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Reduction-responsive multifunctional hyperbranched polyaminoglycosides with excellent antibacterial activity, biocompatibility and gene transfection capability

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ABSTRACT

There is an increasing demand in developing of multifunctional materials with good antibacterial activity, biocompatibility and drug/gene delivery capability for next-generation biomedical applications. To achieve this purpose, in this work series of hydroxyl-rich hyperbranched polyaminoglycosides of gentamicin, tobramycin, and neomycin (HP and SS-HP with redox-responsive disulfide bonds) were readily synthesized via ring-opening reactions in a one-pot manner. Both HP and SS-HP exhibit high antibacterial activity toward *Escherichia coli* and *Staphylococcus aureus*. Meanwhile, the hemolysis assay of the above materials shows good biocompatibility. Moreover, SS-HPs show excellent gene transfection efficiency *in vitro* due to the breakdown of reduction-responsive disulfide bonds. For an *in vivo* antitumor assay, the SS-HP/p53 complexes exhibit potent inhibition capability to the growth of tumors. This study provides a promising approach for the design of next-generation multifunctional biomedical materials.

1. Introduction

Chemotherapy and radiotherapy in standard cancer treatment cause severe damage in patients' immune systems, which may usually result in secondary bacterial infection. As a result, there is a rising demand in developing of multifunctional materials with high antibacterial activity, biocompatibility and drug/gene delivery capability for next-generation biomedical applications [1–7]. Aminoglycosides are active antibacterial therapeutic agents that inhibit the protein synthesis in the cytoplasmic matrix of Gram-negative bacteria (e.g. *Escherichia coli*) and some Gram-positive bacteria (e.g. *Staphylococcus aureus*) [8]. Recently, aminoglycosides and their derivatives have been exploited for developing small interfering RNA (siRNA) vectors due to their high binding affinity to RNAs [9]. Although the complexes of lipid-modified aminoglycosides and siRNA exhibit efficient gene knockdown capability, the native antibacterial activity of aminoglycosides is abolished due to the lipid modification. To overcome this limitation, the aminoglycosides and *N,N′*-methylenebisacrylamide are conjugated to form hyperbranched polymers (HPs) via Michael-addition polymerization [10,11]. Such aminoglycosides-based HPs still exhibit high antibacterial activity and good gene delivery capability due to their highly branched architectures. Nevertheless, their *in vitro* and *in vivo* biodegradation is still challenging, which increases the cytotoxicity and hinders the application of such materials for tumor therapeutics [12].

Polymers containing disulfide bonds provide an effective approach for designing biodegradable polymeric delivery systems [13,14]. For example, polyethylene glycol (PEG) segments were attached to poly(aspartamide) segments via disulfide linkages to resist accumulation in serum and to reduce cytotoxicity. To achieve

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higher gene transfection efficiency, the PEG segments were detach due to the cleavage of disulfide bonds in the intracellular reducing environment containing abundant glutathione (GSH) [13]. The reduction-responsive disulfide bonds are also incorporated in HPs to promote the biodegradation in presence of reducing agents [15–21]. For example, HPs containing disulfide bonds can be engineered as a vector for gene and drug delivery. Upon cleavage of the disulfide bonds, the HPs spontaneously degrade to release drugs in tumor cells, where the presence of GSH is several times higher than that in normal cells. Despite the fast redox-response, their biocompatibility is still a concern because HPs may cause hemolysis of red blood cells (RBCs). It was reported that hydroxyl-rich polymers can significantly reduce cytotoxicity and increase biocompatibility [22–25].

In this paper, series of multifunctional aminoglycosides-based HPs with excellent antibacterial activity, biocompatibility and gene transfection capability are synthesized via the one-pot reaction (Fig. 1). Two sets of aminoglycosides-based HPs, the reduction-responsive HPs (containing disulfide bonds, SS-HP) and non-redox-responsive HPs, are prepared via similar ring-opening reactions. Their antibacterial activity, RBC hemolysis, biodegradation ability and gene transfection efficiency are studied *in vitro*. To explore the *in vivo* anti-tumor activity, p53 gene is loaded to the multifunctional vectors to inhibit tumor growth. This novel approach may shed light on the design of next-generation multifunctional biomedical materials.

