MW) and Ethylene oxide/Propylene oxide (EO/PO) ratios [3] and generally display a good cytocompatibility, without producing significant irritation after topical or parenteral administration [4,5].

The nomenclature of Pluronic[®] includes the letters, F, P, or L, which are associated with the physical states of these polymers, namely solid, paste and liquid, are followed by a two or three digits, which represent a numeric code related with their structural parameters [6,7]. On the other

hand, Tetronic[®]-based copolymers are normally classified as a function of their hydrophilicity as highly hydrophilic (T908, T1107 and T1307), medium hydrophilic (T304, T904 and T1304) and highly hydrophobic (T701, T901, T1301, T90R4 and T150R1) [1].

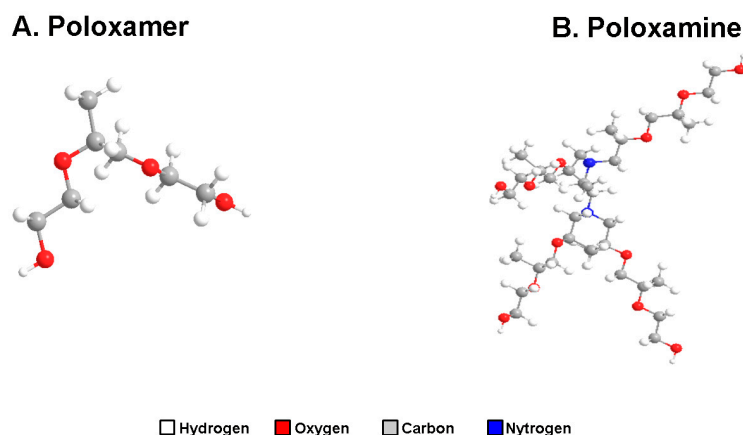


Figure 1. Structure of poloxamers (A) and poloxamines (B). Two-dimensional sequences of Poloxamer P85 (CID: 10145203) and poloxamine 304 (CID: 86278173) were obtained from PubChem. Three-dimensional (3D) structures of the different compounds were drawn with ChemBioOffice 2012 (Chem3D Pro 13; PerkinElmer Informatics, Cambridge, MA, USA).

1.1. General Aspects

A feature of PEO-PPO-PEO copolymers is the ability of the individual block copolymers or “unimers” to self-assemble into micelles in aqueous solution at concentrations higher than the critical micellar concentration (CMC). In general, the lower the lipophilic/hydrophilic balance (HLB) and the higher the MW, the lower the CMC found [8–10]. The temperature-sensitiveness of these copolymers makes the CMC decrease with the increase of temperature. In general, those copolymer varieties with longer PPO blocks require lower concentrations or temperatures for micellization to occur [9].

When the temperature and concentration increase, the micelles form 3D networks (gels) of high viscosity. Chiefly, micellization is noted at much lower concentration (<1 wt %) than those needed for gellation (>15 wt %) [8].

In general, PEO-PPO-PEO copolymers that display a sol-gel transition around 37 °C can be orally administered or injected in the body as liquid solutions at room temperature and, once at the physiological temperature, they become semi-solid to solid gels that sustain drug release [3]. This ability of PEO-PPO-PEO copolymers to self-assemble as micelles and to undergo sol-to-gel transitions upon heating has endowed the denomination of “smart” or “intelligent” systems [11].

Even though PEO-PPO-PEO copolymers do not degrade under physiological conditions, those with molecular weights between 10 and 15 kDa are normally filtered by the kidney and cleared in urine [12]. These features make them powerful candidates to treat different human pathologies by controlling the release of different models of drugs or other bioactives or by providing a platform for the construction of biological structures acting as cell scaffolds in different tissue engineering approaches.

1.2. PEO-PPO-PEO-Based Micellar Systems

Polymeric micelles are nanosized carriers based on amphiphilic PEO-PPO-PEO that can be tailored to fit the physicochemical characteristics of cargo and therapeutic requirements of the pathological process [13]. Structurally, polymeric micelles are based on a hydrophobic core with hydrophobic blocks that are approaching to minimize the contact with the surface, increasing the solubilization and stabilization of poorly water-soluble drugs, and a hydrophilic shell with hydrophilic blocks in contact in the aqueous medium acting as an interface with the core as well as decreasing undesirable drug

interactions with the cells [6,14]. Micellization is a process strongly driven by an entropy gain and the free energy of micellization is mainly a function of the PPO block [15]. Therefore PEO-PPO-PEO copolymers with larger hydrophobic domains form micelles at lower concentrations [15].

Polymeric micelles have a size between 10–100 nm being in the preferred range for pharmaceutical approaches [1,6]. When compared with micelles formed by common low MW surfactants, polymeric micelles have lower CMC, higher thermodynamic and kinetic stability to withstand dilution, and enhanced drugs solubilizing and stabilizing capability [16,17].

PEO-PPO-PEO-based micelles have been used as potential delivery systems of multiple drugs and biomolecules to increase their stability and solubility and afford protection against fast degradation [7,18–21].

1.3. PEO-PPO-PEO-Based Hydrogels

The viscoelastic behavior of PEO-PPO-PEO copolymers as a function of their concentration and the effect of several stimuli such as pH (poloxamines) and temperature on their gelation properties have attracted the attention in recent years for developing controlled delivery systems of diverse biomolecules [22]. As the temperature increases, gel systems based on PEO-PPO-PEO block copolymers become less hydrophilic due to the progressive dehydration of the polyether blocks. This fact promotes the formation of more micelles and ultimately the formation their packing into body centered cubic phase gels [23].

In general for a given content in EO groups, the longer the PO block of the PEO-PPO-PEO copolymer variety, the lower the gel temperature [1]. So far, poloxamers exhibit a sol-gel transition at lower concentration compared with poloxamines. For example, while Pluronic[®] F127 (70 wt % PEO, MW 12.6 kDa) shows a critical gel concentration around 14.8%, Tetronic[®] 1107 (70 wt % PEO, MW 15 kDa) needs a minimal concentration around 30% to undergo sol-to-gel transition [24]. Formulation of aqueous systems based on PEO-PPO-PEO copolymers that can be easily syringeable at room temperature leading to the formation of highly viscoelastic gels at physiological temperature (37 °C) has a great potential for diverse regenerative approaches [18]. In situ gelling systems based on poloxamers or poloxamines have been studied to control the release of growth factors [25,26] and different drugs [27–29] and more recently for 3D cell printing applications in different tissue engineering approaches [30,31].

2. PEO-PPO-PEO Tri-Block Copolymers as Micellar Nanocarriers for Drug Delivery

The ability of PEO-PPO-PEO micelles to host relatively hydrophobic drugs increasing their apparent solubility and protecting them against degradation has already been reported for different models of drugs [7,32–36]. This solubilization capacity from poloxamers and poloxamines relies on the MW and the PEO/PPO ratio and in general the more hydrophobic the molecule, the higher solubilization extension [3]. So far, it has been reported that those copolymers with a similar HLB will display higher solubility efficiency when their MW are higher. On the other hand, a combination of poloxamers with different MW and EO/PO ratios results in mixed micelles with a superior efficiency of solubilization and higher stability upon dilution [8]. Poloxamer micelles have already been shown to increase the solubility of different models of drugs [37].

In the particular case of poloxamines, their dual responsiveness to pH and temperature and the possibility to adjust their aggregation properties by tuning the pH of the medium has attracted strong attention in recent years as a means to increase the solubility of poorly soluble drugs [1]. Chiefly, it has been reported that the higher the pH of the medium, the greater the micellization tendency and the higher the solubilization ability of poloxamines [24]. This fact has already been described for both pH-independent drugs such as griseofulvin [24] and efavirenz [38] and pH-dependent drugs such as triclosan [19].

