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A smart aminoglycoside hydrogel with tunable gel degradation, on-demand drug release, and high antibacterial activity

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ABSTRACT

Aminoglycosides are a family of critically important antibiotics for the treatment of serious infections including multidrug-resistant pathogens. However, clinical use of aminoglycoside antibiotics is compromised by bacterial biofilm formation at subinhibitory concentrations or adverse side effects such as ototoxicity and nephrotoxicity at high antibiotic doses. Preparation of aminoglycoside formulation that allows on-demand drug delivery is a solution to this sticky issue. Here, we designed a new type of aminoglycoside hydrogels by cross-linking oxidized polysaccharides such as dextran, carboxymethyl cellulose, alginate, and chondroitin using aminoglycosides as cross-linkers. The hydrogel modulus, degradation rate and release kinetics can be precisely modulated by tailoring the aminoglycoside dose during gel formation. The aminoglycoside hydrogel showed fast gelation, self-healing and on-demand drug release behaviors, and high antibacterial activities *in vitro* and *in vivo* against both Gram-positive and Gram-negative bacteria. This study provides a facile and promising strategy to develop smart hydrogels for topical administration of aminoglycoside antibiotics.

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1. Introduction

Bacterial infection is one of the most challenging tasks in biomedical fields and still remains a serious threat to human lives today [1–3]. Aminoglycoside antibiotics are a family of potent and broad-spectrum antibacterial agents with limited drug allergic reactions. They are listed by the World Health Organization as critically important antimicrobials for the treatment of bacterial infections, including tuberculosis, endocardial and meningeal infections and the plague [4,5]. Despite their clinical effectiveness, aminoglycoside antibiotics are usually compromised by drug resistance or adverse effects. It is reported that bacteria respond specifically and defensively to insufficient concentrations of antibiotics [6,7], *e.g.* aminoglycoside antibiotics at subinhibitory concentrations induce bacterial biofilm formation in Pseudomonas aeruginosa (*P. aeruginosa*) and *Escherichia coli* (*E. coli*). The biofilm formation resists antibacterial treatment and leads to bacterial persistence in chronic infections [8]. Therefore, high dose of aminoglycosides is required to avoid bacterial biofilm formation. On the other hand, excess aminoglycoside antibiotics will cause adverse effects. Aminoglycosides kill bacteria by inhibiting bacterial protein synthesis *via* targeting ribosome [5,9,10]. Since ribosomes are present in all kinds of mammalian cells and have relatively conserved structures, aminoglycosides have limited selectivity to the ribosomes and thus cause adverse effects. High dose of aminoglycosides may cause irreversible hearing loss due to the destruction of inner ear sensory hair cells [4]. In addition, aminoglycoside antibiotics were reported with severe nephrotoxicity [11]. The situation that we face seems like choosing between Scylla and Charybdis: either biofilm formation at low aminoglycoside doses, or adverse effects such as ototoxicity and nephrotoxicity at high doses. Though the discovery of new antibiotics may alleviate this sticky problem, the efforts have met with limited success since the early 1960s [12].

It was our desire to develop an on-demand delivery system in which we could precisely control the release rate of aminoglycoside antibiotics. Hydrogels encapsulated with antibacterial agents are promising formulations for topical administration of antibiotics [13–15]. The gels have exhibited remarkable benefits such as local delivery, tissue bioadhesive property, days or even weeks of continuous drug release which improve bioavailability and minimize adverse side effects of drugs [16–21]. However, general antibiotic hydrogels only allow drug release based on passive diffusion or hydrogel degradation, which cannot satisfy the requirements for on-demand drug delivery with tunable release kinetics. Here, we reported a new type of smart aminoglycoside hydrogels which allow on-demand drug delivery and achieve different gel degradation rates by changing the dose of aminoglycosides.

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2. Materials and methods

2.1. Materials

The aminoglycosides including netilmicin, isepamicin, capreomycin, ribostamycin, apramycin, amikacin, paromomycin, tobramycin, and neomycin were purchased from Meilun Biotech. (Dalian, China). Dextran (45–60 kDa), chondroitin (~50 kDa), branched polyethylenimine (PEI) with a molecular weight of 25 kDa, and rhodamine B were purchased from Sigma-Aldrich (St. Louis, USA). Carboxymethyl cellulose (~100 kDa) was obtained from TCI (Shanghai, China). Sodium alginate (~250 kDa), sodium periodate, ethylene glycol, hydroxylamine hydrochloride, phenol red, methyl orange, methylene blue, 3-mercaptoacetic acid, *o*-phthalaldehyde, and boric acid were obtained from Aladdin (Shanghai, China). Tryptone, yeast extract, tryptic soy broth, and agar were purchased from Thermo Fisher Scientific. *E. coli* (DH5a), *Staphylococcus epidermidis* (*S. epidermidis*, 1457), *Staphylococcus aureus* (*S. aureus*, USA300), and *P. aeruginosa* (PAO1) were obtained from ATCC.

2.2. Synthesis and characterization of polysaccharide aldehyde

The oxidized dextran was termed dextran aldehyde (Dex-CHO), which was synthesized by adding 8 mL sodium periodate (0.5 M) solution into a dextran solution (1 g, 10% w/v, the molar ratio of sodium periodate to dextran is 0.65) in a dropwise manner, and the solution was stirred in the dark for 4 h at room temperature. The reaction was stopped by the addition of ethylene glycol (10% v/v). After that, the solution was intensively dialyzed against deionized water, and lyophilized to obtain white powders, which were kept under nitrogen atmosphere before use. Chondroitin aldehyde (Cho-CHO) was synthesized by the same procedure. For carboxymethyl cellulose aldehyde (Cmc-CHO) and alginate aldehyde (Alg-CHO), different amounts of sodium periodate were used to oxidize the polysaccharides. 8 mL or 16 mL sodium periodate (0.5 M) was added into the carboxymethyl cellulose or alginate solution (1 g, 5% w/v). The oxidization degrees of the polysaccharides were determined by a colorimetric hydroxylamine titration analysis [13]. The oxidation degrees for Dex-CHO and Cho-CHO are measured to be 48% and 45%, respectively. The degree for Cmc-CHO is 35%, and the value for Alg-CHO is 58%, respectively. The molecular weights of the oxidized polysaccharides were measured by gel permeation chromatography (GPC, Waters Breeze 2, USA).

