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The use of poly(methacrylic acid) nanogel to control the release of amoxycillin with lower cytotoxicity

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ABSTRACT

In order to control the release of amoxycillin (AM) with lower cytotoxicity and higher activity, ethylene glycol dimethacrylate was used as the cross-linker, and a series of poly(methacrylic acid) (PMAA) nanogels were prepared to load the AM. Then, the morphology, size, in vitro release property, long-term antibacterial performance, cytotoxicity, stability and activity of this novel AM/PMAA nanogel were investigated. The results showed that the AM/PMAA nanogel sustainably released AM with long-term antibacterial activity. Moreover, the AM/PMAA nanogel could improve the stability of AM. More importantly, this AM/PMAA nanogel showed slighter cytotoxicity than AM alone, suggesting that the AM/PMAA nanogel was a more useful dosage form than AM for infectious diseases.

1. Introduction

Amoxicillin (AM, $C_{16}H_{19}N_3O_5S \cdot 3H_2O$, CAS: 26787-78-0, Fig. 1), a partial hydrophilic β-lactam antibiotic, has been used widely for the treatment of infectious diseases. It has an excellent activity against Gram positive bacteria, anaerobes, chlamydia and mycoplasma [1–3]. However, as a lactam, AM is easily degraded and consequently loses its antibacterial activity [4,5]. Moreover, traditional drug formulation (such as solution, micelles, and emulsion) cannot sustainably release drug, which results in much loss in bioavailability. So, much and repeat usage during the therapeutic process is a must. Nevertheless, frequent usage may cause serious side effect or decline the patient's compliance. To overcome these problems, some delivery manners have been designed for AM, which attempt to improve the stability, prolong the release, and reduce the side effect [6–10].

In recent years, nanogel represents a promising class of soft materials for delivery of drug and controlled release of bioactive molecule. It exhibits several attractive features over other particulate delivery systems, such as good stability, ease of synthesis, and good control over particle size. The nanogel based on poly(methacrylic acid) (PMAA) has been explored extensively for the drug delivery and tissue engineering. For example, Argentiere et al. prepared a pH stimuli-responsive nanogel based on PMAA for the uptake and controlled release of bioactive molecule [11]. Shi et al. prepared a uniform molecularly-imprinted PMAA nanosphere for the load and sustained release of gatifloxacin [12]. Pan et al. prepared a redox/pH dually stimuli-responsive biodegradable nanohydrogel using PMAA for the study of cancer therapy [13].

In this study, to reduce the side effect of AM and to make the AM become a more useful dosage form than AM alone for infectious diseases, PMAA nanogel was used as the carrier for AM. The nanogel based on PMAA for the load and sustained release of AM was prepared through precipitation polymerization. Shortly, 2,2′-azobisisobutyronitrile (AIBN, $C_8H_{12}N_4$, CAS: 78-67-1, Fig. 1) was used as a catalyzer, and monomers were activated by AIBN to process radical polymerization. Methacrylic acid (MAA, $C_4H_6O_2$, CAS: 79-41-4, Fig. 1) and ethylene glycol dimethacrylate (EGDMA, $C_{10}H_{14}O_4$, CAS: 97-90-5, Fig. 1) were monomers. Thereinto, MAA with monofunctional group was used in chain growth polymerization, and EGDMA with bifunctional group was used in chain termination polymerization. The size and morphology of the prepared PMAA were observed by a scanning electron microscope (SEM). The loading of AM on PMAA and the in vitro release of AM

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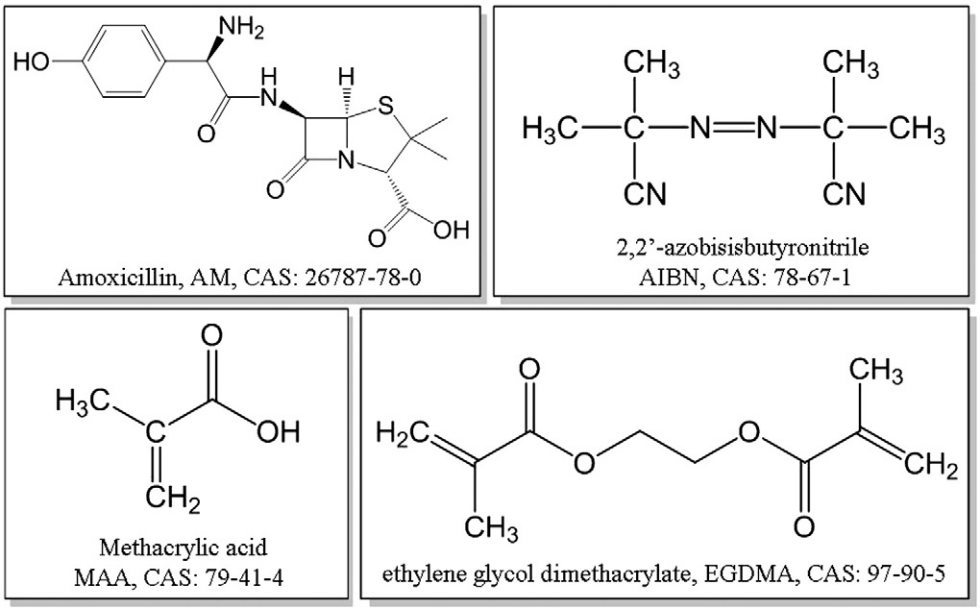

Fig. 1. The chemical structure of amoxicillin, 2,2′-azobisisobutyronitrile, methacrylic acid and ethylene glycol dimethacrylate.

from PMAA were also studied. Moreover, the long-term antibacterial performance, cytotoxicity, stability and activity of this novel AM-loaded PMAA (AM/PMMA) nanogel were also investigated.

