Biomimetic Nanoarchitectures of Peptide Dendrimers for Drug/Gene Delivery

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ABSTRACT SUMMARY

Novel peptide dendrimers with biomimetic nanoarchitectures were developed for drug and gene delivery. The anti-cancer effects of the nanomaterials were studied in details.

INTRODUCTION

Peptide dendrimers have attracted much interest to biomaterials scientists and pharmacist for their excellent biocompatibility and functionality. They are promising carrier candidates via self-assembly to fabricate biomimetic nanoarchitectures for drug and gene delivery.

In the past decade, self-assembly inspired from nature has been evolved as an effective and practical strategy for nanoarchitecture fabrication. Tremendous advances were achieved in the self-assembly polymeric nanoparticles, however, the regulation of dispersive dendrimers into ordered nanoarchitectures as bionanomaterials remains challenges. Peptide dendrimers possessed not only the general characteristics of typical dendrimers, but also certain unique globular protein like properties. Initiating hierarchical self-assembly of peptide dendrimers and polypeptide provides an approach to fabricate supramolecular structures as virosomes in cellular environment.

Herein, we reported a novel approach to regulate the cooperative self-assembly of peptide dendrimers and linear polypeptides into hierarchical nanostructure. The end-functionalized linear polypeptides were utilized to self-assemble with peptide dendrimers primarily to form amphiphiles driven by electronstatic interaction and/or hydrogen binding. The amphiphiles secondarily self-assembled virus like nanoarchitectures with controllable morphologies and biological functions. The self-assembly and disassembly of the peptide-based nanoarchitectures were investigated. The virus-mimicking architectures were used as cargos for anti-cancer drug and gene delivery. The in vitro tests demonstrate that the biomimetic nanovehicles are not only responsive to the pH value in the tumor microenvironment but also achieve intelligent drug release as well as high gene transfection.

EXPERIMENTAL METHODS

The synthesis of POSS-cored poly(L-lysine) dendrimer and carboxyl-terminated poly(L-leucine) was according to the approaches previously reported. The self-assembly of the peptide dendrimers and polypeptides were carried out in DMF. Peptide dendrimers and polypeptides were weighed and dissolved in DMF, respectively, the polypeptides solution was mixed with peptide dendrimers solution in the condition of ultrasound. Distilled water was added dropwise into the mixture. The solution was freeze dried to receive the self-assembly nanobiomaterials. The peptide dendrimers, polypeptides and doxorubicin were dissolved in DMF. Deionized water was added into the solution under ultrasound to prepare the DOX-loaded biomimetic nanoparticles (BNs). The DOX-loaded BNs were dialyzed in PBS buffer solution (pH=7.4) to remove the free drug and organic solvent at 4 °C for 24 h, the DOX-loaded-BNs were obtained after freeze-dry.

HepG2 cells were seeded in three 96-well plates (with the cell density of 1×10^5 cells) and incubated for 48 h, the medium was removed and free medium was added with the DOX-loaded BNs. The final concentration of drug in each well was 10 μg/mL. After incubated for 48 h, 10 μL of CCK-8 kit was added for cell viability measurement.

The BNs were condensed with pEGFP-C1 to evaluate the gene transfection in HEK293 cells. The complexes were prepared with N/P ratio ranging of 35, 45 and 55. HEK 293 cells were seeded in 24-well plates at an initial density of 8×10^4 cells per well and incubated for 24h, then the complexes were added into each well and incubated at 37°C. The cells were analyzed for green fluorescence protein expression with a fluorescence microscope and Confocal Laser Scanning Microscope.

RESULTS AND DISCUSSION

Figure 1. The pH-dependent assembly and disassembly of the BNs, a: Buffer capacity of BNs (1.0 mg/mL) at the pH range from 3.0 to 12.0; b: AFM and TEM images for the structures of BNs at the representative pH, the scale bars represented 100 nm.

The biomimetic nanoarchitectures with 1:1 weight ratio of poly(L-lysine) dendrimers and poly(L-leucine)
were used for investigation. To determine the pH-dependent nature of the BNs in wide range, the titration analysis was carried out to perform the isoelectric points (pI) and buffer capacity (Figure 1). The titration curve could be divided into four stages to disclose how the non-covalent dendritic-polypeptides self-assembled and disassembled at different pH conditions. The non-covalent amphiphiles tended to form different nanoarchitectures with the pH value variation, which led to pH-responsive assembly and disassembly behaviours.

Figure 2. The cytotoxicity of DOX-loaded BNs (red), DOX·HCl (blue), DOX (green) and BNs (grey) to HepG2 cells (a). The flow cytometric profiles of HepG2 cells with 3 h incubation (b), DOX-loaded BNs (red), DOX·HCl (blue), DOX (green), BNs (grey) and control group (black). CLSM images of HepG2 cells treated with DOX-loaded BNs (c1), DOX·HCl (c2) and DOX (c3) with 3 h incubation, the scale bars represented 25 μm.

In Figure 2a, the cytotoxicity of DOX-loaded-BNs was comparable to that of DOX·HCl. As shown in the flow cytometric profiles (Figure 2b), the fluorescence intensity of the DOX-loaded BNs group not only surpassed the fluorescence intensity of hydrophobic DOX group but also exceeded that of DOX·HCl group after 3 hour incubation. It revealed that the BNs could enhance the endocytosis of DOX, which could be attributed to their biomimetic nanoarchitectures. The CLSM images (Figure 2c) further illustrated that the BNs facilitated the delivery of DOX to cellular nuclei.

The gene transfection of GFP expression was presented in Figure 3. Strong green emission of GFP expression and flow cytometry analysis of the peptidomes exhibited that the transfection efficacy in blood serum was comparable to that of PEI 25 kDa in serum-free condition. The biocompatibility of the biomimetic nanoparticles and BNs/DNA complex was excellent as shown in Figure 3d.

CONCLUSION

We have successfully demonstrated a versatile strategy for cooperative self-assembly of globular poly(L-lysine) dendrimers with linear poly(L-Leucine). After two steps self-assembly, the hierarchical nanoparticles exhibited capsid-like biomimetic nanoarchitecture. The pH-sensitivity mechanism of the BNs is disclosed and attributed to the contribution of the weak interactions within the amphiphiles. The pH-responsive BN was fabricated as an intelligent drug/gene delivery platform. The in vitro BNs-mediated DOX delivery demonstrates that the BNs exhibited excellent biocompatibility, nanoscale size, efficient endocytosis and delivery. The BNs also exhibited high gene transfection efficiency as non-viral gene vectors. This work is expected to construct an intelligent drug/gene delivery system via the cooperative self-assembly of polypeptides based biomaterials and provide a strategy for drug/gene delivery platform fabrication.

REFERENCES


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