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COMMUNICATION

Self-assembled fluorodendrimers in the co-delivery of fluorinated drugs and therapeutic genes

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Fluorinated drugs now make up about 25% of all pharmaceuticals. However, versatile carriers are currently lacking for the delivery of fluorinated drugs. Here, we developed fluorodendrimer as a versatile and multifunctional carrier for fluorinated drugs. The fluorodendrimer enables the co-delivery of fluorinated anticancer drugs and therapeutic genes for synergistic cancer therapy.

Although fluorous compounds are nearly absent from biology, they have gained particular attention in medicinal chemistry and diagnosis during the past decades.[1] Fluorine is the second smallest and the most electronegative element in the periodic table of elements. It has distinct chemical reactivity with respect to hydrogen.[2] Fluorine incorporation into the chemical structure of a drug may dramatically change its physiochemical properties such as lipophilicity, solubility and partition coefficient, and lead to much improved pharmacokinetic and pharmacodynamic behaviors.[1a, 2] For example, fluorine substitution is capable of enhancing the metabolic stability of a drug by lowering the susceptibility of nearby moieties to cytochrome P450 enzymatic oxidation.[3] The fluorine substitution also produces more stable C-F bonds than C-H bonds, rendering the drug molecules resistance to chemical or thermal degradation.[4] Introducing a fluorine atom to a drug may significantly reduce the basicity of neighbouring functional groups, thus resulting in improved membrane permeation and bioavailability.[5] In addition, the presence of fluorine atoms in a drug may enhance its binding affinity towards the target protein by hydrogen bonding interactions between fluorine and the protein, or by alternation in the conformation of the drug molecule.[2] Up to now, hundreds of fluorinated drugs have come to the market and make up about 25% of all pharmaceuticals.[1a, 3] With the advancement of fluorine chemistry, a variety of fluorinating methodologies and reagents for selective fluorine substitution become available for medicinal chemists.[6] The percentage of fluorinated drugs in the launched pharmaceuticals will further increase as a result.

Most of the fluorinated drugs are insoluble in aqueous solution, which represents a major obstacle for drug formulation and administration. Generally, a biocompatible carrier is needed to improve the aqueous solubility and bioavailability of hydrophobic drugs.[7] Considering that a large percentage of the marketed drugs containing one or more fluorine atoms, it is crucially important to develop versatile carriers for the delivery of fluorinated drugs.

Fluoroalkyl substances are both hydrophobic and lipophobic. Conjugation of fluoroalkyls to a polymer enhances its ability to self-assemble into nanostructures via a fluorophilic effect compared to non-fluorinated homologues.[8] Fluorinated drugs can be loaded by fluoroalkyl-grafted materials via specific fluorine-fluorine interactions.[9] Dendrimer is a class of smart polymers with unique properties such as monodispersity, well-defined chemical structure, and high density of surface functional groups.[10] Conjugation of heptafluorobutyric acid to low molecular weight dendrimers yields biocompatible fluorodendrimers, which assemble into various nanostructures in aqueous solutions depending on the fluorination degree.[11] The fluorodendrimers possess high gene transfection efficacy and combine the features of lipid and polymeric vectors in gene delivery. Here, we reported the use of fluorodendrimers as versatile carriers for both hydrophobic (sorafenib) and hydrophilic (5-fluorouracil, 5-Fu) fluorinated drugs via the fluorophilic effect. The gene delivery efficacy of the fluorodendrimers after drug loading is investigated. We hope to develop a promising carrier for the co-delivery of fluorinated

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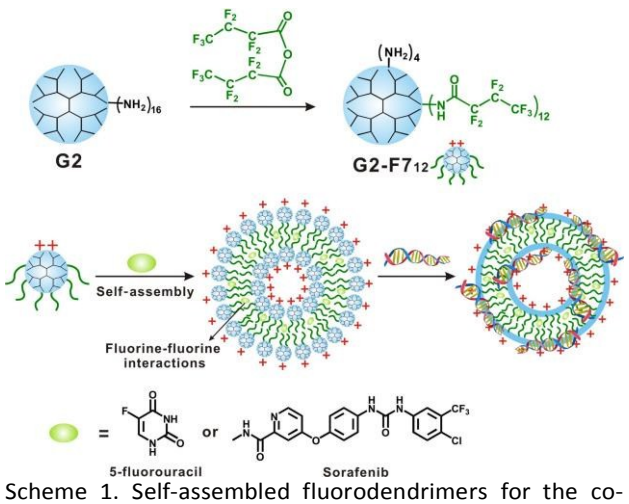
drugs and therapeutic genes in combination cancer therapy (Scheme 1).



