MRS Communications (2018), *8*, 303–313

doi:10.1557/mrc.2018.49

MRS Communications

Prospective Article

Perfluorocarbon-based nanomedicine: emerging strategy for diagnosis and treatment of diseases

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(Received 23 January 2018; accepted 21 March 2018)

Abstract

Nanotechnology has been considered as a promising strategy for diagnosis and treatment of various diseases. However, the stability and circulation times of the conventional nano-carriers, such as liposomes and micelles, are still unsatisfied. Perfluorocarbons (PFCs) are biologic inert synthetic materials, which are highly hydrophobic and have a tendency to self-aggregation. Additionally, PFCs themselves can act as ^{19}F magnetic resonance imaging agents and oxygen carriers. Thus, the construction of the fluorinated carriers will not only improve the stability of the carriers, but also endow them with additional functions. Here we review the recent advances of PFC-based nanosystems for diagnosis and treatment of diseases.

Introduction

Nanomedicine strategies for diagnosis and treatment of human diseases are of growing interest in recent years, owing to their unique and appealing properties, such as prolonged circulation lifetimes, reduced side effects of the drugs.[1,2] Until now, various nanoparticles (NPs) platforms have been developed including liposomes, micelles, dendrimers, gold, silica, and other inorganic NPs, etc.[3] Although some of the NPs have been used in clinical trials, few of them have been proved by the US Food and Drug Administration (FDA) for diagnosis or treatment of human diseases so far.[3,4] One of the main barriers stems from the safety problems of the materials and undesirable therapeutic efficiencies.[5] Therefore, it is highly desired to develop safer and more effective NP platforms.

Perfluorocarbons (PFCs) show biologic inertness and low toxicity even at high doses.[6] In contrast to the hydrocarbons, PFCs are more hydrophobic as well as capable of dissolving oxygen, detection and diagnosis of diseases by ^{19}F magnetic resonance imaging (MRI).[7] In addition, PFCs are also lipophobic and have a strong tendency to self-aggregation regardless of the solvent polarity.[8] Initially, the PFCs were mostly fabricated as the oxygen carriers because of their superior oxygen-dissolving capability.[9,10] They thereby have been widely explored for lung injury, emergency transfusion, and traumatic brain injury.[11] Due to the extremely favorable nuclear magnetic resonance (NMR) properties and virtual absence in the human body of fluorine, the PFCs can also be used for ^{19}F MRI.[12] In recent years, the PFCs were also used for gene/drug vectors, displaying excellent serum-resistant capability for gene delivery and good stability for drug delivery.[13,14] This review aims to give an overview of recent achievements on PFC-based nanomedicine for diagnosis and treatment of diseases (Fig. 1). This review is expected to provide inspiration for the design of PFC-based carriers and prompt the application of PFCs in nanomedicine.

PFC-based nano-carriers

Nanotechnology has been considered as an effective strategy to improve aqueous solubility, decrease side effects, and enhance bioavailability of the conventional drugs.[15] According to the physicochemical properties of the drugs, the specific types of the nano-carriers can be chosen.[16] Until now various NPs have been developed, including liposome, micelle, dendrimer, metal NPs, etc.[17]

Liposomes, as a kind of conventional nano-carrier, have proven advantageous for chemotherapeutic drugs or biopharmaceutical drugs delivery to treat the diseases.[18,19] One paper reported by the FDA described that liposomal drug formulations were the most prevalent category (33% of the submissions) in nanomaterial-based drug products from 1973 to 2015, and more than ten of them have been approved by the FDA.[20,21] For example, the first FDA-approved nano-drug, Doxil®, is mainly composed of doxorubicin and PEGylated liposomes.[22] The Doxil® has been used for the treatment of Kaposi's sarcoma, ovarian, and breast cancer. Typically, liposomes are formed by the self-assembly of the phospholipids

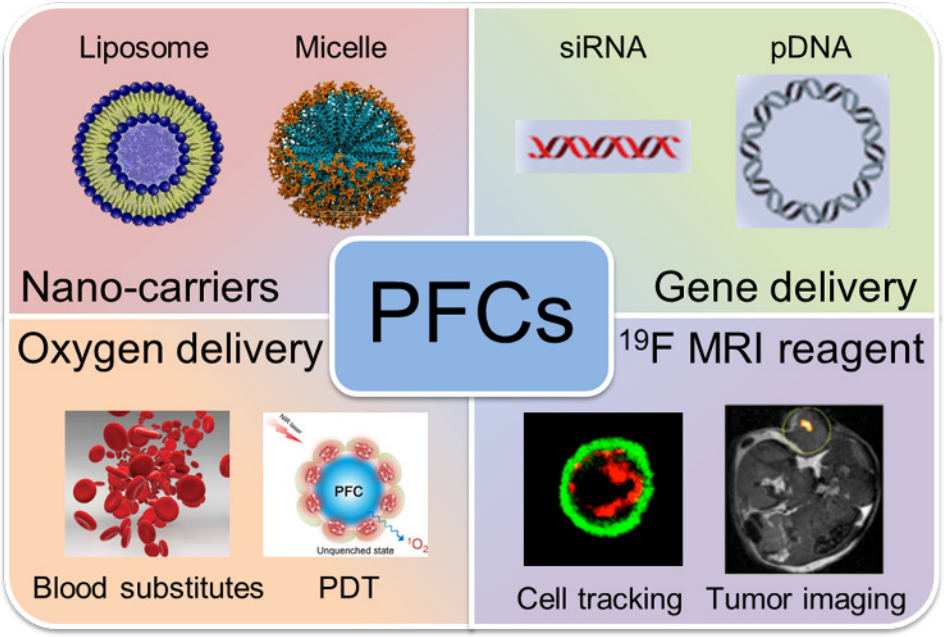

Figure 1. Schematic illustration of the typical applications of perfluorocarbons (PFCs) in the fabrication of nano-carriers, gene delivery, oxygen delivery, and ^{19}F MRI.

