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The effect of fluorination on the transfection efficacy of surface-engineered dendrimers

Mingming Wang, Yiyun Cheng*

Shanghai Key Laboratory of Regulatory Biology, School of Life Sciences, East China Normal University, Shanghai 200241, PR China

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ABSTRACT

Dendrimers have shown great promise in the design of high efficient and low cytotoxic gene vectors. In this study, we synthesized a list of fluorobenzoic acid-modified dendrimers by a facile synthetic route and explored their potential applications as non-viral gene vectors. Fluorination on the aromatic rings significantly improves the transfection efficacy of benzoic acid-modified dendrimers. The transfection efficacy increases with increasing number of fluorine atoms on the aromatic rings of the conjugated benzoic acid. The most efficient conjugate shows superior efficacy to polymer-based commercial reagents such as SuperFect and PolyFect, and comparable efficacy to lipid-based commercial reagents such as Lipofectamine 2000. In addition, the fluorobenzoic acid-modified dendrimers show low cytotoxicity on the transfected cells. The improved transfection efficacy of fluorobenzoic acid-modified dendrimers is due to enhanced cellular uptake and/or easier DNA unpacking behavior compared to non-modified dendrimers. These results provide a new fluorination strategy to generate a library of highly efficient and non-cytotoxic polymeric gene vectors.

1. Introduction

Gene therapy represents a promising option for the treatment of various diseases such as viral infections, inherited disorders and cancers [1,2]. Successful gene therapy in clinical trials relies to a great degree on the appropriate delivery system [3]. Viral vectors are efficient to delivery DNA or siRNA into target cells with inherent shortcomings such as immunogenicity, genotoxicity, limited loading capacity, and incapability of industrial production and long-term gene therapy [4]. Researchers are developing alternatives to viral vectors in an effort to circumvent the safety and production problems associated with viral vectors [5,6]. A variety of non-viral vectors have been developed for gene delivery, including cationic proteins or peptides, lipids, polymers, and nanoparticles [7–14]. Among these vectors, polymers are the most promising candidates in gene therapy due to their advantages including lack of immunogenicity, easy-to-be-manufactured, flexibility, degradability, capacity in delivering large-size DNA, and availability to be conjugated with appropriate functional groups [5,15–17].

Dendrimers are a class of synthetic polymers with well-defined nanostructure, globular shape, excellent monodispersity, and high density of surface functionalities [18–24]. Cationic dendrimers such as amine-terminated poly (amido amine) (PAMAM) are able to condense plasmid DNA into nanoparticles and protect it from enzymatic degradation [25]. The high density of tertiary amine groups within dendrimer interior contributes to the possible “proton sponge” effect during polyplex endosomal escaping [26]. However, these polymers usually exhibit low transfection efficacy and severe cytotoxicity even on easy-to-transfect cells such as HEK293. Therefore, the surface of dendrimer was usually engineered with different ligands such as amino acids, lipids, cyclodextrins, oligoamines, polyethylene glycol (PEG), sugars, proteins, peptides and inorganic nanoparticles to improve transfection efficacy and/or to reduce cytotoxicity [27–37]. Though these functionalized dendrimers show improved efficacy in independent studies, the structure-function relationships of these functionalized dendrimers in gene delivery are rarely reported. In addition, new modification strategies which can dramatically improve the transfection efficacy and reduce the cytotoxicity of dendrimers are urgently needed.

Fluorination is a new strategy to effectively improve the efficacy of polymeric gene vectors [38]. Fluorine is an amazing element. There is a high tendency for fluorinated molecules to assemble into a fluorous phase which is both hydrophobic and lipophobic. Therefore, self-assembly of fluorinated PAMAM dendrimers into nanoscale or microscale particles was observed [39]. PAMAM

* Corresponding author.
E-mail address: yycheng@mail.ustc.edu.cn (Y. Cheng).

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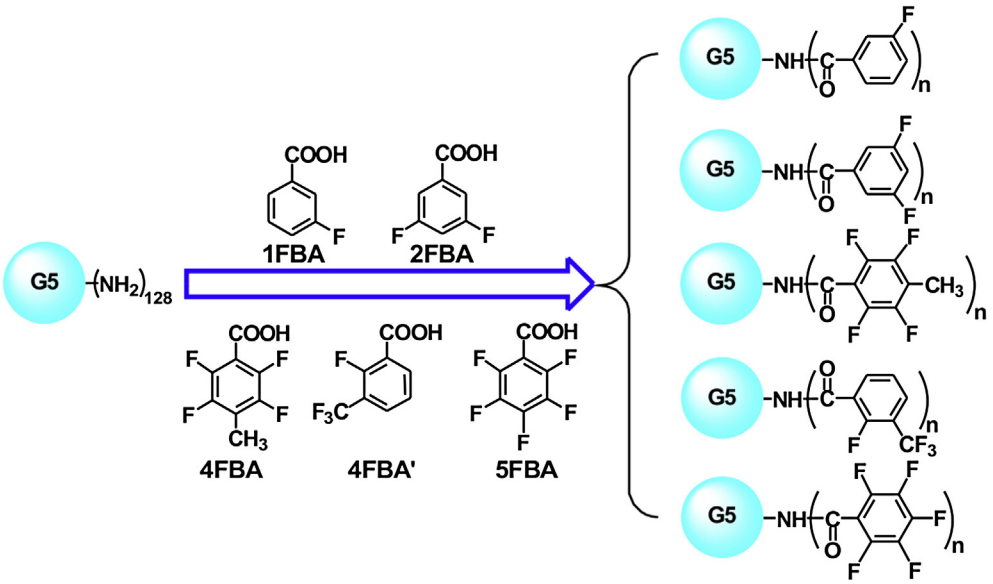