3. PEO-PPO-PEO Tri-Block Copolymers for Passive Micellar Targeting

Direct administration of small free anticancer drugs generally results in a rapid distribution in and out of all tissues, leading to a short residence time and rapid clearance by renal filtration [39]. A valuable strength of PEO-PPO-PEO micelles is their spontaneous and preferential accumulation at biological places with vascular abnormalities leading to enhanced permeability and retention (EPR), a common fact from all tumor types except the vascular ones [3]. To take advantage of the EPR effect, the drug needs to be included into a macromolecular structure that can selectively enter the tumoral tissue through the enhanced pores and avoid its elimination by the lymphatic drainage. Generally, a more prolonged circulation time, ideally over 6 h for polymeric micelles, results in a greater possibility of reaching the cancerous tissue [40]. Polymeric micelles (10–100 nm) are large enough nanocarriers to avoid renal excretion (>50 KDa), but still small to bypass the filtration by inter-endothelial cells slits in the spleen [3]. Of further note, the hydrophilic corona from polymeric micelles plays an important role in preventing the opsonization and successive clearance by the mononuclear phagocyte system in the spleen and liver [41]. Both poloxamers and poloxamines can lead to the formation of micelles with an adequate size for the EPR effect and resist the strong dilution occurring upon contact with physiological fluids [3,10,42–44].

4. PEO-PPO-PEO Tri-Block Copolymers for Human Gene Therapy

4.1. Gene Transfer Vectors: Current Limitations

The identification of gene products that are involved in supporting the underlying cause of pathology has offered the biopharmaceutical industry an opportunity to develop compounds that can specifically target these molecules to improve therapeutic responses and lower the risk of unwanted side effects that are commonly seen in traditional small chemical-based medicines [45]. In this scenario, the development of gene delivery vehicles have emerged as a promising technology to treat different pathologies by directly transferring of genes encoding for therapeutic factors into the places of injury that result in a temporarily and spatially defined delivery of a candidate agent [46]. Current gene transfer vectors used for gene transfer of target cell populations in regenerative medicine approaches include nonviral [47] and viral vehicles [48] such as adenoviral [49], retro-/lentiviral [50,51] herpes simplex viral vectors (HSV) [52], and recombinant adeno-associated viral (rAAV) vectors [53]. Nonviral gene delivery has various advantages as it is considered a safe method that does not provoke immune responses in the host while it can be prepared in large amounts at relatively low expense [54]. While plasmid DNA (pDNA) permits transfection in vivo, packaging DNA with cationic lipids or polymers may further facilitate material uptake and transfection in vitro and in vivo [47]. Therefore, complexation of DNA with cationic polymers (polyplexes) or lipids (lipoplexes) may protect DNA against degradation by nucleases and serum components by creating a less negative surface charge and can be designed to target specific cell types through receptor-ligand interactions [55]. Yet, the main limitation of such vectors is their tendency towards aggregation and their low transfection efficiencies and short-term transgene expression levels (some days) [56]. Nonionic water-soluble polymers that do not bind or condense with DNA but significantly enhance the expression of transgenes in vitro and in vivo have noticeably received increased attention in recent years [6,57–60]. Of note, PEO-PPO-PEO copolymers based on Pluronic[®] were reported to enhance the expression of both naked pDNA [58,60–64] and genes delivered using polycation-DNA complexes [65–69] both in vitro and in vivo. While depicting a higher efficiency, viral gene transfer is limited by the toxicity associated with some types of vectors (adenovirus), a possible diffusion of the vectors to nontarget places, and the existence of patient-associated factors and physiological barriers (existence of neutralizing antibodies against the viral capsid, inhibition of transduction in the presence of specific anticoagulants) that may interfere with the effective delivery, processing, and expression of transgene inside the target cells [46]. The use of PEO-PPO-PEO copolymers has already been described to increase the efficiency

of viral gene transfer by providing a localized delivery into the targets or protecting the vectors against physiological barriers [46], including of adenoviral [70–74], lentiviral [75,76], and rAAV vectors [77–82].

4.2. PEO-PPO-PEO Copolymers: Applications for Nonviral Gene Transfer

Skeletal muscle is a common target for gene therapy due to its accessibility and ability for production of proteins as systemic therapeutic reagents [83]. Likewise, injection of naked pDNA into muscle was already shown to provide an expression for up 19 months [84]. Still, the levels of gene expression achievable with naked DNA are often insufficient to ensure a therapeutic effect. To increase the efficiency of transgene expression, a combination of two poloxamers (SP1017: Pluronic® L61 and F127) was involved to intramuscularly deliver pDNA encoding for reporter β -galactosidase gene activity (*lacZ*) in mice [58,64] (Table 1). An estimation of the levels of *lacZ* expression revealed that SP1017 considerably increased pDNA diffusion through the tissue [58,64]. A micellar solution of a PEO-PPO-PEO copolymer with an average MW of 8400 was involved as a carrier for eye-drop gene delivery of pDNA with *lacZ* in vivo [62]. Topical delivery of pDNA via PEO-PPO-PEO micelles for 48 h demonstrated *lacZ* expression was detected around theiris, sclera, conjunctiva, and lateral rectus muscle of rabbit eyes and also in the intraocular tissues of nude mice [62]. Polyelectrolyte complexes formed between DNA and polycations constitute one of the gold standards for nonviral gene delivery. So far, one of the main problems encountered using these techniques is the relatively low efficacy of DNA (or complex) release from endocytic compartments in the cytoplasm and nucleus of cells [85]. Likewise, due to charge neutralization, these complexes are often unstable in aqueous solutions and precipitate, thus hindering their application in gene delivery [65,85]. To solve these issues, a micellar solution of Pluronic® P85 has been employed to deliver DNA encoding for the chloramphenicol acetyltransferase (CAT) gene complexed with poly(*N*-ethyl-4-vinylpyridiniumbromide) (PEVP) [65]. Results from this study showed an increase of CAT gene internalization and expression in different cell lines when provided via polymeric Pluronic® P85 micelles [65]. Polyethyleneimine (PEI) constitutes one of the most widely used cationic polymers for nonviral gene transfer [86]. This polymer spontaneously forms interpolyelectrolyte complexes with DNA as a result of cooperative electrostatic interactions between the ammonium groups of the polycation and phosphate groups of the DNA [66]. However, one of the main drawbacks of PEI is its poor solubility upon complexation with DNA that may considerably reduce its transfection efficiency. Grafting of PEI with PEO-PPO-PEO copolymers (Pluronic® P123) has been reported as a potential approach to solve such a limitation by optimizing the size of the polyplexes [67], achieving an increased expression of the reporter Firefly luciferase (*luc*) transgene, with a uniform distribution in the liver when administered intravenously in mice [66]. So far, the highest efficiency was reported to be achieved when using PEO-PPO-PEO copolymers at high HLB [68]. Similarly to PEI, polylysine (PLL) is one of the best known cationic polymers to strongly interact with pDNA, resulting in a compact polymer/DNA complex for an increased uptake of foreign genes to mammalian cells [87]. Yet, its use as a DNA carrier is still limited by its low transfection efficiency. Conjugation of PLL with Pluronic® F127 showed a 2-fold increase in transfection efficiency compared with unmodified PLL [69]. Although less explored than their poloxamer counterparts, poloxamines have also been studied as integrants of scaffolds for the controlled delivery of nonviral vectors [88]. A fibrin/T904 hydrogel was synthesized to incorporate either a naked plasmid encoding for the green fluorescent protein (GFP) or polyplex vectors based on such a reporter gene sequence. When N2A neuroblastoma cells were encapsulated in these systems, an increased transgene expression profile was noted over 2 weeks [88].

