Self-assembly of Peptide dendrimers and their bio-applications in theranostics

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1. Introduction

Early disease diagnosis and effective treatment are of great significance to patients’ physical recovery [1]. Peptides with various functionalities, such as specific targeting, stimuli-responsive, cell penetration and therapeutic potential, can be delicately incorporated in a nano-system to achieve disease diagnosis and therapy [2–6]. Compared to non-peptide compounds, peptide-based nanomaterials possess huge potential applications due to its programmability, biological activity and good biocompatibility [7,8]. In the past decades, the rapid development of peptide dendrimers in the field of bio-applications, especially in cancer diagnostics and therapy, has revolutionized conventional health care and medical technology [9,10]. Peptide dendrimers are hyperbranched biomacromolecules with a peptidyl core, dendrons, or surface functional groups [11]. Their molecular size ranges from low molecular weight structures (about 2 kDa) to large protein-like structures with molecular weights over 100 kDa, depending on their adjustable generation numbers [12]. Generally, peptide dendrimers can be classified into three categories according to their composition: 1) composed of branching poly amino acids, representing by the poly-lysine dendrimeric structures [13]; 2) contained a branching core with either unnatural amino acids or organic groups (e.g., poly-amidoamine (PAMAM) core) and surface groups modified by peptide or proteins [14]; 3) comprised the core consisting of amino acids and attached peptide chains on surface functional groups, such as the most typical example multiple antigen peptides (MAPs) [15]. The three types of peptide dendrimers have been frequently applied in biological and biochemical applications, and there have been many reviews on the synthesis and bio-applications of peptide dendrimers [12,16]. Herein, we describe some details concerning the self-assembly behaviors of peptide dendrimers. Self-assembly, bringing in novel structure elements, is valued in material science, biology and chemistry. As an ideal biomaterial, peptide dendrimers tend to assemble into well-defined structures in solutions with novel physicochemical properties, benefiting from their large size, controllable functionalities and secondary structure from the polypeptide chain [17]. Specifically, for self-assembled peptide dendrimers, their functionalities can be tuned through meticulous design using multiple synthesis methods, and their architectures can be sensitively adjusted by varying temperature, pH and medium in selective solutions [18–22]. The fundamental driving forces for the assembly behavior are various non-covalent interactions, including hydrogen bonding, hydrophobic, van der Waals and electro-static interactions, resulting in different architectures such as spherical micelle, vesicles and nanofibers. Due to their highly tunable compositions and structures, the self-assembly of peptide dendrimers has attracted widespread attention in many applications involving imaging [23], inhibitors [24], biomimetic collagen [25], gelation [26] and drug/gene delivery [27,28]. When used in the theranostics field, the self-assembled peptide dendrimers own novel physicochemical properties through the synergistic effects of the polypeptide chain, dendrimer and nano-structure, including: 1) polyvalence that can improve the biological activity of grafted peptides; 2) multiple binding interactions with many receptors simultaneously; 3) protein-like structural features that
imitate the role of multiple biomolecules; 4) better biocompatibility and biodegradability; 5) highly resistant to proteolytic digestion and effectively delay renal clearance [29].

This minireview mainly summarizes the self-assembly of peptide dendrimers and their bio-applications for disease diagnosis and therapy. We briefly sum up the self-assembly behaviors of peptide dendrimers from their nanosize, morphology and composition, in which the self-assembled nanoparticles not only have higher stability but also have more functional groups available for modification on the periphery. We further introduce the theranostic applications based on the specific imaging and efficient delivery recently. Finally, we make a brief prospect in this area.