such as phosphatidylethanolamine, phosphatidylcholine, and phosphatidylglycerol, as well as cholesterol, displaying the hydrophilic core and hydrophobic lipid bilayer. Thus, liposomes can encapsulate hydrophilic, hydrophobic, and even amphiphilic drugs. In addition, the surface of liposomes can also be modified with the target-specific ligands or antibodies to improve their accumulation in the target tissue. Compared with the conventional drugs, the liposomes display reduced toxicity and higher accumulation efficiency of drugs that can reach the targeted tissue or pathology. However, delivery efficiency of the liposomes is still of depression. A latest statistical work reported by Chan and co-workers demonstrated that the average delivery efficiency of liposome-based drug delivery systems was only 0.5%.[23] This suggests that just 0.5% of administered liposomes are able to reach the targeted diseased tissue. The low delivery efficiency of the liposomes is mainly attributed to their short circulation lifetimes.[24]

In the aspect of the enhancement on the circulation times of the liposomes, the most widely used strategy is to construct the PEGylated liposome.[25,26] It is reported that half-life of the conventional liposome increased from 30 min to 5 h via the introduction of PEG-based amphiphilic materials.[27] Alternatively, the construction of fluorinated liposomes has also emerged as an attractive strategy to prolong the circulation lifetimes of liposomes with PFCs as hydrophobic component.[8] The fluorinated liposomes were more stable and displayed longer circulation lifetimes, due to their unique properties of both hydrophobicity and lipophobicity, high phase-separation tendency, and biomolecules adsorption resistance.[28] As early as 1993, Riess and co-workers have demonstrated that the fluorinated liposomes have much longer circulation lifetimes compared with the conventional liposomes composed of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol.[29]

The researchers also developed a series of liposome-like fluorinated nano-carriers to pursue the efficient drug/gene vectors.[30] Via the self-assembly of the amphiphilic fluorinated dendrimers, a library of liposome-like nano-assemblies named dendrimersomes were developed by Percec and co-workers (Fig. 2).[31] The morphology of the dendrimersomes could be unilamellar or onion-like depending on the composition of the hydrophobic chains [Figs. 2(b) and 2(c)]. Additionally, the potential imaging properties of dendrimersomes for ^{19}F MRI and fluorescence imaging make them interesting tools for nanomedicine. With the aim of developing novel nano-carriers that can co-deliver therapeutic genes and fluorinated drugs, Cheng and co-workers synthesized a library of amphiphilic materials composed of heptafluorobutyric anhydride and polyamidoamine (PAMAM) dendrimers with low generation.[32,33] The fluorodendrimer-based nano-assemblies with the features of both lipids and polymers can readily encapsulate the fluorinated drugs regardless of hydrophilicity and hydrophobicity as well as efficiently deliver therapeutic genes.

The fluorinated catansomes were also developed by Mouritsen and co-workers aimed at obtaining alternative vectors to liposomes.[34] The fluorinated catansomes were constructed by the self-assembly of the oppositely charged surfactants with the diameter of 100–200 nm. The catansomes were pretty stable for over 5 months and could efficiently load the fluorescent molecule calcein as a model drug.