Fig. 1. Synthesis of FBA-modified G5 PAMAM dendrimers.

dendrimers modified with perfluoroaliphatic acids such as heptafluorobutyric acid showed superior efficacy to representative cationic polymers such as polyethylenimine (PEI) and commercial transfection reagents such as Lipofectamine 2000 and SuperFect on a list of commonly used cell lines. The conjugated heptafluorobutyric acid groups with hydrophobic and lipophobic properties improved the cellular uptake and endosomal escape of dendrimer/DNA polyplexes. In addition, fluorination made the optimal nitrogen-to-phosphorus (N/P) ratio of PAMAM dendrimer decreased from 8:1 to 1.5:1 [38]. This meant that fluorinated dendrimers could achieve efficient gene transfection at low vector dose or low charge density in the polyplex, ensuring low cytotoxicity during gene transfection. Fluorinated ligands on the dendrimers also allowed ^{19}F magnetic resonance imaging during gene

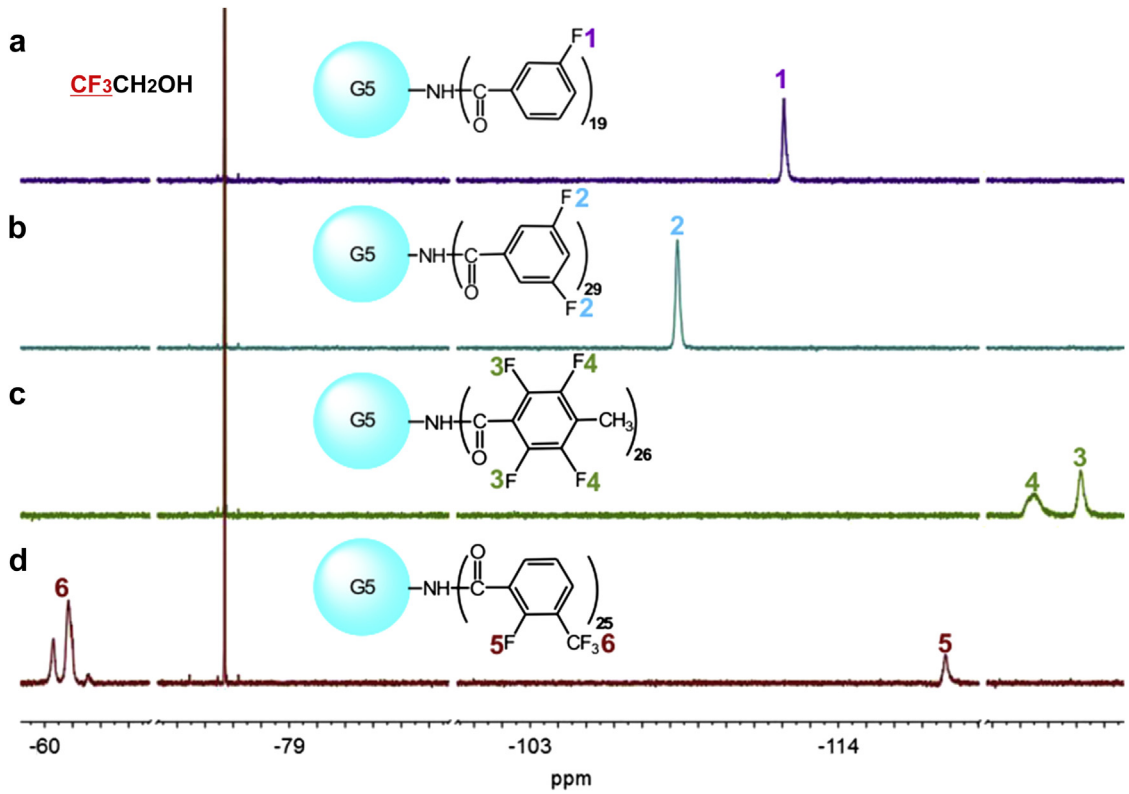

Fig. 2. ^{19}F NMR spectra of (a) G5-1FBA$_{19}$, (b) G5-2FBA$_{29}$ (c) G5-4FBA$_{26}$ and (d) G5-4FBA′$_{25}$. 2,2,2-trifluoroethanol was used as an internal standard.

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