2. Self-assembly of peptide dendrimers

2.1. Self-assembly of peptide dendrimers contained a branching core with non-amino acids

The branching core in the peptide dendrimers plays a vital role in self-assembly. For example, unnatural amino acid or organic groups as branching units can assist the formation of many intermolecular or intramolecular non-covalent interactions, promoting the self-assembly of peptide dendrimers. In 2000, Aida et al. firstly reported that poly (benzyl ether) dendrimers carrying dipeptides were able to self-assemble into a dendritic physical gel in organic solvents via hydrogen-bonding interactions [30]. Van der Waals forces derived from the large dendritic wedges probably stabilized the hydrogen bonds among the dipeptides. To further study the structural parameters for efficient gelation, this group synthesized eleven different peptide-core dendritic macromolecules, each containing a [G2] poly (benzyl ether) dendritic wedge at the peptide side chain, N-terminal or C-terminal, and investigated their gelation properties [31]. The results indicated that dipeptides, ester functionalities on the surface of the dendritic wedge, side-chain dendronization or N-dendronization of dipeptides and higher generation numbers of the dendritic wedge are favorable for the gelation. The Heiney group depicted a collection of amphiphilic dendritic dipeptides (amphiphilic benzyl ether dendrons functionalized at their apex with a dipeptide) that could self-assemble into supramolecular helical pores in solution and the solid-state through a molecular recognition process [32]. A trans conformation of the dendron is required to mediate self-assembly and provide a new hydrogen-bonding mechanism of a parallel and partially interdigitated array of dipeptides. They further demonstrated that the internal structure and the stability of the self-assembled helical porous columns are programmed by the structure and stereochemistry of the dipeptide, the protective groups of the dipeptide, and the structure and chirality of the dendron attached to the dipeptide [33–35].

PAMAM dendron can be modified with functional peptides, resulting in self-assembled macromolecules with desired properties for diverse applications. Peng et al. designed copolymers with both asymmetrical and symmetrical topologies, using PAMAM dendron-like poly(γ-benzyl-γ-glutamate) (Dm-PBLG) and linear poly (ethylene oxide) (PEO) as building blocks [36]. Both spherical and wormlike micelles self-assembled from these Dm-PBLG-b-PEO copolymers in aqueous solution, and the PBLG unit mainly guided the morphology of nanostructures. Kojima and co-workers designed an ethylenediamine-core PAMAM dendrimer fully modified with collagen model peptides (Pro-Hyp-Gly)10 to self-assemble into hydrogels at 40 °C upon heating. The hydrogels were denatured around 75 °C, much higher than nature collagen denatured at 40–45 °C, indicating that self-assembly stabilized the triple helical structure of (Pro-Hyp-Gly)α-den [19]. In addition to the linear peptides, cyclic arginine-glycine-aspartic acid (cRGD) peptide decorated on the surface of G3 PAMAM could also self-assemble into nano-sized spherical structures with an average size of 80.86 nm [37]. More interestingly, dendrimers can be modified with two oppositely charged polypeptides, leading to "schizophrenic" self-assembly behaviors. Through a combination of click chemistry and ring-opening polymerization (ROP), the Lin group designed a PAMAM dendrimer carrying poly-(l-lysine) at the focal point and poly-(l-glutamic acid) as the surface functional group. The obtained PLL-b-D2-(PLGA)4 can perform "schizophrenic" self-assembly by varying the solution pHs, in which PLGA-core aggregates were preferred at acidic pH and PLL-core aggregates were formed at alkaline pH, respectively. Nanoscale changeable particles such as worm-like micelles, large compound micelles, large compound vesicles, simple vesicles and tubular vesicles were obtained in "schizophrenic" aggregation process by simply increasing the solution pH (Fig. 1) [22].

Amide dendritic peptides exhibited distinct self-assembly behavior through adjusting the peptides and solvent type. Kim's group introduced a larger cyclic CVVLLC peptide onto the amide dendron (2G), and the presence of intramolecular disulfide bonds facilitated the formation of vesicular structure with diameters ranging from 50 to 400 nm [38]. For the linear counterpart, no regular structure in the aqueous phase was observed, indicative of the importance of peptide structure organization in self-assembly. Lee et al. synthesized a series of 2nd generation amide dendron-dipeptide conjugates to investigate their self-assembly behavior in different solvents [30]. All conjugates formed vesicular structures in aqueous solutions because of a dominant role played by the amide dendrons and fibrous structures in organic media. For the organic-aqueous solution (e. g. HFIP/H2O (1:100 v/v)), the dipeptide moiety became extremely important, in which dendron-diglycine shaped rod-like structures while dendron-diphenylalanine formed donut-like structures due to the strengthened π-π interactions from the additional phenyl moiety.