Table 1. Use of PEO-PPO-PEO copolymers for nonviral gene transfer.

Nonviral Systems	Copolymers	Genes	Targets	Administration	Observations	References
pDNA	SP1017: Pluronic® L61 + F127	<i>lacZ, luc</i>	muscle	i.m. (rat)	10-fold increased transgene expression	[58]
		<i>lacZ</i>			increased transgene expression after electroporation	[64]
	PE6400	<i>lacZ</i>	muscle	cranial muscle (mouse)	long-term expression similar to electrotransfer	[63]
	Pluronic® F68 and F127	<i>luc</i>	n.s.	in vitro BL-6 cells	increased activity in transfecting cells in the presence of 20% serum	[61]
	Pluronic® P85 and L61	<i>luc, GFP</i>	n.s.	in vitro NIH3T3, C2C12 and C166 cells	increased transgene expression	[60]
	PEO-PPO-PEO copolymers average MW 8400	<i>lacZ</i>	Eye	ocular (rabbit, mouse)	higher transgene expression at 2 and 3 days	[62]
Polycation DNA and poly(<i>N</i> -ethyl-4-vinylpyridinium)	Pluronic® P85	CAT	n.s.	in vitro NIH 3T3, MDCK, and Jurkat cell lines	enhanced transfection	[65]
P123-g-PEI(2K)polyplex	Pluronic® P123	<i>luc</i>	n.s.	in vitro Cos-7 cells, i.v. (mouse)	more uniform distribution of transgene, significant improvement of gene expression in liver	[66]
			n.s.	in vitro prostate cancer cells (PC-3)	optimization of polyplex size	[67]
PEI-DNA complex	Pluronic® F68, F127, P105, P94, L122, L61	<i>lacZ</i>	n.s.	in vitro NIH/3T3 cells	Pluronic® with higher HLB showed marked improvement of gene expression levels in serum media compared with PEI-DNA complexes alone	[68]
PEI-DNA complex or pDNA	Tetronic® 904	GFP	n.s.	in vitro N2A cells	sustained transgene expression for over 2 weeks	[88]
PLL-g-Pluronic®	Pluronic® F127	<i>lacZ</i>	n.s.	in vitro HeLa cells	higher transfection efficiency with polymer:DNA at 1:1	[69]

Abbreviations: pDNA: plasmid DNA; *lacZ*: *E. coli* β -galactosidase; *luc*: luciferase; PE6400: poly(ethyleneoxide)(13)-poly(propyleneoxide)(30)-poly(ethyleneoxide)(13) block copolymer; n.s.: not specified; GFP: green fluorescent protein; CAT: chloramphenicol acetyltransferase; i.m.: intramuscular; i.v.: intravenous; P123-g-PEI(2K)polyplex: Pluronic® 123 grafted with 2KDa polyethyleneimine; PEI-DNA complex: polyethyleneimine-DNA complex; PLL-g-Pluronic®: poly-L-lysine grafted with Pluronic®.

4.3. PEO-PPO-PEO Copolymers: Applications for Viral Gene Transfer

Development of strategies for in vivo transfer of therapeutic genes relevant to these specific vascular pathophysiologic processes offers new therapeutic possibilities for the treatment of this type of disorders. Specifically, adenoviral vectors appear to be the most efficient vectors for vascular gene transfer [70]. However, transduction of vascular vessels requires enough time (20–45 min) to permit sufficient vector uptake. Administration of adenoviral vectors with hydrogel systems based on Pluronic® F127 has been reported as a potential approach to reduce the transfection time of the blood vessels with significant improvement of transgene expression both in vitro [70,71] and in vivo [72,73] (Table 2). A 10–100-fold increase on *lacZ* expression was seen upon administration of adenoviral vectors with a hydrogel system based on Pluronic® F127 to vascular smooth muscle cells [70], reducing the incubation time of adenoviral vectors from 20 to 10 min without compromising transfection efficiency [71]. Intratumoral infusion of adenoviral vectors with Pluronic® F127 gels was also described as a strong approach for cancer treatment by reducing the dissemination of the viral vectors from tumor to normal tissues during and after the infusion [74,77]. Pluronic®-based gel systems are potent adjuvants to increase the transduction efficiency of lentiviral vectors in vitro [76] and in vivo [75]. Effective transduction of astrocytes has been reported by stereotaxic delivery of a HIV-1-based lentiviral vector in a 15% PF127 gel [75]. rAAV exhibit potential advantages over other viral vectors due to their small size, ability to transduce dividing and nondividing cells, and absence of immunogenicity, constituting the most adapted vector to treat musculoskeletal disorders [89,90]. Still, although gene transfer via viral vectors is highly efficient, the existence of patient-associated factors and physiological barriers (existence of neutralizing antibodies against the viral capsid, inhibition of transduction in the presence of specific anticoagulants) may interfere with the effective delivery, processing, and transgene expression in the target cells [90]. Also, a possible dispersion of the rAAV viral particles to nontarget places may reduce their gene transfer efficiency [91]. Encapsulation of rAAV in poloxamer PF68 and poloxamine T908 polymeric micelles was shown to increase the levels of transgene expression of both mesenchymal stem cells (MSCs) [79,80] and osteoarthritic chondrocytes in vitro or in an in situ model of osteochondral defect [81,82] either with the reporter *lacZ* gene [80,81] or with highly chondrogenic genes (the sex-determining region Y-type high mobility group box 9 transcription factor—*SOX9*, the transforming growth factor beta—*TGF-β*) [79,82] (Table 3). Of further note, delivery of rAAV using such systems resulted in the restoration of the transduction of MSCs or chondrocytes with rAAV in conditions of gene transfer inhibition, i.e., in the presence of heparin or of a specific antibody directed against the rAAV capsid [79,81]. These features were attributed to the X-shaped structure of the poloxamines, rendering a positive charge at the physiological pH and protective effect exerted by PEO shell masking the antibody-specific rAAV capsid epitopes binding and thus the rAAV neutralization. Despite their potential advantages and simplicity, in situ gel systems based on these self-assembled copolymers remain partially hydrophilic and thus the gel depot erodes quite rapidly. To solve these drawbacks, we recently developed pseudopolyrotaxanes hydrogels by combining PEO-PPO-PEO copolymers with alpha cyclodextrins (α -CD) [92]. Results from these studies showed that incorporation of α -CD into gels based on mixtures of chondroitin sulfate (CS) or hyaluronic acid (HA) with PEO-PPO-PEO copolymer PF68 gels resulted in higher rAAV concentrations and sustained levels of transgene expression over time. Still, and while addition of α -CD resulted in the formation of more structured networks with greater elastic and viscous moduli compared with the pristine systems, a higher mechanical stability may be necessary to provide support for load-bearing functions of the cartilage tissue.

Table 2. Use of PEO-PPO-PEO copolymers for viral gene transfer (part I).

