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Research Paper

Biocompatible fluorinated poly(β-amino ester)s for safe and efficient gene therapy

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ABSTRACT

Cationic polymers have been widely used as one of the most promising non-viral vehicles for gene delivery due to their potential safety and ease of large-scale production. Here, we report the design and synthesis of a series of novel biodegradable fluorinated poly(β-amino ester)s (FPBAEs) by simple Michael-addition reaction as safe and efficient gene carrier. The results of transfection efficacy assay demonstrated the optimal FPBAE could mediated much higher GFP expression than the commercial transfection agents, polyethyleneimine (PEI, M_w = 25K) and Lipo 2000, as well as the non-fluorinated poly(β-amino ester)s (PBAE) on both HeLa and HEK-293T cell lines (higher than 70 and 90%, respectively), which was largely attributed to fluorination. Moreover, MTT and hemolysis assay indicated a preferable biocompatibility of FPBAE compared with PEI 25K owing to the low molecular weight and the presence of cleavable ester bonds. Taken together, the novel polymer FPBAE with both excellent gene transfection efficacy and much lower toxicity could serve as a desirable gene vector.

1. Introduction

Gene therapy is considered to have great potential to treat various inherited and acquired diseases at the genetic level (Draghici and Ilies, 2015; Friedmann, 1989). A pivotal challenge posed to successful gene therapy is the exploit of safe and efficient delivery systems (Wei et al., 2013; Yin et al., 2013). Currently, the major categories of gene vectors include viral and synthetic carriers. Viral vectors allow highly efficient gene transfection, but are commonly associated with immunogenicity and production problems which in turn inspired the evolution of synthetic vectors based on cationic polymers (Huang and Kamihira, 2013).

Among the numerous polymeric carries that have already been investigated, poly(β-amino ester) (PBAE) especially came into our sight as a promising biodegradable cationic polymer with relative low molecular weight (LMW), giving rise to a much better tolerance and biocompatibility compared with high molecular weight (HMW) materials (Akinc and Langer, 2002; Anderson et al., 2005, 2004; Green et al., 2006). Furthermore, the synthesis of PBAE is commonly simple and versatile, which is based on the Michael-addition reaction of polyamine and a diacrylate, making it easy to manufacture (Akinc et al., 2003; Anderson et al., 2003). Despite the promising advantages, PBAE is much less efficient compared with viral vectors and HMW materials. Thus, approaches aimed at improving the transfection efficiency of LMW PBAE are in imperative demand, which would open up more probabilities for PBAE in gene delivery, making it become a both highly efficient and prominently biocompatible vector.

Fluorination stands out from a host of methods and captures much attention from researchers in the field of chemistry and materials as a result of its rosy performance on circumventing a slice of physiological obstacles inclusive of poor Serum resistance ability, finite cellular uptake efficiency and restricted endosomal escape capability (Liu et al., 2014; Wang and Cheng, 2014). Meanwhile, a series of these behaviors are endowed by the structural features of fluorine. Fluorinated materials are both hydrophobic and lipophobic, and thus exhibited an extraordinary phase-separation tendency in polar as well as non-polar environment (Patrick et al., 2013; Xiong and Li, 2010). Consequently, fluorination can realize an improvement in the affinity between fluorinated compounds and bio-membranes including cell membranes (Kasuya et al., 2011) and endosome/lysosome membranes (Neil and Marsh, 2000), helping transverse the lipid bilayer of the cells and

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endosome membranes, eventually inducing efficient cellular uptake and successful endosomal escape. Accordingly, enhanced transfection efficacy can be achieved by means of breaking through the intrinsic barriers. Several attempts have been carried out to support the hypothesis. Take an example, Wang et al. synthesized a fluorocarbon chains-modified polyethyleneimine (PEI, M_n = 10,000) via a disulfide exchange reaction and demonstrated high DNA-binding ability and gene transfection efficacy with the help of fluorine (Wang et al., 2016). Besides, impacts of fluorination on dendrimers were also manifested by Cai et al. (2016). These researches could indeed certify the function of fluorination and provide new platforms for gene delivery. However, these polymers investigated previously all belong to HMW cationic polymers, and have non-negligible higher toxicity and inferior biocompatibility compared with their LMW counterparts behind superior transfection efficacy. Thus, to achieve safe and efficient gene delivery, seeking the balance between gene transfection efficacy and cytotoxicity is of great significance. And in this term, construction of a degradable cationic polymer with LMW may be a decent alternative for gene delivery.

Hence, on the basis of above challenges, we designed and synthesized a series of biodegradable LMW PBAEs with fluorinated side chains by Michael-addition polymerization of three monomers: diacrylate, fluoroamine and relatively hydrophilic amine to achieve both high transfection efficacy and low cytotoxicity (Scheme 1). The final obtained FPBAE possessed several unique properties. Firstly, the relative LMW could endow FPBAE with a preferable tolerance, and the ester bonds in FPBAE could also be degraded via hydrolytic cleavage in the cytoplasm, which could further reduce the cytotoxicity as well as promote the release of the genes. Secondly, fluorination could be beneficial to the cellular uptake and endosomal escape, helping break through the cellular barriers during the process of gene delivery, and eventually realize excellent gene transfection efficacy. Given these characteristics, LMW FPBAE could serve as a high-performance gene vector with low toxicity and our strategy could also provide a versatile method for other LMW cationic polymers to perform better efficiency.