Hydrophilic-hydrophobic interaction is another driving force for the self-assembly of grafted peptide dendrimers. Toth et al. synthesized a dendritic structure consisting of a hydrophobic polycarbonate core and peripheral hydrophilic B-cell epitope J14 (KQAEDKVKASREAKKQVE-KALEQLEDVKV) [39]. By dialysis of a solution of the peptide dendrimer in DMF against water, the self-assembly strategy generated nanoparticles of 20 nm. Guo and co-workers prepared the hydrophobic naproxen (Nap) conjugated amphiphilic peptide dendrimers, in which Nap and oligo-aspartic acid were attached to G1, 2, 3 dendrimers [40]. The three conjugates were self-assembled to nearly 30–40 nm spherical in shape, where Nap acted as the hydrophobic core of micelles. The varying Nap ratio in the three peptide dendrimers endowed their different solubility in organic or inorganic solutions. Monteiro et al. also constructed three polymeric dendrimers peripherally coated with varying l-lysine density using one-pot method through the nitroxide radical coupling (NRC) and the azide-alkyne cycloaddition (CuAAC) reactions. The particle sizes increased from 8.38 to 11.07 nm for dendrimers with increasing number of l-lysine on the periphery [41]. Additionally, Rudick and his partners presented a graft-to strategy for synthesizing poly (alkyl ether) dendron-LK peptide via CuAAC reactions, self-assembling as α-helical bundles. The elongation of peptide length to 14 residues made it more stable for self-assembled α-helical bundles [42].

2.2. Self-assembly of peptide dendrimers with branching poly amino acids

Dendrimers using amino acids as branching blocks with amide linkages have a tendency for self-assembly by virtue of some peptide bonds in their structure [43]. Aspartic acid and glutamic acid are appealing branching blocks for synthesizing peptide dendrimers due to their inherent extra carboxylic acid groups [44,45]. Three aspartic acid-based dendrimers with different generations have been synthesized through a convergent coupling methodology to investigate the organogels formation, in which gel forming propensity increases from first to second generation dendrimer and decreases from second to third generation [46]. The hydrogen bonding interaction from the multiple amide group is the main driving force for the formation of the fibrillar network that responsible for gelation. Besides choosing suitable amino acid as branching block, Verma et al. designed a series of aspartic acid-based peptide dendrimers with urea and urea triazole cores to reveal the role of well-designed core played in self-assembly [47]. The introduction of
urea or urea triazole at the central core of the peptide dendrimer could
inculcate peculiar self-assembly morphologies, exhibiting fibrillar and
vesicular assemblies, respectively.