Viral Systems	Copolymers	Genes	Targets	Administration	Observations	References
Adenovirus	Pluronic® F127	<i>lacZ</i>	cardiovascular	in vitro vascular smooth muscle cells	high pericellular concentrations of vector and 10- to 100-fold increase of transduction	[70]
	Pluronic® F127	<i>lacZ</i>	vascular	in vitro vascular smooth muscle cells; in vivo balloon injured carotid arteries (rat)	improved gene transfer efficiencies	[71]
	Pluronic® F127	<i>lacZ, luc</i>	vascular	in vivo percutaneous administration in iliac arteries (rabbit)	increased efficacy of percutaneous gene transfer and reduced transfection time	[72]
	Pluronic® F127	<i>gax</i>	vascular	in vivo external iliac artery with channel balloon catheter	<i>gax</i> overexpression inhibits neointimal hyperplasia and lumen loss in atheromatous stented rabbit iliac arteries	[73]
	Pluronic® F127	GFP, <i>luc</i>	solid tumors	in vivo intratumoral infusion (mouse)	blocked convection of viral vectors in the interstitial space and the lumen of microvessels in the vicinity of the infusion site	[74]
Lentivirus	Pluronic® F127	GFP	CNS	in vivo injection to the thalamus (rat)	increased transduction of astrocytes at injection site	[75]
	Pluronic® F108	GFP, <i>luc</i>	n.s.	in vitro HEK293T, KARPAS-299, SUDHL-1, SR-786, SUP-M2, and PANC-1 cell lines	specific contribution to efficiency of each adjuvant; polybrene: charge protector and poloxamer synperonic F108: membrane modulator	[76]

Abbreviations: *lacZ*: *E. coli* β -galactosidase; *luc*: luciferase; *gax*: growth arrest homeobox; GFP: green fluorescent protein; rAAV: recombinant adeno-associated viral vectors; CNS: central nervous system; DC: dendritic cells; n.s.: not specified.

Table 3. Use of PEO-PPO-PEO copolymers for viral gene transfer (part II).

Viral Systems	Copolymers	Genes	Targets	Administration	Observations	References
rAAV	Pluronic® F127	GM-CSF	solid tumors	in vivo intratumoral infusion (mouse)	higher efficiency by combining DC, local tumor irradiation and controlled supply of recombinant mGM-CSF with Pluronic®	[77]
	Pluronic® F68	<i>lacZ</i>	adipose tissue	in vivo inguinal (mouse)	increased transgene expression after 4 weeks	[78]
	Pluronic® F127	<i>lacZ</i>	cartilage	in vitro hMSCs	controlled release of rAAV for high efficiencies over time and gene expression levels similar to those achieved by direct vector application	[80]
	Pluronic® F68, Tetronic® 908	RFP, <i>lacZ</i> , and SOX9	cartilage	in vitro hMSCs	encapsulation of rAAV in polymeric micelles for effective, durable, and safe modification of hMSCs; restoration of hMSC transduction in conditions of gene transfer inhibition; effective chondrogenesis	[79]
	Pluronic® F68, Tetronic® 908	<i>lacZ</i>	cartilage	in vitro hOACs in situ human osteochondral model	micellar encapsulation for increased stability and bioactivity of rAAV; high levels of safe transgene expression in vitro and in experimental osteochondral defects in situ	[81]
	Pluronic® F68, Tetronic® 908	TGF- β	cartilage	in vitro hOACs in situ human osteochondral model	increased levels of transgene expression compared with free vector treatment; high proteoglycan deposition and increased cell numbers in hOACs in vitro; high deposition of type-II collagen and reduced hypertrophy in osteochondral defects models in situ	[82]
	Pluronic® F68, Tetronic® 908	<i>lacZ</i>	cartilage	in vitro hMSCs	high concentrations of rAAV; sustained levels of transgene expression over time	[92]

Abbreviations: rAAV: recombinant adeno-associated viral vectors; *lacZ*: *E. coli* β -galactosidase; GM-CSF: granulocyte-macrophage colony-stimulating factor; hMSCs: human mesenchymal stem cells; RFP: red fluorescent protein; SOX9: sex-determining region Y-type high mobility group box 9; hOACs: human osteochondral chondrocytes; TGF- β : transforming growth factor beta.

5. Conclusive Remarks

The application of responsive “smart” PEO-PPO-PEO polymers has a broad potential for drug delivery in different regenerative medicine approaches. While less explored, the use of these compounds as gene delivery systems represents a potential tool in gene therapy approaches. In this regard, the versatility of these molecules possibly forming micelles or gels in response to changes in temperature and/or pH makes them new gene transfer systems with a superior performance relative to the currently used gene shuttles. Diverse mechanisms have been proposed to explain the ability of these polymers to increase the levels of transgene expression. While micellar solutions from PEO-PPO-PEO copolymers were reported to increase the levels of DNA internalization [65] by interacting with the cell plasma membrane [76,93], thus overcoming potential natural barriers to vector transfection/transduction [68,79,81], gel systems were described to act as local reservoirs of vector release [70,75], increasing DNA distribution through tissues and reducing the transfection time compared with classical gene transfer vector [71]. Also, PEO-PPO-PEO copolymers have been reported to promote membrane resealing and to decrease trauma via electroporation [64,94], increasing the stability in solution of polyplexes [6,67]. There is also evidence showing that PEO-PPO-PEO copolymers may play a role in increasing transgene expression. For instance, copolymers with higher HLB are able to produce the highest improvement of gene expression levels in serum media from 10% to 50% fetal bovine serum compared with PEI-DNA complexes alone [68]. A similar trend was noted when combining such copolymers with viral vectors, promoting the highest gene transfer efficiencies when used at highly hydrophilic varieties (HLB > 24) [79]. Yet, despite potential advantages, gel systems formed by PEO-PPO-PEO copolymers are still characterized by a weak mechanical strength, a short residence time, and a high permeability which limit their applicability in gene/drug delivery [95]. Stabilization of these systems may however be promoted by crosslinking the gels [96] or by combining them with other polymers [92]. While less explored than their poloxamer counterparts, poloxamines have been also tested as gene delivery systems of both nonviral [88] and viral systems [79,81,82,92]. The X-shaped structure of these copolymers, rendering a positive charge at the physiological pH, may have an additional feature in conditions of gene transfer inhibition [79]. Altogether, the use of PEO-PPO-PEO for gene copolymers represents an emerging field in gene delivery approaches. Despite the considerable number of studies reporting the efficiency of such copolymers as adjuvants to increase the levels of transgene expression, more work is needed to elucidate their benefit-to-risk ratio for translational regenerative medicine approaches. In this scenario, limited work has been performed in relevant animal models of human diseases. Finally, and to our best knowledge, only a limited number of clinical trials reporting the application of PEO-PPO-PEO copolymers with gene transfer vectors have been reported for the treatment of intermittent claudication in patients with moderate to severe peripheral arterial disease [97] and as preventive vaccines against cytomegalovirus-associated disease [98].