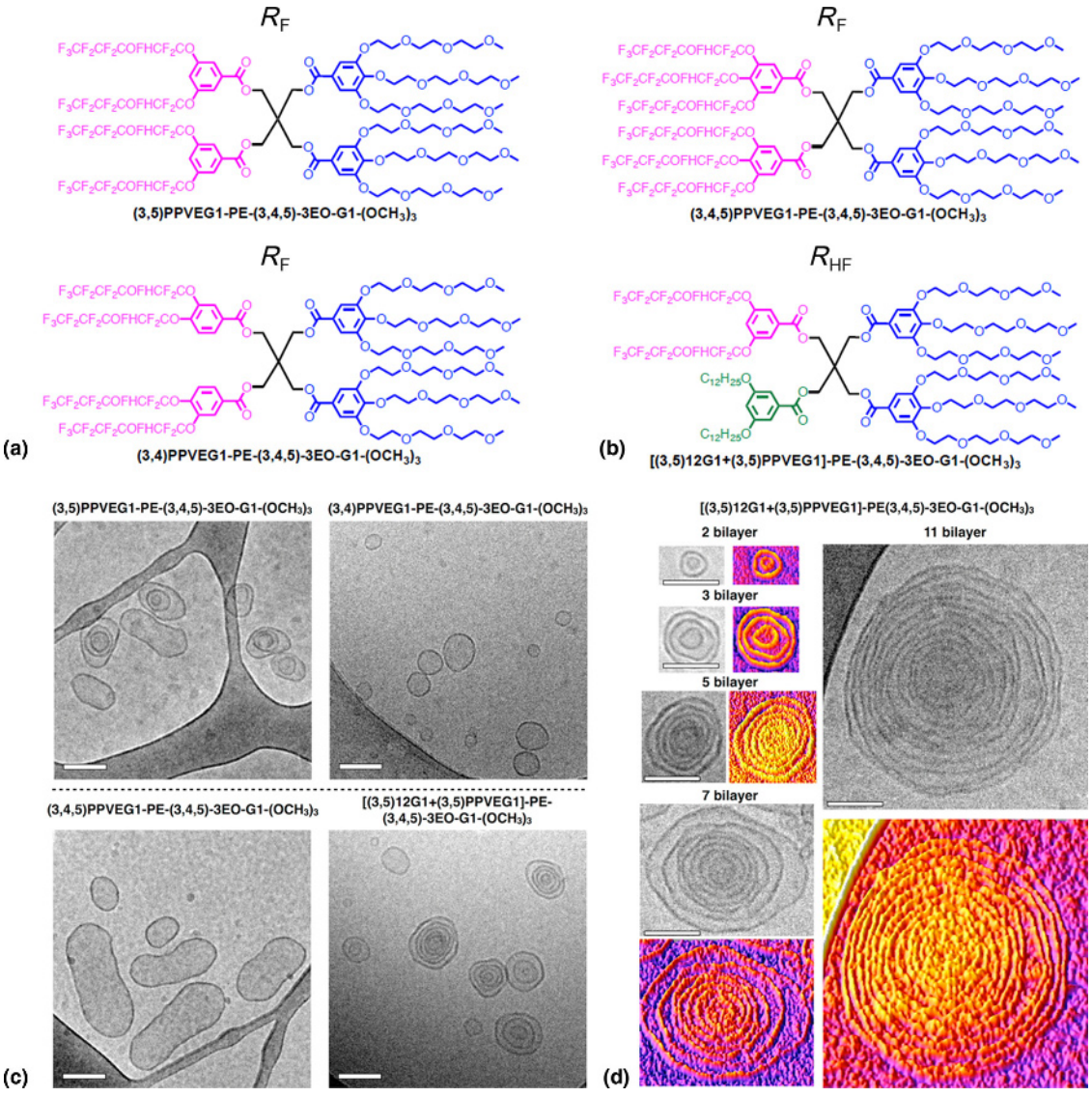

Figure 2. (a) Library containing three janus dendrimers with fluorinated dendrons in their hydrophobic part (RF), one hybrid with a combination of chiral-racemic fluorinated and achiral hydrogenated dendrons (RHF). (b) Cryo-transmission electron microscopy (TEM) images of dendrimersomes assembled by RF and RHF Janus dendrimers. (c) Selected cryo-TEM images of onion-like dendrimersomes self-assemblies and their three-dimensional intensity-plotting images with different numbers of bilayers and diameters. Scale bar, 100 nm. Adapted with permission from Ref. 31 (American Chemical Society, 2016).

Micelles, another traditional nano-carrier, have been widely used for drugs and genes delivery.[35,36] The micelles are typically constructed via the self-assembly of the amphiphilic molecules into the core–shell architectures. The hydrophilic shell can impart them steric stability and prolong their circulation lifetimes, while the hydrophobic core can load the cargoes and further enhance their stability.[37] Until now, various kinds of micelles have been developed, such as PEG-b-PLGA, PEG-b-PCL, and PVP-b-PDLLA, for the drugs or genes delivery.[38–40] But few of them have been approved by the FDA. One of the major problems is the poor stability of the micelles in blood.[41,42] In comparison with hydrocarbons, the PFCs are more hydrophobic with lower van der Waals interactions,[43] which will enhance the cohesion of the hydrophobic core and make the micelles more stable.[28,44] Thus, the development of fluorinated micelles will be a promising strategy to combat the stability problems of micelles. A kind of bioreducible fluorinated micelle was reported by You and co-workers for gene delivery (Fig. 3).[13] The reported micelles were prepared via the self-assembly of PFC-modified poly(ethylenimine) (Mn = 10,000 g/mol). The introduction of the PFCs vastly enhanced the stability of the vectors and DNA complexes. Surprisingly, they can maintain high transfection efficiencies even in the presence of 50% fetal bovine serum (FBS).