2. Materials and methods

2.1. Materials

Gentamicin sulfate (98%), tobramycin (98%), neomycin sulfate (98%), 2-hydroxyethyldisulfide (>98%), glycidyl methacrylate (GMA) (98%), tetrabutylammonium bromide (98%), ethylene glycol diglycidyl ether (EGDE, 98%), ethylenediamine (ED, 98%), DL-1,4-dithiothreitol (DTT, 98%), triethylamine (TEA, 98%), branched polyethylenimine (PEI, $M_w = 25$ kDa), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich. The HEK293 and C6 cell lines were purchased from American Type Culture Collection (ATCC). The plasmid DNA (pDNA, pRL-CMV and pEGFP-N1) were prepared as described in our earlier work [26].

2.2. Synthesis of hydroxyethyl disulfide diglycidyl ether (HDDE)

2-Hydroxyethyldisulfide (5 mmol, 0.59 mL), sodium hydroxide (15 mmol, 600 mg) and tetrabutylammonium bromide (0.05 mmol, 16 mg) were slowly added into a three-necked flask. The reaction mixture was stirred at 40 °C. Epichlorohydrin (15 mmol, 1.18 mL) was added dropwisely into the above mixture using a dropping funnel. After reacting for 3 h with constant stirring, the crude product of HDDE was purified by using column chromatography (ethyl acetate/n-hexane = 4/1).

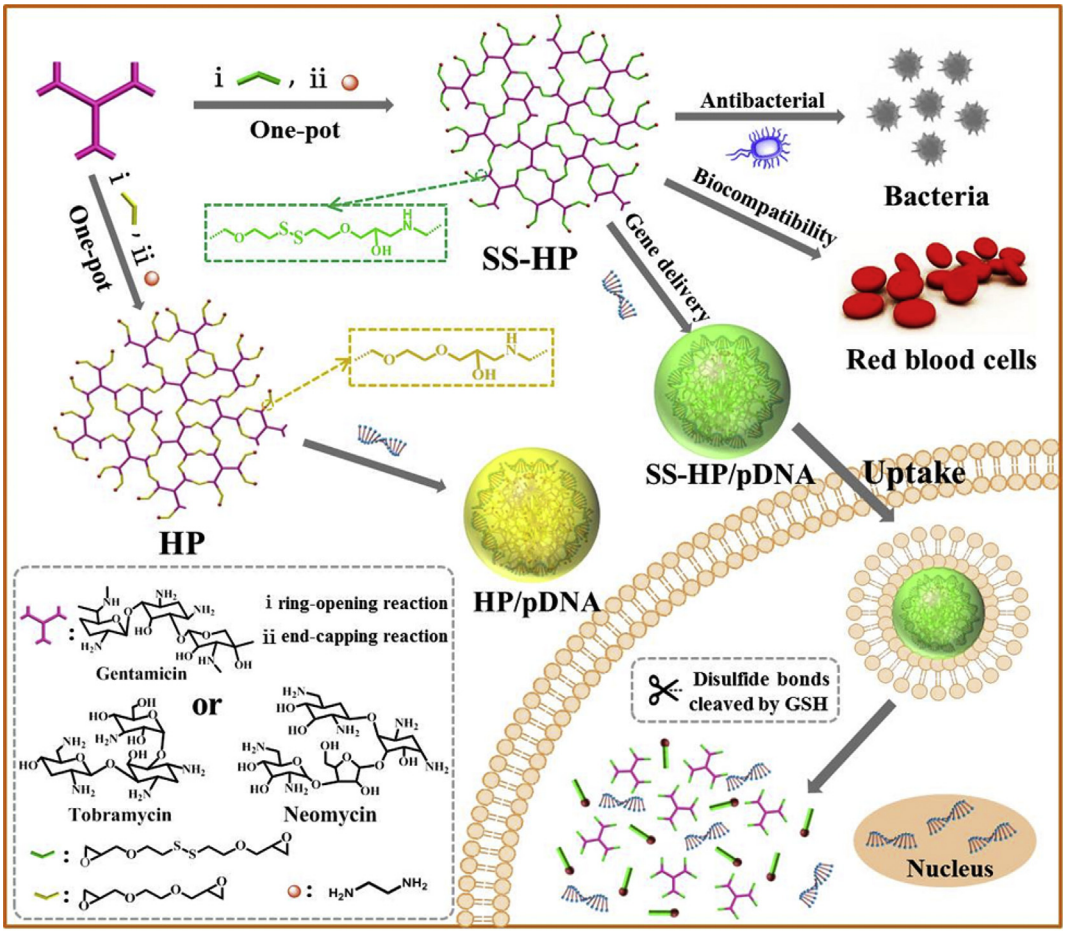

Fig. 1. Schematic illustration for multifunctional aminoglycosides-based hyperbranched polymers with antibacterial activity, biocompatibility, and gene transfection capability.