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References

1. Alvarez-Lorenzo, C.; Rey-Rico, A.; Sosnik, A.; Taboada, P.; Concheiro, A. Poloxamine-based nanomaterials for drug delivery. *Front. Biosci.* **2010**, *2*, 424–440. [[CrossRef](#)]
2. Sosnik, A.; Sefton, M.V. Methylation of poloxamine for enhanced cell adhesion. *Biomacromolecules* **2006**, *7*, 331–338. [[CrossRef](#)] [[PubMed](#)]

3. Alvarez-Lorenzo, C.; Sosnik, A.; Concheiro, A. Peo-ppo block copolymers for passive micellar targeting and overcoming multidrug resistance in cancer therapy. *Curr. Drug Targets* **2011**, *12*, 1112–1130. [[CrossRef](#)] [[PubMed](#)]
4. Cao, Y.; Rodriguez, A.; Vacanti, M.; Ibarra, C.; Arevalo, C.; Vacanti, C.A. Comparative study of the use of poly(glycolic acid), calcium alginate and pluronics in the engineering of autologous porcine cartilage. *J. Biomater. Sci. Polym. Ed.* **1998**, *9*, 475–487. [[PubMed](#)]
5. Singh-Joy, S.D.; McLain, V.C. Safety assessment of poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, poloxamer 105 benzoate, and poloxamer 182 dibenzoate as used in cosmetics. *Int. J. Toxicol.* **2008**, *27* (Suppl. S2), 93–128. [[PubMed](#)]
6. Kabanov, A.V.; Lemieux, P.; Vinogradov, S.; Alakhov, V. Pluronic block copolymers: Novel functional molecules for gene therapy. *Adv. Drug Deliv. Rev.* **2002**, *54*, 223–233. [[CrossRef](#)]
7. Chiappetta, D.A.; Sosnik, A. Poly(ethylene oxide)-poly(propylene oxide) block copolymer micelles as drug delivery agents: Improved hydrosolubility, stability and bioavailability of drugs. *Eur. J. Pharm. Biopharm.* **2007**, *66*, 303–317. [[CrossRef](#)] [[PubMed](#)]
8. Oh, K.T.; Bronich, T.K.; Kabanov, A.V. Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic pluronic block copolymers. *J. Control. Release* **2004**, *94*, 411–422. [[CrossRef](#)] [[PubMed](#)]
9. Attwood, D.; Zhou, Z.; Booth, C. Poly(ethylene oxide) based copolymers: Solubilisation capacity and gelation. *Expert Opin. Drug Deliv.* **2007**, *4*, 533–546. [[CrossRef](#)] [[PubMed](#)]
10. Gonzalez-Lopez, J.; Alvarez-Lorenzo, C.; Taboada, P.; Sosnik, A.; Sandez-Macho, I.; Concheiro, A. Self-associative behavior and drug-solubilizing ability of poloxamine (tetronic) block copolymers. *Langmuir* **2008**, *24*, 10688–10697. [[CrossRef](#)] [[PubMed](#)]
11. Alvarez-Lorenzo, C.; Concheiro, A. Intelligent drug delivery systems: Polymeric micelles and hydrogels. *Mini Rev. Med. Chem.* **2008**, *8*, 1065–1074. [[CrossRef](#)] [[PubMed](#)]
12. Pec, E.A.; Wout, Z.G.; Johnston, T.P. Biological activity of urease formulated in poloxamer 407 after intraperitoneal injection in the rat. *J. Pharm. Sci.* **1992**, *81*, 626–630. [[CrossRef](#)] [[PubMed](#)]
13. Aliabadi, H.M.; Lavasanifar, A. Polymeric micelles for drug delivery. *Expert Opin. Drug Deliv.* **2006**, *3*, 139–162. [[CrossRef](#)] [[PubMed](#)]
14. Anderson, R.A. Micelle formation by oxyethylene-oxypropylene polymers. *Pharm. Acta Helv.* **1972**, *47*, 304–308. [[PubMed](#)]
15. Alexandridis, P.; Hatton, T.A. Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modeling. *Colloids Surf. A Physicochem. Eng. Asp.* **1995**, *96*, 1–46. [[CrossRef](#)]
16. Torchilin, V.P. Structure and design of polymeric surfactant-based drug delivery systems. *J. Control. Release* **2001**, *73*, 137–172. [[CrossRef](#)]
17. Bae, Y.H.; Yin, H. Stability issues of polymeric micelles. *J. Control. Release* **2008**, *131*, 2–4. [[CrossRef](#)] [[PubMed](#)]
18. Barreiro-Iglesias, R.; Bromberg, L.; Temchenko, M.; Hatton, T.A.; Concheiro, A.; Alvarez-Lorenzo, C. Solubilization and stabilization of camptothecin in micellar solutions of pluronic-g-poly(acrylic acid) copolymers. *J. Control. Release* **2004**, *97*, 537–549. [[CrossRef](#)] [[PubMed](#)]
19. Chiappetta, D.A.; Degrossi, J.; Teves, S.; D’Aquino, M.; Bregni, C.; Sosnik, A. Triclosan-loaded poloxamine micelles for enhanced topical antibacterial activity against biofilm. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 535–545. [[CrossRef](#)] [[PubMed](#)]
20. Fuentes, I.; Blanco-Fernandez, B.; Alvarado, N.; Leiva, A.; Radic, D.; Alvarez-Lorenzo, C.; Concheiro, A. Encapsulation of antioxidant gallate derivatives in biocompatible poly(epsilon-caprolactone)-b-pluronic-b-poly(epsilon-caprolactone) micelles. *Langmuir* **2016**, *32*, 3331–3339. [[CrossRef](#)] [[PubMed](#)]
21. Shi, C.; Zhang, Z.; Shi, J.; Wang, F.; Luan, Y. Co-delivery of docetaxel and chloroquine via peo-ppo-pcl/tpgs micelles for overcoming multidrug resistance. *Int. J. Pharm.* **2015**, *495*, 932–939. [[CrossRef](#)] [[PubMed](#)]
22. Habas, J.P.; Pavie, E.; Perreur, C.; Lapp, A.; Peyrelasse, J. Nanostructure in block copolymer solutions: Rheology and small-angle neutron scattering. *Phys. Rev. E Stat. Nonlinear Soft Matter. Phys.* **2004**, *70*, 061802. [[CrossRef](#)] [[PubMed](#)]