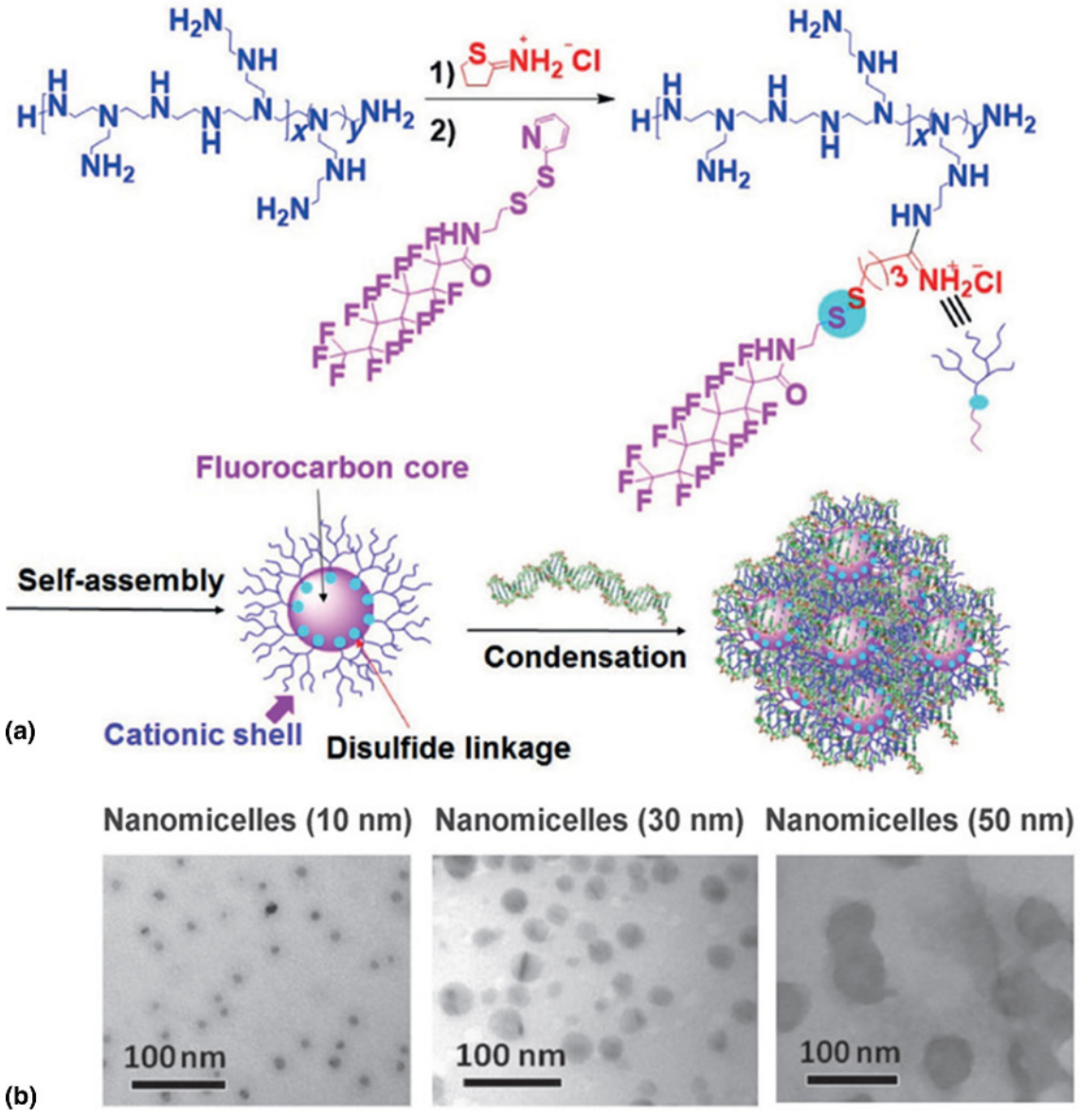

Figure 3. (a) Scheme outlining the conjugation of PFCs to PEI, nanomicelle formation, nanomicelle@DNA complexes. (b) TEM images of the nanomicelles. Adapted with permission from Ref. 13 (Wiley, 2016).

PFC-based gene delivery

Gene-based therapy has been recognized as the promising approach to treat the diseases by the modulation of gene expression in the targeted cells.[45,46] The modulation can be realized by using various exogenous nucleic acids, including DNA, mRNA, siRNA, and CRISPR.[47–49] Although the mechanisms of these nucleic acids are different, it is essential to realize efficient gene transfection with the aid of gene vectors.[50,51] Viral-based vectors are able to escape the sequestration by the mononuclear phagocyte system and display high gene transfection efficiency both in vitro and in vivo.[52,53] However, the potential immunogenicity and carcinogenesis limit the application of them in gene delivery.[54,55] Unlike viral-based vectors, non-viral vectors, such as polycations and liposomes, are safer and more feasible to prepare.[19,56] While the low gene transfection efficiency in the presence of serum is undesirable, due to the attack of serum proteins leading to the disassembly or aggregation of the vectors.[57,58]

To overcome the poor delivery efficiency of the non-viral vectors in the presence of serum, researchers have developed various strategies, for example, the construction of virus-like vectors and the development of charge-reversal nanosystems.[59–62] Although these strategies can improve their delivery efficiency in serum-containing medium, the complex synthetic

procedures and cytotoxicity issue limit their clinical use.[63–65] PFCs are synthetically and biologically inert, displaying special properties of both hydrophobicity and lipophobicity, low surface energy, and high phase-separation tendency.[6] In recent years, researchers have found that the PFC-modified vectors could well maintain their gene transfection efficiency in serum-containing medium.[14,66]

The strategy of functionalizing generation 5 (G5) PAMAM dendrimers with perfluoro acid to construct the pDNA vectors was explored by Cheng and co-workers [Fig. 4(a)].[14] Upon the fluorination of the dendrimers by heptafluorobutyric acid (G5-F7$_{68}$), both the cellular uptake and endosomal escape were enhanced. The gene transfection efficiency of the fluorinated dendrimer was improved from 21.8% to 97.1% in HEK293 cells [Fig. 4(b)]. Moreover, the G5-F7$_{68}$ achieved 55.4% in HeLa cells even in the presence of 50% FBS, whereas Lipo 2000, a commercialized vector, almost lost its gene delivery capability under the same conditions, indicating the good

