Biomaterials 113 (2017) 243–252

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Precision combination therapy for triple negative breast cancer *via* biomimetic polydopamine polymer core-shell nanostructures

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ARTICLE INFO

Article history:
Received 3 August 2016
Received in revised form
29 October 2016
Accepted 30 October 2016
Available online 2 November 2016

Keywords:
Spatiotemporal-controlled drug release
Thermo-responsive
Photothermal therapy
Chemotherapy
Small interfering RNA

ABSTRACT

Photothermal-based combination therapy using functional nanomaterials shows great promise in eradication of aggressive tumors and improvement of drug sensitivity. The therapeutic efficacy and adverse effects of drug combinations depend on the precise control of timely tumor-localized drug release. Here a polymer-dopamine nanocomposite is designed for combination therapy, thermo-responsive drug release and prevention of uncontrolled drug leakage. The thermo-sensitive co-polymer poly (2-(2-methoxyethoxy) ethyl methacrylate-*co*-oligo (ethylene glycol) methacrylate)-*co*-2-(dimethylamino) ethyl methacrylate-*b*-poly (D, L-lactide-*co*-glycolide) is constructed into core-shell structured nanoparticles for co-encapsulation of two cytotoxic drugs and absorption of small interfering RNAs against survivin. The drug-loaded nanoparticles are surface-coated with polydopamine which confers the nanoformulation with photothermal activity and protects drugs from burst release. Under tumor-localized laser irradiation, polydopamine generates sufficient heat, resulting in nanoparticle collapse and instant drug release within the tumor. The combination strategy of photothermal, chemo-, and gene therapy leads to triple-negative breast cancer regression, with a decrease in the chemotherapeutic drug dosage to about 1/20 of conventional dose. This study establishes a powerful nanoplatform for precisely controlled combination therapy, with dramatic improvement of therapeutic efficacy and negligible side effects.

1. Introduction

Clinical practices have revealed that combination therapy is the future for treatment of deadly cancers with heterogeneous complexity in nature, due to the great potential in enhancing therapeutic efficacy, lowering drug dosage, and overcoming drug resistance [1–4]. Combination regimen is complicated since the administration time, dosage and sequence of different drugs should be coordinated to achieve desirable efficacy and tolerable side effects [5–9]. Co-delivery of several drugs using functional nanocarriers shows promises in improving the pharmacokinetics and therapeutic efficacy while reducing toxicity of drug combinations by specific targeting and controlled release of drugs in tumor tissues [7,10–16]. In recent years, strategies for combination of photothermal therapy and chemotherapy or gene therapy have been extensively investigated for suppression of solid tumors based on functional nanomaterials [3,17–21]. Previously we have designed a porphyrin self-assembled micelle for simultaneous photothermal and chemotherapy [22]. We have also integrated photothermal agents, cytotoxic drugs and nucleic acids in a thermo-sensitive nanoparticle that was constructed from the amphiphilic co-polymer poly (2-(2-methoxyethoxy) ethyl methacrylate-*co*-oligo (ethylene glycol) methacrylate)-*co*-2-(dimethylamino) ethyl methacrylate-*b*-poly (D, L-lactide-*co*-glycolide) (P(MEO$_2$MA-*co*-OEGMA-*co*-DMAEMA)-*b*-PLGA) [23]. These strategies effectively suppressed triple-negative breast cancer growth,

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http://dx.doi.org/10.1016/j.biomaterials.2016.10.053
0142-9612/

significantly lowered cytotoxic drug dosage, and avoided possible adverse effects. However, a critical concern of these nanomaterials is that about 30% of chemotherapeutic drugs were uncontrollably released within a short period. These runaway drugs have low bioavailability and may cause latent toxicity to normal tissues. Thereby, optimized nanoplatforms with spatiotemporal-controlled drug release property and high photothermal sensitivity need to be explored for precise combination therapy.

Dopamine, a melanin-like mimic of mussel adhesive proteins, can self-polymerize to form surface-adherent polydopamine (PDA) films onto a wide range of materials [24–28]. PDA has recently emerged as a promising photothermal therapeutic agent due to its unique characteristics such as strong near-infrared (NIR) light absorption, high photothermal conversion efficiency, excellent biocompatibility and biodegradability [29–32]. A Fe_3O_4@PDA nanocomposite has been reported as a theranostic agent for intracellular mRNA detection and multimodal imaging-guided photothermal therapy [33]. Polymerization of PDA onto gold nanorods has enabled surface functionalization of targeting biomolecules and elicited photothermal killing of cancer cells [32]. Recently, we have constructed a doxorubicin-loaded micelle with surface modification of PDA for combined photothermal-chemotherapy [34]. Since the surface modification of PDA based on oxidized dopamine polymerization is a simple, mild and efficient reaction, we wonder whether coating of the thermo-sensitive nanoparticle P(MEO_2MA-*co*-OEGMA-*co*-DMAEMA)-*b*-PLGA with compact PDA is capable of photothermal therapy under NIR irradiation, and most importantly, will refrain from initial burst release of encapsulated drugs.