L-lysine has been extensively used as building blocks to construct the
dendrons with diverse functional groups and then self-assembled into
nanoparticles with various driving forces. Gu group has designed the
lysine-based dendron with heparin on the focal point and doxorubicin
(DOX) as a surface functional group [48]. The designed dendron
self-assembled into uniform nanoparticles with the size of 80 nm, mainly
forced by hydrophilic-hydrophobic interactions between lysine polymers
and dendronized heparin-DOX blocks. To increase the biocompatibility,
polyethylene glycol (PEG) was also introduced to lysine dendrons to
self-assembled into nanoscale particles with neutrally charged surface
and a size of ~80 nm [49]. The primary driving force responsible for
the self-assembly behavior is the minimization of the interfacial energy
governed by the balance between the hydrophilic interaction of the linear
mPEG and the hydrophobic interaction of the dendron DOX block.
The amphilic dendron could be further integrated with tetra-peptide
GFLG (Gly-Phe-Leu-Gly) as an enzyme-sensitive linker, and synthesized
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The primary driving force responsible for the self-assembly behavior is the minimization of the interfacial energy governed by the balance between the hydrophilic interaction of the linear mPEG and the hydrophobic interaction of the dendron DOX block. The amphilic dendron could be further integrated with tetra-peptide GFLG (Gly-Phe-Leu-Gly) as an enzyme-sensitive linker, and synthesized mPEGylated dendron-GFLG-DOX conjugate, which self-assembled into nanoparticles of 80–100 nm with a negative charge [50]. The DOX moiety also influenced self-assembly because of its multiple domains with different chemical compositions like hydrophobic, aliphatic and aromatic groups. The peptide sequence in this amphilic dendron could be tuned to fit various extracellular environment in tumor therapy. For example, a matrix metalloproteinase sensitive peptide PVGLIG (Pro-Val-Gly-Leu-Ile-Gly) was selected to design the mPEGylated dendron-PVGLIG-DOX that could self-assembled into spherical structures with a size of 90–150 nm [51]. Furthermore, the DOX in the amphilic dendron could be self-assembled into cylindrical micelles and vesicles, respectively (Fig. 2B and C). For comparison, the traditional JPD system with the same weight percent alkyl chain (wt%) tends to self-assembled into different nanostructures. Nevertheless, this strange phenomenon requires further research for its mechanism.

Gu group reported three cationic twin head lipids, and each of them contains a dioleoyl-glutamate hydrophobic tail and a twin polar head of lysine, arginine or histidine [55]. Such lipids were proven to self-assemble in aqueous solution with well-defined nanostructures and residual amino-, guanidine- or imidazole-rich periphery, showing strong buffering capacity and good liquidity. In 2008, Nie group designed different-generation arginine-rich dendritic lipopeptides (R1LS, R2LS, R3LS) to form nanoparticles with different morphologies, sizes, surface potentials via self-assembly [56]. Their surface potential and hydrophilicity both gradually increased with the generations increasing for the three dendrimers. The arginine-rich dendritic lipopeptides could be further integrated with the hydrophobic drugs to increase their solubility. In detail, the lipimated Arg4+ dendrimers were assembled into discrete micelles in an aqueous environment, and the bufalin-micelle inclusion complex was prepared by the co-precipitation method [57]. These peptide-dendrimer hybrids are of fundamental interest to investigate the role of polymer architecture on self-assembly, and can exhibit advantages in applications.

3. Bio-applications of self-assembled peptide dendrimers

3.1. Bio-imaging

Early detection and accurate tumor localization using molecular imaging have shown potential to decrease tumor mortality. Nanoscale system obtained from imaging agent-peptide dendrimer conjugates promise the early cancer detection due to the multivalency effect of dendrimers and the amplification property of the surface functionalities [58]. Several research groups have coupled the nanoscale system design with important imaging modalities, including radionuclide-based imaging and fluorescence-based optical imaging. Combination the advantages of the peptide dendrimer and heparin, Luo et al. reported a peptide dendronized heparin-gadolinium (Gd III) polymer that could be used as a potential magnetic resonance imaging (MRI) contrast agent for breast tumor diagnosis [59]. The conjugates self-assembled into a nanoscale system in a compact spherical shape, where it was found that the nanoscale system had a 7-fold increase in the T1 relaxivity than the clinical agent Magnevist (Gd-DTPA) in tumor imaging. Targeting peptides are usually introduced into imaging contrast agents to achieve targeted diagnosis. Almutairi et al. reported a dendritic positron emission
tomography (PET) imaging nanoprobe with a multivalent core-shell architecture including a radioactive core (^{125}\text{I} or ^{76}\text{Br}), a protective shell from polyethylene oxide (PEO) chains and a specific targeting peptide cyclic arginine-glycine-aspartic acid (RGD) towards $\alpha_v\beta_3$ integrin receptors [60]. Cell-based assays of the $^{125}\text{I}$-labeled dendritic nanoprobes using $\alpha_v\beta_3$-positive cells showed a 6-fold increase in $\alpha_v\beta_3$ receptor-mediated endocytosis of the targeted nanoprobe compared with the non-targeted nanoprobe. Notably, this PET nanoprobe radiolabeled with ^{76}\text{Br} showed enhanced bioavailability and in vivo radiostability.