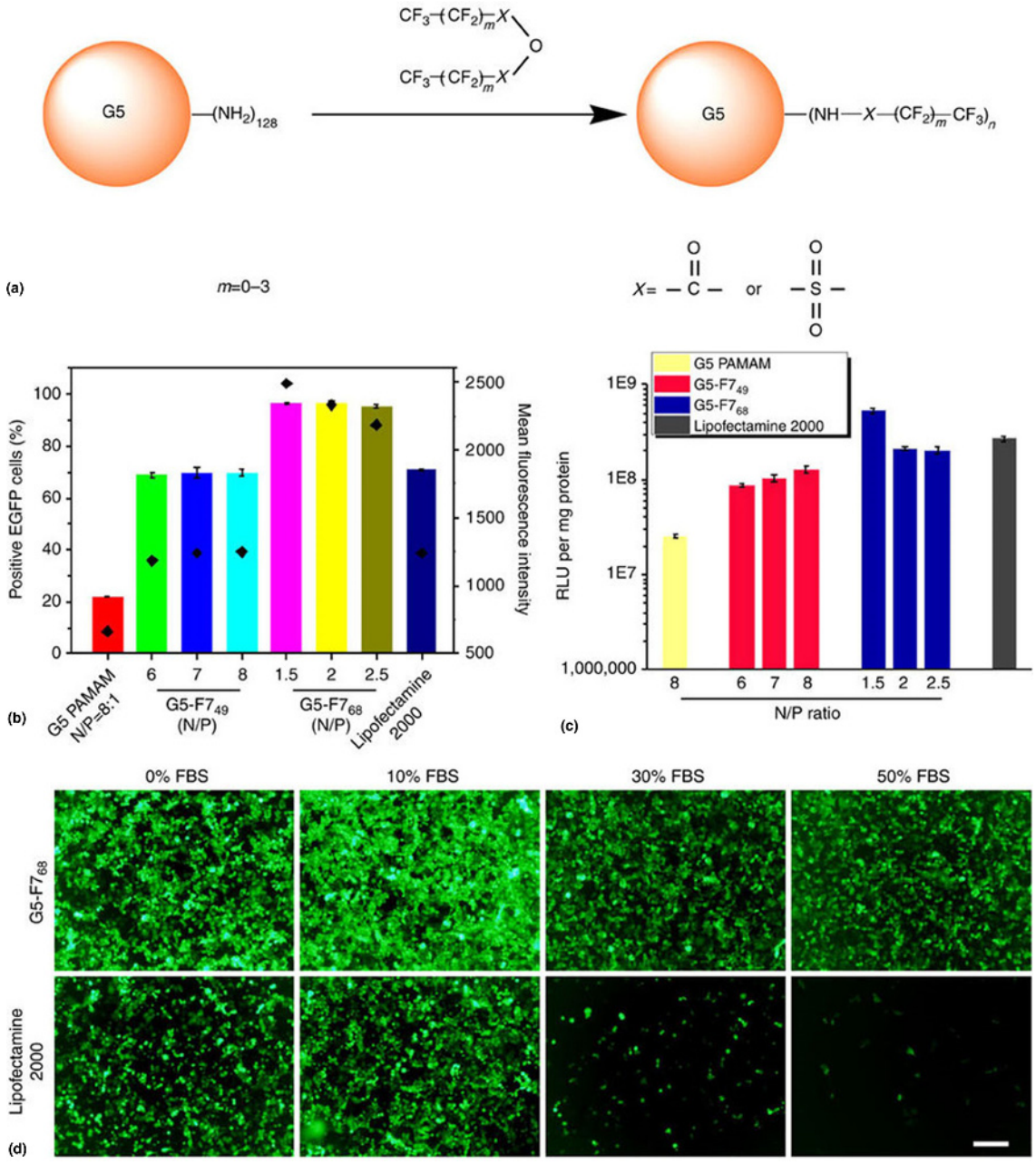

Figure 4. (a) Schematic illustration of the synthetic process of fluorinated dendrimers. (b) Comparison of the ability of G5-F7$_{49}$, G5-F7$_{68}$, G5, and Lipofectamine 2000 to mediate in vitro enhanced green fluorescent protein (EGFP) (b) and luciferase (c) gene transfection in HEK293 cells after 24 h. (d) Serum-resistance capabilities of fluorinated dendrimers on EGFP gene transfection in HeLa cells. Scale bar, 200 μm. Adapted with permission from Ref. 14 (Springer Nature, 2014).

serum-resistant capability of fluorinated vectors [Fig. 4(c)]. The fluorination strategy also worked on polypropylenimine dendrimer and other cationic polymers such as bPEI 25k.[67,68] Considering the cytotoxicity problems of the polycations and their complexes with nucleic acids in vivo environment, the biodegradable fluorinated peptide dendrimers with low generation were developed by Gu and co-workers for pDNA/siRNA delivery.[69,70] The biodegradable and biocompatible G2 poly (L-lysine) dendrimer with 32 peripheral amino groups is served as positive charged component. Fluorination of the dendrimer by heptafluorobutyric acid can create an inactive surface to suppress protein adsorption and improve cellular delivery efficiency. The reversible crosslinking of the vectors by disulfide-contained linker is able to decrease the cytotoxicity of the vectors, and simultaneously enhance their stability in vivo.[71] The vectors also well preserved the gene transfection efficiency in HEK293 and HepG2 cells in the presence of 30% FBS and achieved considerable gene transfection efficiency in HepG2 tumor xenografts. As discussed before, the fluorinated micelles/liposomes with the PFCs as hydrophobic component were also prepared for gene delivery.[13,33] These carriers can well maintain their gene delivery efficiency in vivo, and simultaneously encapsulate chemotherapy drugs such as 5-fluorouracil, displaying high efficiency in tumor inhibition.[32]