23. Perreur, C.; Habas, J.P.; Peyrelasse, J.; Francois, J.; Lapp, A. Rheological and small-angle neutron scattering studies of aqueous solutions of branched peo-ppo-peo copolymers. *Phys. Rev. E Stat. Nonlinear Soft Matter Phys.* **2001**, *63*, 031505. [[CrossRef](#)] [[PubMed](#)]
24. Alvarez-Lorenzo, C.; Gonzalez-Lopez, J.; Fernandez-Tarrio, M.; Sandez-Macho, I.; Concheiro, A. Tetronic micellization, gelation and drug solubilization: Influence of pH and ionic strength. *Eur. J. Pharm. Biopharm.* **2007**, *66*, 244–252. [[CrossRef](#)] [[PubMed](#)]
25. Rey-Rico, A.; Silva, M.; Couceiro, J.; Concheiro, A.; Alvarez-Lorenzo, C. Osteogenic efficiency of in situ gelling poloxamine systems with and without bone morphogenetic protein-2. *Eur. Cells Mater.* **2011**, *21*, 317–340. [[CrossRef](#)] [[PubMed](#)]
26. Del Rosario, C.; Rodriguez-Evora, M.; Reyes, R.; Simoes, S.; Concheiro, A.; Evora, C.; Alvarez-Lorenzo, C.; Delgado, A. Bone critical defect repair with poloxamine-cyclodextrin supramolecular gels. *Int. J. Pharm.* **2015**, *495*, 463–473. [[CrossRef](#)] [[PubMed](#)]
27. Miyazaki, S.; Tobiyama, T.; Takada, M.; Attwood, D. Percutaneous absorption of indomethacin from pluronic f127 gels in rats. *J. Pharm. Pharmacol.* **1995**, *47*, 455–457. [[CrossRef](#)] [[PubMed](#)]
28. Fernandez-Tarrio, M.; Yanez, F.; Immesoete, K.; Alvarez-Lorenzo, C.; Concheiro, A. Pluronic and tetronic copolymers with polyglycolized oils as self-emulsifying drug delivery systems. *AAPS PharmSciTech* **2008**, *9*, 471–479. [[CrossRef](#)] [[PubMed](#)]
29. Marcos, X.; Perez-Casas, S.; Llovo, J.; Concheiro, A.; Alvarez-Lorenzo, C. Poloxamer-hydroxyethyl cellulose- α -cyclodextrin supramolecular gels for sustained release of griseofulvin. *Int. J. Pharm.* **2016**, *500*, 11–19. [[CrossRef](#)] [[PubMed](#)]
30. Kolesky, D.B.; Truby, R.L.; Gladman, A.S.; Busbee, T.A.; Homan, K.A.; Lewis, J.A. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv. Mater.* **2014**, *26*, 3124–3130. [[CrossRef](#)] [[PubMed](#)]
31. Chang, C.C.; Boland, E.D.; Williams, S.K.; Hoying, J.B. Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. *J. Biomed. Mater. Res. B Appl. Biomater.* **2011**, *98*, 160–170. [[CrossRef](#)] [[PubMed](#)]
32. Kabanov, A.V.; Chekhonin, V.P.; Alakhov, V.; Batrakova, E.V.; Lebedev, A.S.; Melik-Nubarov, N.S.; Arzhakov, S.A.; Levashov, A.V.; Morozov, G.V.; Severin, E.S.; et al. The neuroleptic activity of haloperidol increases after its solubilization in surfactant micelles. Micelles as microcontainers for drug targeting. *FEBS Lett.* **1989**, *258*, 343–345. [[CrossRef](#)]
33. Zhang, T.; Zhou, S.; Liu, Y.; Luo, X.; Di, D.; Song, Y.; Liu, X.; Deng, Y. Polysialic acid and pluronic f127 mixed polymeric micelles of docetaxel as new approach for enhanced antitumor efficacy. *Drug Dev. Ind. Pharm.* **2017**, *43*, 1827–1835. [[CrossRef](#)] [[PubMed](#)]
34. Zhang, S.S.; Xia, W.T.; Xu, J.; Xu, H.L.; Lu, C.T.; Zhao, Y.Z.; Wu, X.Q. Three-dimensional structure micelles of heparin-poloxamer improve the therapeutic effect of 17 β -estradiol on endometrial regeneration for intrauterine adhesions in a rat model. *Int. J. Nanomed.* **2017**, *12*, 5643–5657. [[CrossRef](#)] [[PubMed](#)]
35. Cagel, M.; Bernabeu, E.; Gonzalez, L.; Lagomarsino, E.; Zubillaga, M.; Moretton, M.A.; Chiappetta, D.A. Mixed micelles for encapsulation of doxorubicin with enhanced in vitro cytotoxicity on breast and ovarian cancer cell lines versus doxil(r). *Biomed. Pharmacother.* **2017**, *95*, 894–903. [[CrossRef](#)] [[PubMed](#)]
36. Grimaudo, M.A.; Pescina, S.; Padula, C.; Santi, P.; Concheiro, A.; Alvarez-Lorenzo, C.; Nicoli, S. Poloxamer 407/tpgs mixed micelles as promising carriers for cyclosporine ocular delivery. *Mol. Pharm.* **2018**, *15*, 571–584. [[CrossRef](#)] [[PubMed](#)]
37. Bodratti, A.M.; Alexandridis, P. Formulation of poloxamers for drug delivery. *J. Funct. Biomater.* **2018**, *9*, 11. [[CrossRef](#)] [[PubMed](#)]
38. Chiappetta, D.A.; Hocht, C.; Taira, C.; Sosnik, A. Efavirenz-loaded polymeric micelles for pediatric anti-hiv pharmacotherapy with significantly higher oral bioavailability [corrected]. *Nanomedicine* **2010**, *5*, 11–23. [[CrossRef](#)] [[PubMed](#)]
39. Modi, S.; Prakash Jain, J.; Domb, A.J.; Kumar, N. Exploiting epr in polymer drug conjugate delivery for tumor targeting. *Curr. Pharm. Des.* **2006**, *12*, 4785–4796. [[CrossRef](#)] [[PubMed](#)]
40. Harada, A.; Togawa, H.; Kataoka, K. Physicochemical properties and nuclease resistance of antisense-oligodeoxynucleotides entrapped in the core of polyion complex micelles composed of poly(ethylene glycol)-poly(l-lysine) block copolymers. *Eur. J. Pharm. Sci.* **2001**, *13*, 35–42. [[CrossRef](#)]

41. Kwon, G.S. Polymeric micelles for delivery of poorly water-soluble compounds. *Crit. Rev. Ther. Drug Carr. Syst.* **2003**, *20*, 357–403. [[CrossRef](#)]
42. Husseini, G.A.; Myrup, G.D.; Pitt, W.G.; Christensen, D.A.; Rapoport, N.Y. Factors affecting acoustically triggered release of drugs from polymeric micelles. *J. Control. Release* **2000**, *69*, 43–52. [[CrossRef](#)]
43. Fan, W.; Zhang, L.; Li, Y.; Wu, H. Recent progress of crosslinking strategies for polymeric micelles with enhanced drug delivery in cancer therapy. *Curr. Med. Chem.* **2017**. [[CrossRef](#)] [[PubMed](#)]
44. Wang, J.; Li, Y.; Wang, L.; Wang, X.; Tu, P. Comparison of hyaluronic acid-based micelles and polyethylene glycol-based micelles on reversal of multidrug resistance and enhanced anticancer efficacy in vitro and in vivo. *Drug Deliv.* **2018**, *25*, 330–340. [[CrossRef](#)] [[PubMed](#)]
45. Nicolaidis, N.C.; Sass, P.M.; Grasso, L. Advances in targeted therapeutic agents. *Expert Opin. Drug Discov.* **2010**, *5*, 1123–1140. [[CrossRef](#)] [[PubMed](#)]
46. Rey-Rico, A.; Cucchiari, M. Controlled release strategies for raav-mediated gene delivery. *Acta Biomater.* **2016**, *29*, 1–10. [[CrossRef](#)] [[PubMed](#)]
47. Niidome, T.; Huang, L. Gene therapy progress and prospects: Nonviral vectors. *Gene Ther.* **2002**, *9*, 1647–1652. [[CrossRef](#)] [[PubMed](#)]
48. Rey-Rico, A.; Cucchiari, M. Recent tissue engineering-based advances for effective raav-mediated gene transfer in the musculoskeletal system. *Bioengineered* **2016**, *7*, 175–188. [[CrossRef](#)] [[PubMed](#)]
49. Breyer, B.; Jiang, W.; Cheng, H.; Zhou, L.; Paul, R.; Feng, T.; He, T.C. Adenoviral vector-mediated gene transfer for human gene therapy. *Curr. Gene Ther.* **2001**, *1*, 149–162. [[CrossRef](#)] [[PubMed](#)]
50. Chang, L.J.; Gay, E.E. The molecular genetics of lentiviral vectors—Current and future perspectives. *Curr. Gene Ther.* **2001**, *1*, 237–251. [[CrossRef](#)] [[PubMed](#)]
51. Yi, Y.; Noh, M.J.; Lee, K.H. Current advances in retroviral gene therapy. *Curr. Gene Ther.* **2011**, *11*, 218–228. [[CrossRef](#)] [[PubMed](#)]
52. Lachmann, R. Herpes simplex virus-based vectors. *Int. J. Exp. Pathol.* **2004**, *85*, 177–190. [[CrossRef](#)] [[PubMed](#)]
53. Daya, S.; Berns, K.I. Gene therapy using adeno-associated virus vectors. *Clin. Microbiol. Rev.* **2008**, *21*, 583–593. [[CrossRef](#)] [[PubMed](#)]
54. Ibraheem, D.; Elaissari, A.; Fessi, H. Gene therapy and DNA delivery systems. *Int. J. Pharm.* **2014**, *459*, 70–83. [[CrossRef](#)] [[PubMed](#)]
55. De Laporte, L.; Cruz Rea, J.; Shea, L.D. Design of modular non-viral gene therapy vectors. *Biomaterials* **2006**, *27*, 947–954. [[CrossRef](#)] [[PubMed](#)]
56. Wang, W.; Li, W.; Ma, N.; Steinhoff, G. Non-viral gene delivery methods. *Curr. Pharm. Biotechnol.* **2013**, *14*, 46–60. [[PubMed](#)]
57. Mumper, R.J.; Duguid, J.G.; Anwer, K.; Barron, M.K.; Nitta, H.; Rolland, A.P. Polyvinyl derivatives as novel interactive polymers for controlled gene delivery to muscle. *Pharm. Res.* **1996**, *13*, 701–709. [[CrossRef](#)] [[PubMed](#)]
58. Lemieux, P.; Guerin, N.; Paradis, G.; Proulx, R.; Chistyakova, L.; Kabanov, A.; Alakhov, V. A combination of poloxamers increases gene expression of plasmid DNA in skeletal muscle. *Gene Ther.* **2000**, *7*, 986–991. [[CrossRef](#)] [[PubMed](#)]
59. Kabanov, A.V.; Alakhov, V.Y. Pluronic block copolymers in drug delivery: From micellar nanocontainers to biological response modifiers. *Crit. Rev. Ther. Drug Carr. Syst.* **2002**, *19*, 1–72. [[CrossRef](#)]
60. Sriadibhatla, S.; Yang, Z.; Gebhart, C.; Alakhov, V.Y.; Kabanov, A. Transcriptional activation of gene expression by pluronic block copolymers in stably and transiently transfected cells. *Mol. Ther.* **2006**, *13*, 804–813. [[CrossRef](#)] [[PubMed](#)]
61. Liu, F.; Yang, J.; Huang, L.; Liu, D. Effect of non-ionic surfactants on the formation of DNA/emulsion complexes and emulsion-mediated gene transfer. *Pharm. Res.* **1996**, *13*, 1642–1646. [[CrossRef](#)] [[PubMed](#)]
62. Liaw, J.; Chang, S.F.; Hsiao, F.C. In vivo gene delivery into ocular tissues by eye drops of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (peo-ppo-peo) polymeric micelles. *Gene Ther.* **2001**, *8*, 999–1004. [[CrossRef](#)] [[PubMed](#)]
63. Pitard, B.; Pollard, H.; Agbulut, O.; Lambert, O.; Vilquin, J.T.; Cherel, Y.; Abadie, J.; Samuel, J.L.; Rigaud, J.L.; Menoret, S.; et al. A nonionic amphiphile agent promotes gene delivery in vivo to skeletal and cardiac muscles. *Hum. Gene Ther.* **2002**, *13*, 1767–1775. [[CrossRef](#)] [[PubMed](#)]