2.3. Preparation of aminoglycoside hydrogels and rheology characterization

Aminoglycoside antibiotic hydrogels were prepared by simply mixing the polysaccharide aldehyde solution with aminoglycosides at different aminoglycoside contents (0.21–2.8 wt%). Hydrogels with different shapes were prepared by using poly(tetrafluoroethylene) molds. The rheology properties of the hydrogels at different drug contents were evaluated by a rheometer at 37 °C (TA Instrument, USA). A time-dependent rheology measurement was conducted at 1% strain and 1 Hz.

2.4. Cell culture and cytotoxicity evaluation

NIH 3T3 cells (a mouse embryonic fibroblast cell line, ATCC) were plated in a 96-well plate at a density around 10^4 cells per well, and cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin sulfate and 100 units/mL streptomycin, and incubated at 37 °C under a humidified 5% CO_2. The prepared amikacin/Dex-CHO hydrogels (0.71–2.14 wt% amikacin) were immersed in DMEM for 24 h, and then the gel soaking solution was added to the wells at different concentrations and the cells were cultured for another 24 h. The cell viability was measured by a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Five repeats were conducted for each sample.

2.5. In vitro drug release studies

200 μL amikacin/Dex-CHO hydrogels (1.43 wt% amikacin) was prepared in a vial and 2 mL PBS buffer (pH 7.4 or pH 5.0) was added into the vial. The vial was incubated at room temperature and 300 μL samples were collected at scheduled time intervals. For comparison, the release rate of amikacin from an alginate/calcium hydrogel physically encapsulated with amikacin was conducted as a control. Generally, 100 μL sodium alginate and amikacin solution was mixed with 100 μL $CaCO_3$/gluconolactone suspension to form the alginate/calcium hydrogel. The amikacin content in the hydrogel is kept constant at 1.43 wt%. The drug release studies for amikacin/Dex-CHO or netilmicin/Dex-CHO hydrogels at different drug contents were conducted by the same procedure.

The concentration of amikacin or netilmicin was determined by a derivatization method using *o*-phthalaldehyde. Generally, 268 mg *o*-phthalaldehyde was dissolved in 10 mL methanol, and then the solution was supplemented with 1.4 mL 3-mercaptoacetic acid, and 38.6 mL boric acid (0.2 M, pH 10.5 adjusted by potassium hydroxide). The derivatization solution was freshly prepared before measurement. 600 μL of the prepared derivatization solution was added into 300 μL aminoglycoside samples and incubated for 15 min. Absorbance of the samples at 333 nm was recorded for colorimetric analysis. The calibration curves for amikacin and netilmicin are $y = 0.2447 \times -0.0031$ ($R^2 = 0.998$) and $y = 0.4436 \times -0.0143$ ($R^2 = 0.999$), respectively, where x is the aminoglycoside concentration (mg/mL), and y is the absorbance of samples at 333 nm. Three repeats were conducted for each sample.

2.6. Bacteria culture and pH sensing

Fresh culture of *E. coli* was prepared by isolating a single colony from a Luria-Bertani (LB) plate and suspended in 5 mL sterile LB medium. *S. epidermidis*, *S. aureus*, and *P. aeruginosa* were prepared by inoculating 5 mL sterile TSB medium with a single bacteria colony from a tryptic soy agar (TSA) plate. The counts of bacteria were quantified by measuring the optical density of medium at 600 nm (OD_{600}) using a microplate plate (Thermo Fisher). For all the assays, the bacteria were allowed to grow to logarithmic phase ($OD_{600} = 0.6$–0.8) before use. To determine the pH condition of the bacteria during growth, 0.0025% (*w*/w) phenol red and 1% (*w*/w) glucose were added in a TSA plate. The *S. epidermidis* was diluted to 10^8 CFU/mL and locally inoculated on the plate, and then incubated for 24 h before observation.

2.7. In vitro antibacterial assay

70 μL antibiotic hydrogels were directly formed in a 96-well plate by mixing Dex-CHO (50 mg/mL) with amikacin solutions at different drug contents (0.71, 1.43, 2.14 and 2.8 wt%, respectively). The control alginate/calcium hydrogel was prepared by mixing 35 μL alginate and amikacin solution (1.43 wt% amikacin) with 35 μL $CaCO_3$/gluconolactone suspension. The PEI/Dex-CHO hydrogel (2.8 wt% PEI) was prepared by a similar method as the amikacin/Dex-CHO hydrogels. Antibacterial activities of commercial antibacterial gels including Nano-Ag, erythromycin (1 wt%), and achromycin (3 wt%) gels were also tested at the same mass of bioactive contents with the amikacin/Dex-CHO (1.43 wt% amikacin) gel. Bacteria without any treatment and bacteria-free medium without any bacteria were also tested as controls.

The bacteria culture was diluted to 10^4 CFU/mL with sterile LB or TSB media and 100 μL of the bacteria solutions were added to the wells. After 24 h incubation at 37 °C, 50 μL of the supernatant was mixed with another 50 μL sterile medium, and the bacteria counts in the tested samples were measured as described above.

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