PFC-based oxygen storage and transport

The PFCs have also attracted much attention due to their properties in dissolving oxygen.[9,72] As early as 1966, researchers have found that PFC liquids could support respiration of mice and cats.[73] Afterward, a series of PFC-based oxygen carriers have been developed as blood substitutes, organ preservation solutions, and oxygen suppliers for photodynamic therapy (PDT).[10,74,75]

The PFCs are initially explored for artificial blood fabrications.[76,77] There is increasing demand of blood throughout the world because of wars, natural disasters, and aging population issues.[78] In hospitals, especially for the emergency services, the patients are urgently needed the matched blood to live.[79] However, the amount of blood supply is not sufficient to meet the demand of blood, and the chasm is continued to increase.[80] In addition, complex process of blood transfusion and strict storage condition of blood further threaten the human life.[81] Thus, it is highly desirable to develop the available and safe blood substitutes. PFCs with comparable oxygen solubility capability to hemoglobin are promising candidates to overcome the drawbacks of human blood.[9,82] Until now, many PFC-based blood substitutes have been developed, for example, Fluosol-DA as a blood substitute in hemorrhage and emergency transfusion, and Oxygent as a blood substitute in hemorrhagic shock and cerebral ischemia.[10,11] However, there is no FDA-approved blood substitute to date because of the safety issue.[83] Therefore, it is a prerequisite to develop the next generation of the blood substitutes with the biocompatibility as the first priority consideration.

In recent years, researchers have also extended the applications of the PFCs onto PDT therapy as an oxygen supplier.[84,85] The therapeutic efficacy of PDT therapy is mainly determined by the $^1O_2$ generation rate.[86] The oxygen concentration is pretty low in some biophysical circumstances, for example, hypoxia tumors microenvironment, leading to low $^1O_2$ generation rate and poor therapeutic efficacy.[87] PFCs with highly dissolved oxygen capability are promising candidates to overcome the limitation of PDT.[88] Hu and co-workers designed a kind of oxygen self-enriching nanosystem with PFCs and photosensitizer coencapsulated by lipids (Fig. 5).[75] The nanosystem can not only enhance the $^1O_2$ generation rate, but also prolong the half-life of $^1O_2$. In contrast to the traditional PDT, the oxygen self-enriching nanosystem showed higher therapeutic efficacy on the treatment of hypoxia tumors (Fig. 5). Another effective strategy was explored by Liu and co-workers, wherein the oxygen carriers composed of albumin and PFCs were administered to relieve the tumor hypoxia microenvironment firstly, and then the PDT treatment was performed.[89] The carriers can efficiently adsorb oxygen in the lung and the adsorbed oxygen can be rapidly released into the tumor upon the stimulation of ultrasound. In comparison with the conventional PDT treatment using liposomal chlorin e6, the combination of oxygen carriers and PDT treatment remarkably inhibited the growth of tumors.

PFC-based diagnostic reagent

Owing to the negligible fluorine concentration in the human body and extremely favorable NMR properties of fluorine, the ^{19}F MRI techniques have also been employed for various biomedical applications, such as cell tracking and diseases diagnosis.[12,90,91] To meet the requirements of ^{19}F MRI applications, kinds of PFC-based contrast agents have been developed, including the aromatic PFCs, saturated linear PFCs, perfluoroethers, and polyethers.[7] The properties of conventional PFC-based MRI agents have been well discussed in the previous reviews.[12] Herein, this part will focus on the introduction of the recent works related to PFC-based MRI agents.

One of the main barriers of the ^{19}F MRI lies on the low sensitivity. To improve the sensitivity of the probes, Ahrens and co-workers developed a library of paramagnetic fluorinated nanoemulsions with perfluoropolyether (PFPE) as metal chelators, which could incorporate various paramagnetic metal ions in the fluorous phase.[92] The formed nanoemulsions were stable in the intracellular milieu with pretty low rates of metal leakage. Additionally, this formation of the ^{19}F MRI probes had a reduction in spin–lattice relaxation time (T_1), which contributed to improving the cell detection sensitivity. For the iron (III) tris-β-diketonate nanoemulsions with PFPE, their ^{19}F MRI detection sensitivity was improved more than eightfold at clinical field strengths. The strategy to develop the multimodal imaging agents was also adopted to compensate for the low sensitivity of ^{19}F MRI.[93] Thurecht and co-workers developed a multimodal polymer NPs system by combining high-resolution ^{19}F MRI and sensitive fluorescence imaging for diagnosis of disease in vivo [Fig. 6(a)].[94] The hyperbranched polymer (HBP) was