Unimolecular micelles based on PAMAM dendrimer were synthesized as both a cargo delivery vector and an imaging agent for triple-negative breast tumors [61]. With the chemical conjugation of a peptide (F3, against cellular nucleolin) to increase the cellular internalization, these micelles could accumulate potently and specifically in breast cancer cells (Fig. 3A). They discovered $^{64}\text{Cu}$-incorporated PAMAM micelles were suitable for in vivo studies, as shown in Fig. 3B, both $^{64}\text{Cu}$-PAMAM-DOX-F3 and $^{64}\text{Cu}$-PAMAM-DOX had excellent radio-chemical stability in mouse serum. Serial PET imaging demonstrated that the tumor uptake of $^{64}\text{Cu}$-PAMAM-DOX-F3 in MDA-MB-231 tumors was fast, potent and persistent, almost three times higher than that of $^{64}\text{Cu}$-PAMAM-DOX (Fig. 3C).

Fluorescence imaging is also a non-invasive technique for detecting tumors with improved accuracy, which provides real-time information about tumor margins and extent of tumor spread. Various peptide dendrimers-based near infrared fluorescent probes have been developed for high-penetration in vivo imaging. Ma et al. focused on synthesizing RGD-TAT-PEG-PAMAM/MTX (RTPPM) complex that had a round morphology, in which a dual function RGD-TAT peptide was used as the targeting molecule, PEG as the linker, and methotrexate (MTX) as the model drug [62]. Labeled with Cy7 as the fluorescent probe, a gradually increasing RGD-TAT-PEG-PAMAM-Cy7 (RTPPC) fluorescence signal was observed at tumor sites via in vivo imaging. Xu group employed Pep-1 as...
an anchor to decorate PEGylated PAMAM to enhance delivery across the Blood Brain Tumor Barrier (BBTB) and home to glioma [63]. The assembled Pep-PEG-PAMAM particles had a small size and good penetration into the tumor. The in vivo real-time fluorescence imaging with Cy5.5 in U87MG tumor-bearing mice confirmed that the fluorescence intensity at glioma site of the targeted group was 2.02 folds higher than that of the untargeted group. In addition, a single agent-based dendritic theranostic nanoplatform iRGD-cypate-PAMAM-DTX (RCPD) was prepared, in which the fluorescent targeting ligand cypate and a tumor penetration peptide iRGD (CRGDKGPDC) was covalently combined with PAMAM dendrimer [64]. The nanoplatform could be used for NIR imaging due to its maximum emission wavelength at 820 nm. The assembled Pep-PEG-PAMAM particles had a small size and good penetration into glioma site, exhibiting a much higher fluorescence signal and was maintained up to 24 h compared with non-targeting cypate-PAMAM particles.

3.2. Drug delivery

Self-assembled peptide dendrimers forming nano-carriers have an increasing trend in the design of drug delivery systems. Drugs can be bound to peptide dendrimers, producing prodrugs or targeted drugs, also can be encapsulated in these nanostructures via weak interactions [65]. The formed nano-carriers can accumulate and deliver anticancer drugs at tumor sites through enhanced permeability and retention effects.

Stimuli responsive vehicles have been one of the major research topics relating to the tumor microenvironment. She et al. designed the dendronized heparin-PAMAM-DTX (RCPD) was prepared, in which the fluorescent targeting ligand cypate and a tumor penetration peptide iRGD (CRGDKGPDC) was covalently combined with PAMAM dendrimer [64]. The nanoplatform could be used for NIR imaging due to its maximum emission wavelength at 820 nm. The assembled Pep-PEG-PAMAM particles had a small size and good penetration into glioma site, exhibiting a much higher fluorescence signal and was maintained up to 24 h compared with non-targeting cypate-PAMAM particles.