Scheme 1. Self-assembled fluorodendrimers for the co-delivery of fluorinated drugs (sorafenib and 5-fluorouracil) and therapeutic genes.

The representative fluorodendrimer was synthesized by reacting generation 2 (G2) polyamidoamine dendrimer with heptafluorobutyric anhydride at room temperature. According to the ninhydrin assay, an average number of 12.1 heptafluorobutyric acid molecules were conjugated on each G2 dendrimer. The material was defined as G2-$F7_{12}$. Due to the fluorophilic effect of conjugated heptafluorobutyric acids, G2-$F7_{12}$ assembles into nanostructures in aqueous solutions (Figure 1a and 1b, ~130 nm by transmission electron microscopy, TEM and ~151 nm by dynamic light scattering, DLS). After loading with sorafenib, the size of the assembled nanoparticles is slightly increased (Figure 1c and 1d, ~153 nm by TEM and ~168 nm by DLS). Sorafenib is an insoluble drug in aqueous solutions. The unmodified G2 polyamidoamine dendrimer shows poor efficacy in loading sorafenib due to its small molecule size (~2 nm). After conjugation with heptafluorobutyric acid, the yielding fluorodendrimer shows significantly increased drug loading efficacy (~22.9% for G2-$F7_{12}$). In addition, the average number of sorafenib encapsulated within each fluorodendrimer is increased with increasing fluorination degree. For example, the average numbers of sorafenib loaded by each G2-$F7_8$, G2-$F7_{10}$ and G2-$F7_{12}$ are 2.6, 3.2, and 3.6, respectively (Figure 1e and Table S1).

The in vitro release profile of sorafenib from the prepared G2-$F7_{12}$/sorafenib complexes was further investigated. As shown in Figure 1f, sorafenib molecules are slowly released from the G2-$F7_{12}$/sorafenib complexes within 12 h (<12%), in comparion, nearly 100% of the drugs are released from unmodified G2 dendrimer within 6 h. In addition, the non-fluorinated homologue (G2 dendrimer conjugated with 12 butyric acids, G2-BA_{12}, Figure S1) shows a weak ability in the controlled release of sorafenib (~80% of the drug released within 12 h). For the fluorodendrimer G2-$F7_{12}$, fluorophilic effect between the fluorous chains makes the assembled nanostrucutures more stable. The fluorine-fluorine interactions between the fluoromethyl groups in sorafenib and the conjugated heptafluorobutyric acid on G2-$F7_{12}$ further retards the release of sorafenib. These results clearly suggest the essential roles of fluorophilic effect in loading and controlled release of sorafenib by fluorodendrimers.

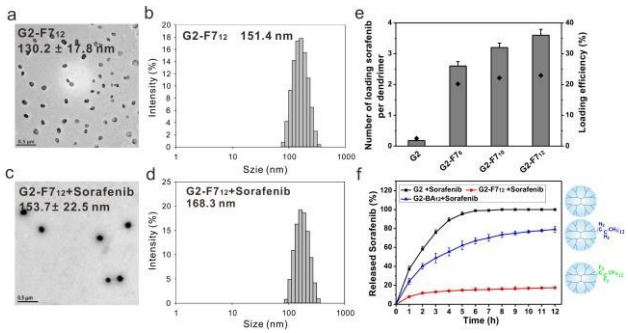


Figure 1. (a-d) Assembled nanostructures of G2-$F7_{12}$ (a, b) and G2-$F7_{12}$/sorafenib complexes (c, d) characterized by TEM (a, c) and DLS (b, d), respectively. (e) Drug loading capacity of unmodified G2 dendrimer and fluorodendrimers G2-$F7_8$, G2-$F7_{10}$ and G2-$F7_{12}$. (f) In vitro release of sorafenib from the G2, G2-$F7_{12}$ and G2-BA_{12}.