In the present study, we utilized the amphiphilic co-polymer P(MEO_2MA-*co*-OEGMA-*co*-DMAEMA)-*b*-PLGA as the building block for construction of a core-shell structured nanoparticle. This co-polymer integrated the advantages of polyethylene glycol (PEG) and poly (*N*-isopropylacrylamide), exhibiting low toxicity, low immunogenicity, and thermo-sensitivity [23]. The lower critical solution temperature (LCST) of the co-polymer could be precisely adjusted to be slightly higher than body temperature by changing the content of the chain transfer agent hydroxyethanethiol and ratios of MEO_2MA to OEGMA during synthesis, and therefore, a small temperature increase by local heating can induce the nanoparticle collapse for effective drug release. Doxorubicin and paclitaxel were loaded into the hydrophilic core and hydrophobic layer of the nanoparticles (named NP-DT), respectively, and small interfering RNAs (siRNA) against survivin were absorbed onto the nanoparticle surface by electrostatic interaction (named NP-DTS). The drug-loaded nanoparticles were ultimately modified with PDA at the outer layer (named NP-DTS-PDA, Scheme 1). The spatiotemporal-controlled drug release profile and dramatically improved combination therapeutic effects of NP-DTS-PDA against triple negative breast cancer were investigated.

2. Materials and methods

2.1. Materials

Materials for synthesis of co-polymer, including 2-(2-methoxyethoxy) ethyl methacrylate, 2-(dimethylamino) ethyl methacrylate, oligo (ethylene glycol) methacrylate, 2-hydroxyethanethiol, tin(II) 2-ethylhexanoate, D, L-lactide, glycolide, pyrene, and benzoyl peroxide, were from Sigma-Aldrich (St. Louis, MO, USA). Dopamine hydrochloride was purchased from J&K (Beijing, China). Survivin siRNA was obtained from RiboBio (Guangzhou, China). Doxorubicin and paclitaxel were purchased from Huafeng United Technology (Beijing, China) and Norzer Pharmaceutical (Beijing, China), respectively. Cell culture agents, including Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS), were from WISENT (Canada). Cell counting kit-8 was from Dojindo Laboratories (Kumamoto, Japan). Matrigel matrix was obtained from Corning (Corning, NY, USA). Paraformaldehyde was from Solarbio (Beijing, China). A continuous-wave diode laser at the wavelength of 808 nm (Daheng Science & Technology, China) was used for the laser irradiation experiments.

2.2. Synthesis of P(MEO_2MA-co-OEGMA-co-DMAEMA)-b-PLGA

According to the previous study [23], the co-polymer precursor, hydroxyl-terminated poly (2-(2-methoxyethoxy) ethyl methacrylate-*co*-oligo (ethylene glycol) methacrylate-*co*-2-(dimethylamino) ethyl methacrylate), was fabricated through the radical co-polymerization in which 2-hydroxyethanethiol and benzoyl peroxide served as chain transfer agent and initiator, respectively. Materials including 2-(2-methoxyethoxy) ethyl methacrylate, oligo (ethylene glycol) methacrylate, 2-(dimethylamino) ethyl methacrylate, 2-hydroxyethanethiol and benzoyl peroxide were dissolved in 30 mL of tetrahydrofuran, followed by reflux under nitrogen at 70 °C for 7 h. Then diethyl ether was used to precipitate the co-polymer precursor which was purified using a slow liquid–liquid diffusion method. P (MEO_2MA-*co*-OEGMA-*co*-DMAEMA)-*b*-PLGA was ultimately synthesized by the ring-opening esterification polymerization of the co-polymer precursor with glycolide and D, L-lactide in the presence of $Sn(Oct)_2$ in toluene, followed by reflux under nitrogen at 120 °C for 24 h.

2.3. Construction of NP-DTS

Based on previous studies [35], the co-polymer P (MEO_2MA-*co*-OEGMA-*co*-DMAEMA)-*b*-PLGA (20 mg), dissolving in 1 mL of methylene chloride, was emulsified with 200 μL of doxorubicin solution under ultrasonication for 5 min, followed by addition of polyvinyl alcohol (2%, 2 mL). The mixture was dropped into 200 μL of paclitaxel using methylene chloride as solvent. After stirring for 3 min, the mixture underwent the second emulsification for 5 min. The solution was further incubated with 10 mL of polyvinyl alcohol (0.6%) for 10 min. Following evaporation, the solution was centrifuged at 12,000 g for 10 min to precipitate NP-DT. For the preparation of NP-DTS, NP-DT was dissolved in nuclease-free water, slowly added to siRNA solution, and incubated for 20 min at room temperature.

2.4. Construction of NP-DTS-PDA

Coating of PDA was achieved by incubation of NP-DTS with dopamine hydrochloride dissolved in Tris buffer (10 mM, pH 8.5) and rotation for 6 h at room temperature. Dopamine concentration was fixed at 0.4 mg mL^{-1} and the concentration of nanoparticles changed for different co-polymer/dopamine mass ratio including 4/1, 2/1, 1/1, 1/1.5, 1/2 and 1/4.

2.5. Characterization of the nanoparticles

All nanoparticles were examined by the dynamic light scattering (Zetasizer Nano ZS90, Malvern Instruments, UK) for detection of particle diameter, zeta potential and polydispersity index. Nanoparticles, negatively stained with uranyl acetate (1%), were evaluated by transmission electron microscopy (TEM, EM-200CX, JEOL, Tokyo, Japan) for morphology analysis. The loading content and encapsulation efficiency of drugs were calculated as follows: loading content = (weight of loaded drugs)/(total weight of nanoparticle and drugs) × 100%; encapsulation efficiency = (weight of loaded drugs)/(weight of initially added drugs) × 100%. The weight