64. Riera, M.; Chillon, M.; Aran, J.M.; Cruzado, J.M.; Torras, J.; Grinyo, J.M.; Fillat, C. Intramuscular sp1017-formulated DNA electrotransfer enhances transgene expression and distributes hhgf to different rat tissues. *J. Gene Med.* **2004**, *6*, 111–118. [[CrossRef](#)] [[PubMed](#)]
65. Astafieva, I.; Maksimova, I.; Lukanidin, E.; Alakhov, V.; Kabanov, A. Enhancement of the polycation-mediated DNA uptake and cell transfection with pluronic p85 block copolymer. *FEBS Lett.* **1996**, *389*, 278–280. [[CrossRef](#)]
66. Nguyen, H.K.; Lemieux, P.; Vinogradov, S.V.; Gebhart, C.L.; Guerin, N.; Paradis, G.; Bronich, T.K.; Alakhov, V.Y.; Kabanov, A.V. Evaluation of polyether-polyethyleneimine graft copolymers as gene transfer agents. *Gene Ther.* **2000**, *7*, 126–138. [[CrossRef](#)] [[PubMed](#)]
67. Gebhart, C.L.; Sriadibhatla, S.; Vinogradov, S.; Lemieux, P.; Alakhov, V.; Kabanov, A.V. Design and formulation of polyplexes based on pluronic-polyethyleneimine conjugates for gene transfer. *Bioconjug. Chem.* **2002**, *13*, 937–944. [[CrossRef](#)] [[PubMed](#)]
68. Kuo, J.H. Effect of pluronic-block copolymers on the reduction of serum-mediated inhibition of gene transfer of polyethyleneimine-DNA complexes. *Biotechnol. Appl. Biochem.* **2003**, *37*, 267–271. [[CrossRef](#)] [[PubMed](#)]
69. Jeon, E.; Kim, H.D.; Kim, J.S. Pluronic-grafted poly-(l)-lysine as a new synthetic gene carrier. *J. Biomed. Mater. Res. A* **2003**, *66*, 854–859. [[CrossRef](#)] [[PubMed](#)]
70. March, K.L.; Madison, J.E.; Trapnell, B.C. Pharmacokinetics of adenoviral vector-mediated gene delivery to vascular smooth muscle cells: Modulation by poloxamer 407 and implications for cardiovascular gene therapy. *Hum. Gene Ther.* **1995**, *6*, 41–53. [[CrossRef](#)] [[PubMed](#)]
71. Feldman, L.J.; Pastore, C.J.; Aubailly, N.; Kearney, M.; Chen, D.; Perricaudet, M.; Steg, P.G.; Isner, J.M. Improved efficiency of arterial gene transfer by use of poloxamer 407 as a vehicle for adenoviral vectors. *Gene Ther.* **1997**, *4*, 189–198. [[CrossRef](#)] [[PubMed](#)]
72. Van Belle, E.; Maillard, L.; Rivard, A.; Fabre, J.E.; Couffignal, T.; Kearney, M.; Branellec, D.; Feldman, L.J.; Walsh, K.; Isner, J.M. Effects of poloxamer 407 on transfection time and percutaneous adenovirus-mediated gene transfer in native and stented vessels. *Hum. Gene Ther.* **1998**, *9*, 1013–1024. [[CrossRef](#)] [[PubMed](#)]
73. Maillard, L.; Van Belle, E.; Tio, F.O.; Rivard, A.; Kearney, M.; Branellec, D.; Steg, P.G.; Isner, J.M.; Walsh, K. Effect of percutaneous adenovirus-mediated gax gene delivery to the arterial wall in double-injured atheromatous stented rabbit iliac arteries. *Gene Ther.* **2000**, *7*, 1353–1361. [[CrossRef](#)] [[PubMed](#)]
74. Wang, Y.; Liu, S.; Li, C.Y.; Yuan, F. A novel method for viral gene delivery in solid tumors. *Cancer Res.* **2005**, *65*, 7541–7545. [[CrossRef](#)] [[PubMed](#)]
75. Strappe, P.M.; Hampton, D.W.; Cachon-Gonzalez, B.; Fawcett, J.W.; Lever, A. Delivery of a lentiviral vector in a pluronic f127 gel to cells of the central nervous system. *Eur. J. Pharm. Biopharm.* **2005**, *61*, 126–133. [[CrossRef](#)] [[PubMed](#)]
76. Hofig, I.; Atkinson, M.J.; Mall, S.; Krackhardt, A.M.; Thirion, C.; Anastasov, N. Poloxamer synperonic f108 improves cellular transduction with lentiviral vectors. *J. Gene Med.* **2012**, *14*, 549–560. [[CrossRef](#)] [[PubMed](#)]
77. Driessens, G.; Nuttin, L.; Gras, A.; Maetens, J.; Mievis, S.; Schoore, M.; Velu, T.; Tenenbaum, L.; Preat, V.; Bruyns, C. Development of a successful antitumor therapeutic model combining in vivo dendritic cell vaccination with tumor irradiation and intratumoral gm-csf delivery. *Cancer Immunol. Immunother.* **2011**, *60*, 273–281. [[CrossRef](#)] [[PubMed](#)]
78. Zhang, F.L.; Jia, S.Q.; Zheng, S.P.; Ding, W. Celastrol enhances aav1-mediated gene expression in mice adipose tissues. *Gene Ther.* **2011**, *18*, 128–134. [[CrossRef](#)] [[PubMed](#)]
79. Rey-Rico, A.; Venkatesan, J.K.; Frisch, J.; Rial-Hermida, I.; Schmitt, G.; Concheiro, A.; Madry, H.; Alvarez-Lorenzo, C.; Cucchiari, M. Peo-ppo-peo micelles as effective raav-mediated gene delivery systems to target human mesenchymal stem cells without altering their differentiation potency. *Acta Biomater.* **2015**, *27*, 42–52. [[CrossRef](#)] [[PubMed](#)]
80. Diaz-Rodriguez, P.; Rey-Rico, A.; Madry, H.; Landin, M.; Cucchiari, M. Effective genetic modification and differentiation of hmscs upon controlled release of raav vectors using alginate/poloxamer composite systems. *Int. J. Pharm.* **2015**, *496*, 614–626. [[CrossRef](#)] [[PubMed](#)]
81. Rey-Rico, A.; Frisch, J.; Venkatesan, J.K.; Schmitt, G.; Rial-Hermida, I.; Taboada, P.; Concheiro, A.; Madry, H.; Alvarez-Lorenzo, C.; Cucchiari, M. Peo-ppo-peo carriers for raav-mediated transduction of human articular chondrocytes in vitro and in a human osteochondral defect model. *ACS Appl. Mater. Interfaces* **2016**, *8*, 20600–20613. [[CrossRef](#)] [[PubMed](#)]