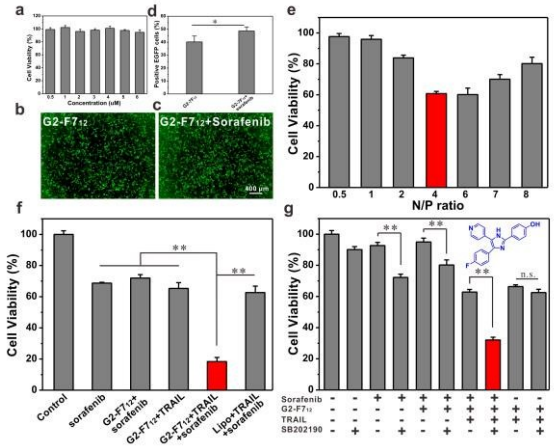


Figure 2. (a) Viability of MDA-MB-231 cells treated with different concentrations of G2-$F7_{12}$, (b, c) EGFP expressions in MDA-MB-231 cells by G2-$F7_{12}$ in the absence (b) or presence (c) of sorafenib. (d) Positive EGFP cells in (b) and (c) determined by flow cytometry. (e) Cytotoxicity of G2-$F7_{12}$/TRAIL plasmid complexes at various N/P ratios on MDA-MB-231 cells. The concentration of G2-$F7_{12}$ is kept constant at 4 μM. (f) Viability of MDA-MB-231 cells treated with sorafenib, G2-$F7_{12}$/sorafenib, G2-$F7_{12}$/TRAIL plasmid and G2-$F7_{12}$/sorafenib/TRAIL plasmid complexes. The sorafenib concentration is 4 μM. Lipo 2000 is a control. (g) The role of SB202190 on the toxicity of sorafenib, G2-$F7_{12}$/sorafenib, G2-$F7_{12}$/TRAIL plasmid, and G2-$F7_{12}$/sorafenib/TRAIL plasmid complexes. The concentration of SB202190 and sorafenib are 20 μM and 2 μM, respectively. The N/P ratio of G2-$F7_{12}$/TRAIL plasmid in (f) and (g) is 4.

The fluorodendrimer also shows minimal toxicity to the treated cells. As shown in Figure 2a, G2-F7$_{12}$ is non-toxic to MDA-MB-231 human breast cancer cells at concentrations up to 6 μM. This is probably due to the relatively low molecular weight of G2 polyamidoamine dendrimer, the low charge density on G2-F7$_{12}$ (4 positive charges per dendrimer), and the biocompatibility of fluorinated materials.[12] We further investigated the transfection efficacies of G2-F7$_{12}$ and G2-F7$_{12}$/sorafenib complexes. G2-F7$_{12}$ efficiently transfects EGFP plasmids in MDA-MB-231 cells (Figure 2b). After loading with sorafenib, the fluorodendrimer even shows slightly improved gene transfection efficacy (Figure 2c and 2d). This result suggests the promising potential of fluorodendrimer in the co-delivery of fluorinated drugs and DNA. G2-F7$_{12}$ is also able to deliver a therapeutic gene encoding tumor necrosis factor-related apoptosis inducing ligand (TRAIL) into MDA-MB-231 cells. TRAIL is an immunosurveillance cytokine that kills cancer cells by apoptosis induction but causes minimal toxity to normal cells.[13] As shown in Figure 2e and Figure S2, G2-F7$_{12}$-mediated TRAIL expression in MDA-MB-231 cells efficiently suppresses the tumor cell growth at an optimal nitrogen-to-phosphorous (N/P) ratio of 4.

Though TRAIL-based gene therapy shows great promise in the treatment of cancer, the comparatively low transfection efficacy of non-viral gene vectors limits its clinical applications. Usually, co-delivery of drugs and TRAIL plasmid were proposed to achieve the synergistic/combined effect of drug and gene therapies.[14] We further investigated the potential of G2-F7$_{12}$ in the co-delivery of sorafenib and TRAIL plasmid to ablate cancer cells. As shown in Figure 2f, the ternay complex consisted of G2-F7$_{12}$, sorafenib, and TRAIL plasmid kills more than 80% of the MDA-MB-231 cells, which is much higher than the binary complexes and free sorafenib (<40% cell death). In addition, G2-F7$_{12}$ is more efficient than the commercial lipid gene vector-Lipofectamine 2000 (Lipo 2000) in the co-delivery of sorafenib and TRAIL plasmid (Figure 2f). The results suggest that combination therapy through the co-delivery of sorafenib and TRAIL plasmid by fluorodendrimer enables increased cytotoxicity on the cancer cells.