Fig. 3. Self-assembling 64Cu-labeled PAMAM-DOX-NODA nanocarriers for bioimaging. A) Schematic illustration of the multifunctional PAMAM-DOX-NODA nano-carriers for tumor-targeted drug delivery and PET imaging. B) Serum stability of 64Cu-PAMAM-DOX-F3 and 64Cu-PAMAM-DOX. C) Coronal PET images of MDA-MB-231 tumor-bearing mice at different time points post injection of 64Cu-PAMAM-DOX-F3 and 64Cu-PAMAM-DOX. Copyright 2018, Elsevier.

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3.3. Gene delivery

Gene therapy requires transport of adequate nucleic acids into the site of cells in an efficient way [67]. Cationic dendrimers, like positively-charged amino acid functionalized peptide dendrimers, bind nucleic acids such as DNA and siRNA via ionic interactions, forming over time under pH control.

The nano-carrier assembled from amphiphilic mPEGylated dendron-GFLG-DOX conjugate has been explored as enzyme-responsive anti-cancer agents. Gu group demonstrated that the nanoparticles showed better results in tumor cells than free DOX, producing lower toxicity and side effects (Fig. 4A and B) [50]. Moreover, the results pointed out a significantly increased tumor growth inhibition (TGI) of 80.3% (the free DOX treatment presenting TGI of 57.3%), and induced apoptosis on breast tumor model (Fig. 4C). Collectively, these results provide a promising perspective for drug delivery based on the self-assembling amphiphilic peptide dendrimer. Another enzyme-sensitive peptide dendrimer carrying gemcitabine has been considered as a targeted drug delivery system in breast cancer therapy [52]. The self-assembled nanoparticles from functionalized dendrimer-gemcitabine improved cancer targeting and reduced toxicity relative to the free gemcitabine, based on the 4T1 breast tumor model.

Self-assembled micelle particles formed from lipid peptide dendrimers are good candidates for the delivery of low solubility therapeutic agents. Parekh et al. designed an arginine-terminated lipid dendrimers for encapsulation of the negatively charged bufalin to improve its solubility [57]. The self-assembled bufalin-containing micelles showed a more than threefold increase in the aqueous solubility (142.9 μg mL⁻¹) of bufalin, when compared with a saturated bufalin aqueous solution (42.4 μg mL⁻¹). Nano carries with dual-drugs might synergically improve their therapeutic activities. Zhao et al. designed a series of naproxen conjugated amphiphilic dendrimers bearing an oligo-aspartic acid tail [40]. The dendrimers could rapidly transfer into micelles via self-assembly and efficiently encapsulate curcumin with a satisfied loading amount. These Cur loaded micelles displayed apoptosis inducing capacity inhibition on the MG-63 osteosarcoma cells.
positively charged polyplexes with superior transfection efficiency [68]. For example, a flexible strategy was suggested that spherical poly (L-lysine) dendrimers and linear poly (L-leucine) were synergistically carried out hierarchical self-assembly to form a nanostructure mimicking the viral capsid. The nanostructure showed high gene-transfection efficiency as non-viral gene vectors [69]. Jiang et al. investigated the self-assembly of three dendritic peptide modified cationic lipids, and found the arginine rich assemblies displayed the best DNA binding ability. In detail, compared to commercial PEI25k and Lipofectamine 2000, the in vitro and in vivo gene transfection of arginine-increased ~190-fold and ~7 fold, respectively [55]. Also, Liang and colleagues designed arginine-rich dendritic molecules with different generations to screen out the optimal structures with the highest DNA transfection efficiency [56]. They found that the second-generation dendrimer assemblies presented the best performance, which was 58.7% greater than that of liposome 2000 and PEI for HeLa cells. According to the simulation results, they were tightly integrated and of better flexibility, which were conducive to interaction with cell membranes. In addition, the attachment of fluorophore to arginine-rich amphiphilic dendritic lipopeptide structure could form near-infrared fluorescence theranostic carrier for image-guided gene delivery. Nie group designed amphiphilic molecules consist of three segments: an arginine rich hydrophilic moiety, a derivative of cyanine dye and a disulfide bond [70]. The complex self-assembled into lipid-based nanoparticles with positive charged surface to condense DNA. The nano-carrier displays glutathione responded gene release, activatable fluorescence recovery and up to 7-fold higher in vitro transfection than Lipofectamine 2000.