82. Rey-Rico, A.; Venkatesan, J.K.; Schmitt, G.; Concheiro, A.; Madry, H.; Alvarez-Lorenzo, C.; Cucchiari, M. Raav-mediated overexpression of tgf-beta via vector delivery in polymeric micelles stimulates the biological and reparative activities of human articular chondrocytes in vitro and in a human osteochondral defect model. *Int. J. Nanomed.* **2017**, *12*, 6985–6996. [[CrossRef](#)] [[PubMed](#)]
83. Lu, Q.L.; Bou-Gharios, G.; Partridge, T.A. Non-viral gene delivery in skeletal muscle: A protein factory. *Gene Ther.* **2003**, *10*, 131–142. [[CrossRef](#)] [[PubMed](#)]
84. Wolff, J.A.; Malone, R.W.; Williams, P.; Chong, W.; Acsadi, G.; Jani, A.; Felgner, P.L. Direct gene transfer into mouse muscle in vivo. *Science* **1990**, *247*, 1465–1468. [[CrossRef](#)] [[PubMed](#)]
85. Kabanov, A.V.; Kabanov, V.A. DNA complexes with polycations for the delivery of genetic material into cells. *Bioconjug. Chem.* **1995**, *6*, 7–20. [[CrossRef](#)] [[PubMed](#)]
86. Ramamoorth, M.; Narvekar, A. Non viral vectors in gene therapy—An overview. *J. Clin. Diagn. Res.* **2015**, *9*, GE01-06. [[CrossRef](#)] [[PubMed](#)]
87. Chattoraj, D.K.; Gosule, L.C.; Schellman, A. DNA condensation with polyamines. II. Electron microscopic studies. *J. Mol. Biol.* **1978**, *121*, 327–337. [[CrossRef](#)]
88. Zhang, J.; Sen, A.; Cho, E.; Lee, J.S.; Webb, K. Poloxamine/fibrin hybrid hydrogels for non-viral gene delivery. *J. Tissue Eng. Regen. Med.* **2017**, *11*, 246–255. [[CrossRef](#)] [[PubMed](#)]
89. Evans, C.H.; Ghivizzani, S.C.; Robbins, P.D. Progress and prospects: Genetic treatments for disorders of bones and joints. *Gene Ther.* **2009**, *16*, 944–952. [[CrossRef](#)] [[PubMed](#)]
90. Rey-Rico, A.; Cucchiari, M. Smart and controllable raav gene delivery carriers in progenitor cells for human musculoskeletal regenerative medicine with a focus on the articular cartilage. *Curr. Gene Ther.* **2017**, *17*, 127–138. [[CrossRef](#)] [[PubMed](#)]
91. Jang, J.H.; Houchin, T.L.; Shea, L.D. Gene delivery from polymer scaffolds for tissue engineering. *Expert Rev. Med. Devices* **2004**, *1*, 127–138. [[CrossRef](#)] [[PubMed](#)]
92. Rey-Rico, A.; Babicz, H.; Madry, H.; Concheiro, A.; Alvarez-Lorenzo, C.; Cucchiari, M. Supramolecular polypseudorotaxane gels for controlled delivery of raav vectors in human mesenchymal stem cells for regenerative medicine. *Int. J. Pharm.* **2017**, *531*, 492–503. [[CrossRef](#)] [[PubMed](#)]
93. Alakhov, V.Y.; Kabanov, A.V. Block copolymeric biotransport carriers as versatile vehicles for drug delivery. *Expert Opin. Investig. Drugs* **1998**, *7*, 1453–1473. [[CrossRef](#)] [[PubMed](#)]
94. Hartikka, J.; Sukhu, L.; Buchner, C.; Hazard, D.; Bozoukova, V.; Margalith, M.; Nishioka, W.K.; Wheeler, C.J.; Manthorp, M.; Sawdey, M. Electroporation-facilitated delivery of plasmid DNA in skeletal muscle: Plasmid dependence of muscle damage and effect of poloxamer 188. *Mol. Ther.* **2001**, *4*, 407–415. [[CrossRef](#)] [[PubMed](#)]
95. Li, Z.; Guan, J. Thermosensitive hydrogels for drug delivery. *Expert Opin. Drug Deliv.* **2011**, *8*, 991–1007. [[CrossRef](#)] [[PubMed](#)]
96. Lee, J.B.; Yoon, J.J.; Lee, D.S.; Park, T.G. Photo-crosslinkable, thermo-sensitive and biodegradable pluronic hydrogels for sustained release of protein. *J. Biomater. Sci. Polym. Ed.* **2004**, *15*, 1571–1583. [[CrossRef](#)] [[PubMed](#)]
97. Grossman, P.M.; Mendelsohn, F.; Henry, T.D.; Hermiller, J.B.; Litt, M.; Saucedo, J.F.; Weiss, R.J.; Kandzari, D.E.; Kleiman, N.; Anderson, R.D.; et al. Results from a phase II multicenter, double-blind placebo-controlled study of del-1 (vlts-589) for intermittent claudication in subjects with peripheral arterial disease. *Am. Heart J.* **2007**, *153*, 874–880. [[CrossRef](#)] [[PubMed](#)]
98. Wloch, M.K.; Smith, L.R.; Boutsaboualoy, S.; Reyes, L.; Han, C.; Kehler, J.; Smith, H.D.; Selk, L.; Nakamura, R.; Brown, J.M.; et al. Safety and immunogenicity of a bivalent cytomegalovirus DNA vaccine in healthy adult subjects. *J. Infect. Dis.* **2008**, *197*, 1634–1642. [[CrossRef](#)] [[PubMed](#)]