A recent study reported that mitogen-activated protein kinase 14 (MAPK14) causes the cancer cells less sensitive to sorafenib.[15] The combination of sorafenib and MAPK14 inhibitor is a promising approach to overcome the cancer resistance against sorafenib. SB202190 is a MAPK14 inhibitor.[16] Co-delivery of sorafenib and SB202190 shows increased activity on the prevention of cancer cell growth. As shown in Figure 2g, the presence of SB202190 does not cause a remarkable effect on the toxicity of G2-F7$_{12}$/TRAIL plasmid complexes, but significantly enhances the toxicity of sorafenib and G2-F7$_{12}$/sorafenib complexes. The combination of SB202190 and the ternay complex shows the highest efficacy in the inhibition of MDA-MB-231 cells. The synergistic effect of SB202190 and sorafenib allows us to kill the cancer cells at a relatively low sorafenib dose (2 μM in Figure 2g). Interestingly, SB202190 also has a fluorine atom in its chemical structure. In this case, we can co-deliver two fluorinated compounds and a therapeutic gene in a single formulation.

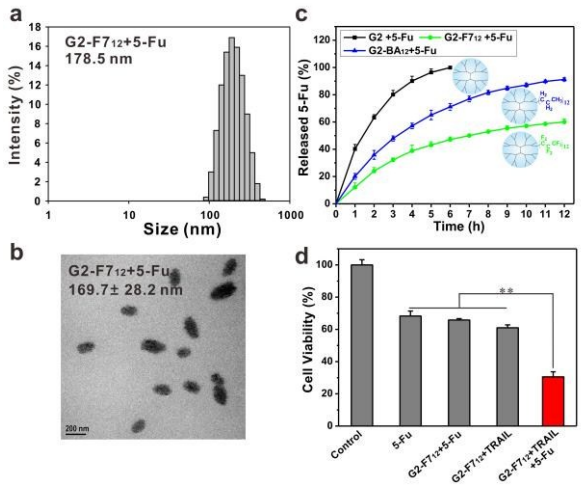


Figure 3. Characterization of G2-F7$_{12}$/5-Fu complexes by DLS (a) and TEM (b). (c) In vitro release kinetics of 5-Fu from the complexes. The molar ratio of 5-Fu and G2-F7$_{12}$ is fixed at 1:1. (d) Viability of MDA-MB-231 cells treated with 5-Fu, G2-F7$_{12}$/5-Fu, G2-F7$_{12}$/TRAIL plasmid and G2-F7$_{12}$/5-Fu/TRAIL plasmid complexes. The concentration of 5-Fu is 64 μM and the N/P ratio of G2-F7$_{12}$/TRAIL plasmid is 4.

The hydrophobic fluorinated drug sorafenib could be also replaced with a hydrophilic one. Here, to test this hypothesis, G2-F7$_{12}$ was loaded with 5-Fu, a hydrophilic anticancer drug with one fluorine atom in its chemical structure. As shown in Figure 3a and 3b, the size of G2-F7$_{12}$/5-Fu complexes (~178 nm by DLS and ~169 nm by TEM) is slightly larger than that of G2-F7$_{12}$/sorafenib complexes. The increased nanoparticle size is probably due to the relatively weak fluorine-fluorine interactions between 5-Fu and the fluorodendrimer. Similarly, 5-Fu shows a much slower release rate from G2-F7$_{12}$ than from unmodified G2 dendrimer and the non-fluorinated material G2-BA$_{12}$ (Figure 3c). The ternary complex formulation consisted of G2-F7$_{12}$, 5-Fu, and TRAIL plasmid shows much higher toxicity on the treated MDA-MB-231 cells than the control formulations (Figure 3d).

Conclusions

In summary, we present a versatile carrier based on low molecular weight fluorodendrimers for the delivery of hydrophobic and hydrophilic fluorinated drugs. The fluorodendrimer G2-F7$_{12}$ shows both high drug loading efficacy and high gene transfection efficacy, and causes minimal toxicity to the cells. Co-delivery of fluorinated anticancer drugs (sorafenib or 5-Fu) and TRAIL causes a synergistic effect on ablating breast cancer cells, probably due to the induced cell apoptosis by TRAIL expression made the cancer cells more sensitive to fluorinated anticancer drugs. Since a large number of drugs containing one or more fluorine atoms, the fluorodendrimers can be developed as versatile carriers for the delivery of these drugs. These carriers also enable the co-

delivery of fluorinated drugs and therapeutic genes for combination cancer therapy.

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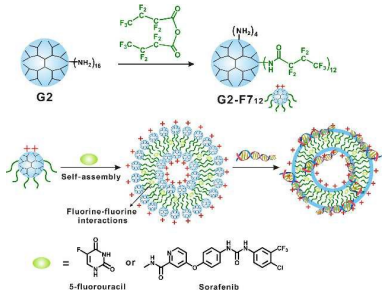

The fluorodendrimer enables the co-delivery of fluorinated anticancer drugs and therapeutic genes for synergistic cancer therapy.