RNA interference (RNAi) holds great promise for therapeutic applications. Peng and co-workers reported an adaptive amphiphilic dendrimer-based nano-assembly acting as versatile vector for effective siRNA delivery and gene silencing, and demonstrated its efficacy for primary and stem cells as well as in vivo [28]. To further enhance the specific targeting, the same group designed a more stable self-assembling amphiphilic dendrimer vector with dual targeting peptide E16,RGDK (Fig. 5), in which RGDK as the targeting warhead, oligo(glutamic acid) E16 as the negatively charged sequence to interact with positively charged siRNA/AD complexes, and the neutral oligo(glycine) G6 as the linker [71]. The designed dendrimer co-assembled with siRNA to form nano-carriers with a 10-fold increased delivery efficiency in vivo compared to the conventional covalent dendrimers. Unlike regular lipid-like amphiphiles, Guan et al. developed dendritic peptide bola-amphiphiles as the cargo to co-assembled with siRNA. The bola-amphiphiles derived nanoparticles disfavor insertion into the cell membrane and offer a more biocompatible alternative to conventional lipids. Both bola-amphiphiles with hydrocarbon (C18) and fluorocarbon cores (F10) exhibited 20–40 times higher uptake than lipofectamine. The optimal vector contained a fluorocarbon core and exhibited enhanced delivery efficiency to a variety of cell lines and improved serum resistance when compared to hydrocarbon analogues and lipofectamine RNAiMAX [72].

3.4. Vaccine delivery

Self-assemble peptide dendrimers are not only used as imaging agents and delivery carriers but may also be fabricated to generate vaccines [73]. In order to mimic the danger signals associated with natural infection and stimulate an adaptive immune response, peptide antigens must be co-delivered with immune adjuvants. When chosen the dendritic
scaffold with adjuvant properties, the nano-carriers can form self-adjuvanting vaccine delivery system when mixed with inactivated antigens. For example, the Toth group has employed a polycrylic core posing adjuvant properties and high affinity for self-assembly as dendritic core to construct the B-cell epitope delivery system [39]. The self-assembled nanoparticles worked as a self-adjuvanting vaccine, allowed maximum possible exposure to the immune system and produced a strong immune response to the group A streptococcus (GAS) M-protein. By selecting the peptide antigen, this platform could further achieve self-adjuvanting vaccines that could induce cellular immune responses. Liu et al. established a synthetic pathway to conjugate a human papillomavirus (HPV) E7 protein-derived peptide antigen to a...
star-polyacrylate polymer to create a macromolecular vaccine candidate to treat HPV-related cancers [74]. These conjugates were able to reduce tumor growth and could eradicate E7-expressing TC-1 tumors in mice after a single immunization, without the help of any external adjuvant. However, these nanocarriers have limited commercial applications due to their inability to biodegrade.