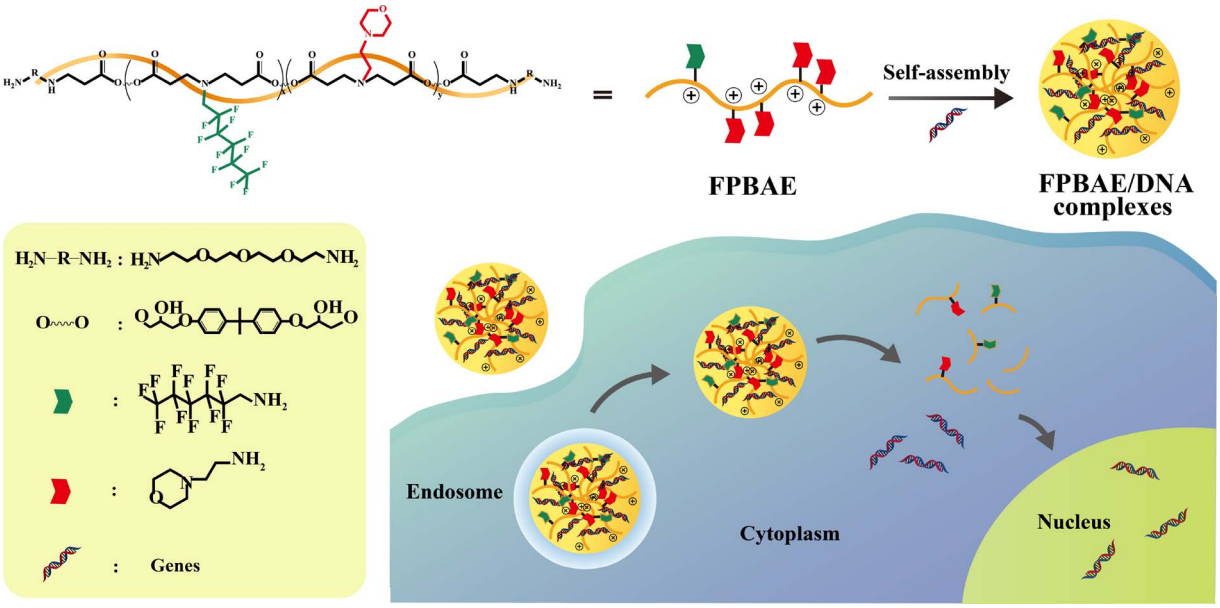
2. Materials and methods

2.1. Materials

Bisphenol A glycerolate (1 glycerol/phenol) diacrylate, branched PEI (Mw = 25 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). *N*-(2-Aminoethyl) morpholine was purchased from J&K (Beijing, China). 1H, 1H-Undecafluorohexylamine was purchased from TCI (Tokyo, Japan). 3,6,9-trioxaundecamethylenediamine was purchased from MERYER (Shanghai, China). Lipofectamine® 2000 (Lipo 2000), fetal bovine Serum (FBS), YOYO-1 iodide and LysoTracker® Red DND-99 were procured from Thermo Fisher Scientific (Waltham, MA, USA). Hoechst 33342, RMPI 1640, Dulbeco's Modified Eagle Medium (DMEM), and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were obtained from KeyGEN BioTECH (Nanjing, China). Trypsin-EDTA solution (0.25%) was purchased from Gibco (Burlington, Canada). Antibodies against p53 and β-actin were purchased from Cell Signaling Technology (Boston, USA). Plasmids were propagated in *Escherichia coli*, extracted by the alkali lysis technique, and purified by an E.Z.N.A.® Fastfilter Endofree Plasmid Maxi kit (Omega, Georgia, USA). All other chemicals and reagents were obtained from commercial sources and used without further purification.

2.2. Synthesis of F_nPBAE and PBAE

A series of F_nPBAE were synthesized by the Michael addition of Bisphenol A glycerolate diacrylate (BG) with the mixture of undecafluorohexylamine (UFA) and *N*-(2-aminoethyl) morpholine (AMP) under different ratios (Fig.1A) using the method developed previously (Eltoukhy et al., 2013; Zhang et al., 2016a). In brief, BG monomer, UFA monomer and AMP monomer were dissolved in 0.5 mL anhydrous DMSO in a total mass of 100 mg and their relative feed molar ratios were varied as 1.2:0.1:0.9, 1.2:0.2:0.8, 1.2:0.3:0.7 and 1.2:0.4:0.6, respectively. The mixture was continuously stirred at 90 °C for 48 h. After the mixture was cooled to RT, 0.2 mmol end-capping amine 3,6,9-trioxaundecamethylenediamine in 0.5 mL anhydrous DMSO was added. The reactions were performed at 40 °C for 24 h, and then stored at −20 °C for later use. The final products were named as $F_{10\%}$PBAE, $F_{20\%}$PBAE, $F_{30\%}$PBAE and $F_{40\%}$PBAE, respectively. And in the following parts, FPBAE was referred to $F_{20\%}$PBAE.



Scheme 1. Schematic illustration of gene delivery mediated by FPBAE. FPBAE could complex genes by electrostatic interaction. After efficient cellular uptake and endosomal escape, genes were released into the cytoplasm due to the cleavage of ester bonds. Then the released genes could perform its function post nuclear entry.