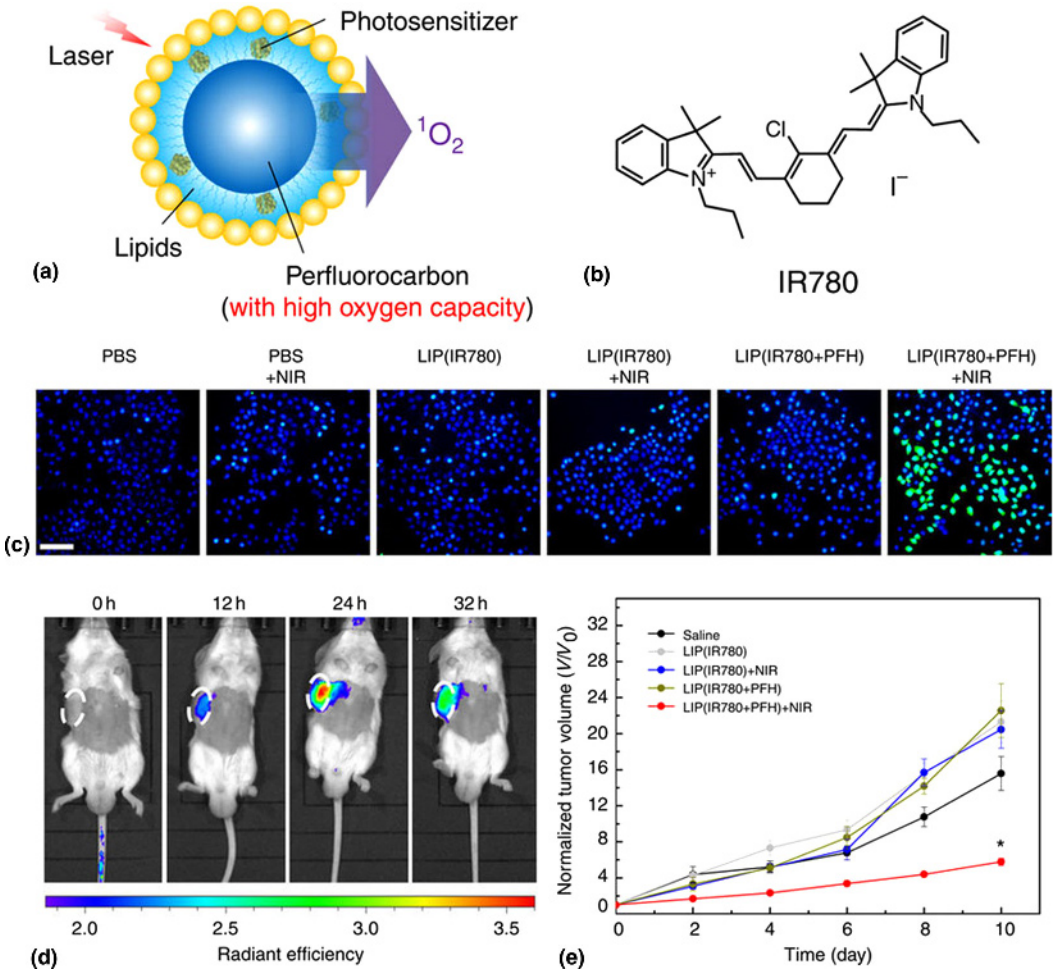

Figure 5. (a) Schematic illustration of the structure and design of the Oxy-PDT agent. PFCs as a hydrophobic core are coencapsulated by lipids. Photosensitizer is uniformly dispersed inside the nanoparticle. (b) The chemical structure of IR780. (c) Enhanced $^1O_2$ production by Oxy-PDT in cells. $^1O_2$ generation was detected using carboxy-H_2DCFDA, showing green fluorescence indicate positive staining for ROS; the cells are stained with Hoechst 33342. Scale bar, 50 mm. (d) Near-infrared imaging of tumor accumulation of IR780 in tumor-bearing mice after intravenous injection of LIP (IR780 + PFH). Images were taken at 0, 12, 24, and 32 h postinjection. Tumors are circled with white dashed lines. (e) Tumor volumes were measured after treated with LIP (IR780 + PFH) and laser irradiation. Values are the means ± SEM. Adapted with permission from Ref. 75 (Springer Nature, 2015).

synthesized by the reversible addition–fragmentation chain transfer (RAFT) polymerization technique, comprising stealth component (PEG), tumor targeting agents (folate), and two imaging modalities (^{19}F MRI and fluorescence). The polymer NPs were capable of fluorescence imaging of individual cells and detection of B16 melanoma tumors by the combination of ^{19}F MRI and fluorescence in vivo [Figs. 6(b) and 6(c)].

Conclusion

In this review, we have systematically described the recent achievements of PFC-based nanomedicine. Compared with hydrocarbons, the PFCs are more hydrophobic and stable because of their high tendency to segregate from the surrounding environment. Besides, the biologic inert PFCs are good candidates for oxygen delivery and ^{19}F MRI because of their great oxygen-dissolving ability, negligible background signal, and extremely favorable NMR properties of fluorine. In recent years, the PFC-based gene/drug carriers have also been developed. These works well demonstrated that fluorination of the carriers, such as liposomes or micelles, was able to vastly improve their stability and serum-resistant capability both in vitro and in vivo. Additionally, the work reported by Riess and co-workers also demonstrated that fluorination of the liposome vastly prolonged the circulation lifetimes. Thus, the construction of fluorinate carriers will be a promising strategy to overcome the limitations of systemic delivery for conventional nano-carriers.