To overcome this drawback, Skwarczynski et al. constructed a biodegradable polymer from natural hydrophobic amino acids (HAAs) [75]. The peptide dendrimers 1 to 5 were self-assembled into nanoparticles driving by the amphiphilic structure, which consisted of the B cell epitope J8 (QAEVKVQSRKQVEKLQELDKVQ), the T helper epitope (PADRE, AKFVAAWTLKAA), and 10 or 15 copies of HAA (Fig. 6A). By analyzing the expression of major histocompatibility complex II (MHC-II) and the CD40 costimulatory molecule in vitro, it is evidenced that those nanoparticles could induce the maturation of antigen-presenting cells (APCs) (Fig. 6B and C). Furthermore, all compounds elicited the high titers of antibodies without using any external adjuvant through subcutaneous immunization in mice. Compound with 15 leucine residues showed superior properties among all examined pHAA based nanoparticles.

The peptide dendrimer can be mixed with other compounds with immunogenicity to produce a more potent self-adjuvanting nanovaccine delivery. Toth et al. reported self-assembly of trimethyl chitosan (TMC) and poly (anionic amino acid)-peptide antigen comprising J8 and T helper P25 epitopes to produce a potent self-adjuvanting vaccine delivery system in outbred mice [76]. Interestingly, nanoparticles made from a peptide with 10 residues of polyglutamic acid and fungal-derived TMC could induce higher systemic antibody titers than those of peptides formulated with a strong mucosal adjuvant cholera toxin subunit B (CTB). Hence, peptide dendrimer design can serve as a promising tool to create safe, biocompatible and immunologically efficient adjuvants in the field of vaccine development.

4. Summary and outlook

Peptide dendrimers in the self-assembled field have aroused an upsurge of interest. This minireview has described the self-assembly behaviors of different peptide dendrimers and their selected bio-applications. The detailed information of self-assembled nanostructures was given from aspects of size, morphology, driving force and regulatory factors. Peptide dendrimers could be assembled into nano-aggregates with desired diameters ranging from 10 nm to 200 nm in water or organic media. There are very few large aggregates with a size of up to 400 nm. Self-assembly of peptide dendrimers have relied heavily on various driving forces (e.g., hydrogen bonding, hydrophobic or electrostatic interactions), and the obtained nanoparticles are primarily spherical, while a few are rod-shaped structures. Besides, the optimization of assembled architectures can be adjusted by regulatory factors like temperature, pH and medium in selective solutions. Through self-assembly, the discrete peptide dendrimers can be cleverly designed into “greater than total” aggregates that bring novel characteristics better than the free state. Nevertheless, the self-assembly of total surface-modified peptides on higher-generation dendrimers remains a challenge due to steric effects. More theoretical investigation on the self-assembly and function- alization of dendrimers should be performed to profoundly understand all the details inside.

Pleasing physicochemical features of nanoparticles make them have great potential, especially for biomedical applications such as diagnosis, cancer therapy, gene therapy and immune therapy. The self-assembly approach to peptide dendrimer synthesis can also be available to other dendrimers in general. Nonetheless, there remain some unsettled problems of self-assembled nanoparticles in biomedicine. Firstly, flexible design of both the dendron core and building blocks is highly demanded to realize a facile synthesis of self-assembled peptide dendrimers for many clinical applications. Secondly, studies have shown that dendrimers have some immunogenicity and unavoidably produce immune responses. Modifying polyethylene glycol (PEG) chains on the surface of dendrimers is an excellent solution to reduce immunogenicity and thus have a longer lifetime in the blood. Thirdly, their biocompatibility and biodegradability are points to be improved in future research to reduce the toxicity towards normal cells.

To sum up, as a creative polymer material with a unique structure, self-assembled peptide dendrimers are now beginning to be explored more methodically, particularly in the field of theranostics. A better understanding of biophysical properties and improved quality control analyses would further promote their diagnostic and therapeutic applications in the recent future.

Credit author statement

Fengjuan Xie: Conceptualization, Writing – original draft. Rongxin Li: Writing – review & editing. Weikang Shu: Writing – review & editing. Liang Zhao: Supervision, Writing – review & editing. Jingying Wan: Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We gratefully acknowledge the financial support from Project 22074044, 22122404 by National Natural Science Foundation of China (NSFC) and Project KP2105 by State Key Laboratory of Oncogenes and Related Genes.

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