The strategy of combining the properties of PFCs with other imaging agents or drugs may be a rational choice to achieve more definitive diagnosis and effective treatment of diseases. For example, to achieve accurate early detection of diseases, the multimodal imaging platforms can be constructed by the combination of ^{19}F MRI with other imaging modalities with a good

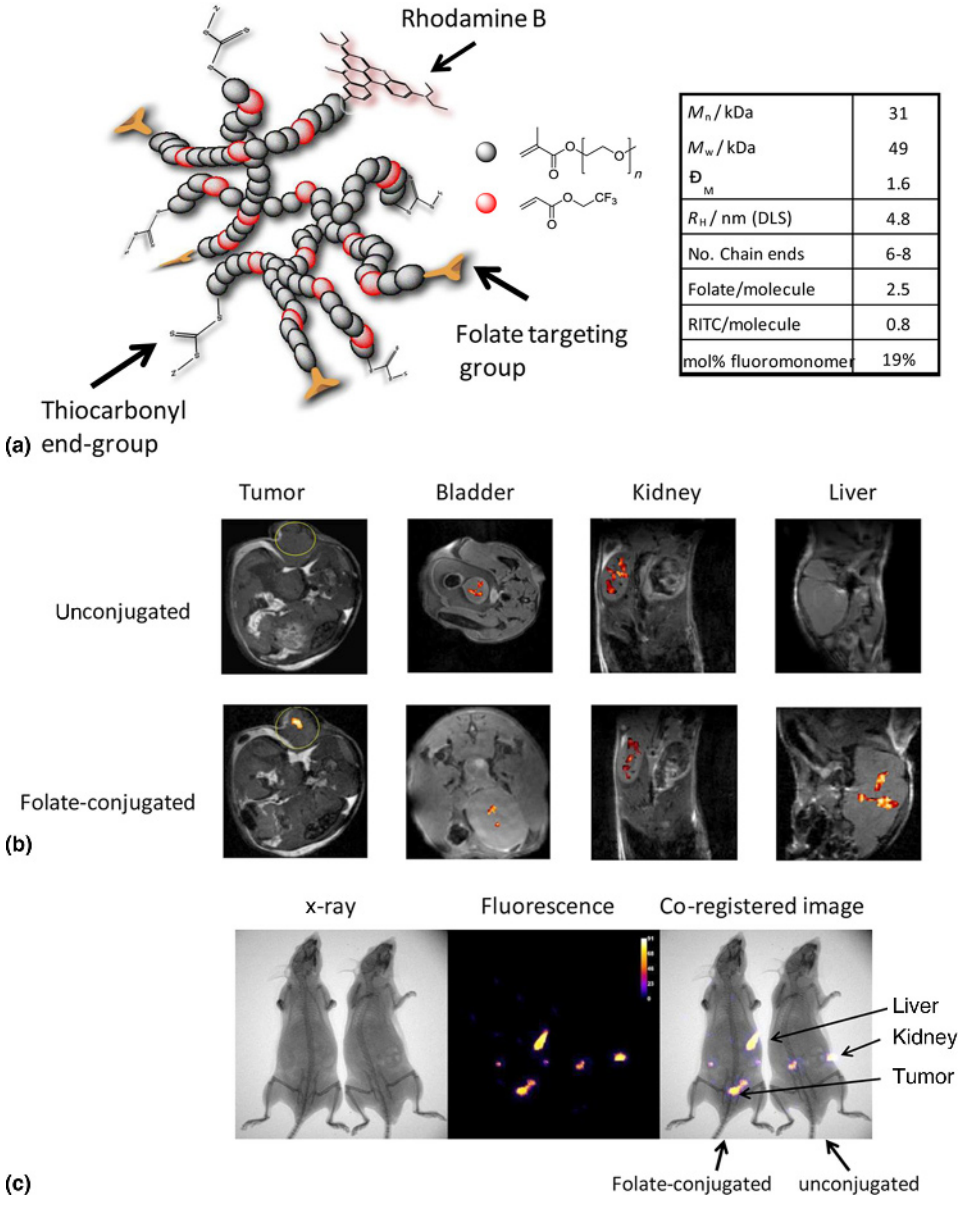

Figure 6. (a) Schematic illustration of the multifunctional hyperbranched polymer used in this work. (b) The application of the HBP for imaging of the mouse subcutaneous B16 melanoma model. MRI images of the tumor (circled in image) bladder, kidney, or liver in the tumor-bearing mice 1 h following intravenous injection of 100 µL of unconjugated or folate-conjugated HBP (20 mg/mL in PBS). The ^{1}H MR image is overlaid with the ^{19}F image. (c) Fluorescence images of mice after injection of two compounds at the same concentration. The fluorescence images are co-registered with x-ray images of the mice 1 h following subcutaneous injection. Adapted with permission from Ref. 94 (American Chemical Society, 2014).

sensitivity such as fluorescence, single-photon emission computed tomography, or positron emission tomography. The highly dissolved oxygen of PFCs can also be applied to promote the efficacy of radiotherapy and PDT for cancer therapies. In addition, the PFC-based theranostics platforms can also be constructed by applying the diagnostic functions of PFC-based ^{19}F MRI and the therapeutic functions of drugs/genes. Therefore, the PFC-based nanomedicines hold appealing potentials as a new strategy to develop stable and efficient nano-carriers for definitive diagnosis and efficacious treatment of diseases.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant numbers 31771094 and 31371014). This work was also supported by the Natural Science Foundation key projects (grant numbers 31630027 and 31430031) and NSFC-DFG project (grant no. 31761133013). The authors also appreciate the support by the "Strategic Priority Research Program" of the Chinese Academy of Sciences (grant no. XDA09030301).

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