2. Experimental section

2.1. Materials

MAA purchased from Sigma-Aldrich was distilled before use. EGDMA from TCI and AM from Jiangsu Yunyang Medicine Company (Jiangsu, China) were used as received. AIBN supplied from Damao Chemical Reagent Factory (Tianjin, China) was recrystallized from ethanol for three times before use. Acetonitrile and methanol were of analytical grade and were used without purification. *Escherichia coli* (*E. coli*) ATCC 25922 and *Staphylococci aureus* (*S. aureus*) ATCC 6538 were supplied by the Guangdong Institute of Microbiology (Guangzhou, China). Thiazolyl blue tetrazolium bromide (MTT) substance was purchased from Sigma-Aldrich (Shanghai, China). Human nasopharyngeal carcinoma CNE1 (CNE1) cells were supplied by the General Hospital of Guangzhou Military Command. Luria–Bertani broth and nutrient agar culture medium were supplied by Huankai Microorganism Co., Ltd (Guangzhou, China). All other reagents and solvents were obtained from commercial suppliers. All aqueous solutions were prepared with ultrapure water (>18 MΩ) from a Milli-Q Plus system (Millipore).

2.2. Preparation of PMAA nanogel

A series of PMAA nanogels with different formula ratios were obtained via thermally initiated precipitation polymerization. For instance, 0.87 mmol MAA was added into 50.0 mL CH_3CN in a 100 mL round bottom flask. Then, 2.03 mmol EGDMA and 25.83 mg AIBN were also added under stirring. The pre-solution was purged with nitrogen for 15 min to drive out oxygen, and then was sealed rapidly. Afterwards, the reaction flask was put in a pre-heated oil bath at 60 °C for polymerization for 24 h under moderate stirring. After that, the product was collected by centrifugation at 8000 rpm, and was purified with methanol for three times to remove unreacted MAA or EGDMA. The purified product was dried in a blast drying oven at 40 °C for 2 days. According to the different contents of EGDMA, the obtained product was designated as PMAA-1 nanogel (containing 33% EGDMA), PMAA-2 nanogel (containing 50% EGDMA) or PMAA-3 nanogel (containing 67% EGDMA).

The density of the obtained nanogel was detected by a densimeter (Alfamirage, MDS-300). The morphology of the obtained nanogel was observed by SEM (PHILIPS, ESEMXL 30, Holand). Each sample was firstly coated with gold by sputter coating (BAL-TEC, SCD 005, Liechtenstein) at 30 mÅ for 90 s. The number-average diameter (D_n) was calculated with an iTEM Soft Imaging System 5.0 (Olympus Soft Imaging Solution GmbH, build 1186). The calculation formula was as follows [12]:

$$D_n = \sum_{i=1}^{n} \frac{n_i D_i}{n}. \quad (1)$$

Here, D_i denoted the diameter of the i nanogel, n_i was the number of nanogel with the diameter of D_i, and n was no less than 100. The results were expressed as the mean ± SD.

2.3. Preparation of AM/PMAA nanogel

In load experiment of drug, AM was loaded into the nanogel via an immersing method. Briefly, 10.0 mg PMAA-1 nanogel, PMMA-2 nanogel or PMMA-3 nanogel was soaked in 4.0 mL 0.9 wt.% NaCl solution which contained saturated AM. The mixture solution was ultrasonicated for 10 min to make the nanogel disperse well, and then was kept in a digital shaking air bath at 37 °C for 24 h to obtain the AM/PMAA-1 nanogel, AM/PMAA-2 nanogel and AM/PMAA-3 nanogel, respectively. The AM-loaded nanogel was collected by centrifugation at 8000 rpm and the supernatant was withdrawn. The concentration of AM in supernatant was dialyzed (immersed in PBS (pH = 7.4) with a dialysis bag whose molecular weight cutoff was 3000) and detected using an analysis of the active ingredient by a HPLC (Agilent 1260) with an external

Table 1
Load content (*LC*), load efficiency (*LE*) and water absorption (*WA*) for AM/PMAA with different EGDMA contents.

Sample	EGDMA content (wt.%)	*LC* (μg·mg^{-1})[a]	*LE* (%)[b]	*WA* (wt.%)[c]
AM/PMAA-1	33	20.6	72.1	127 ± 23
AM/PMAA-2	50	67.8	60.3	221 ± 32
AM/PMAA-3	67	44.6	46.7	184 ± 15

[a] *LC* calculation was based on Eq. (2).
[b] *LE* calculation was based on Eq. (3).
[c] *WA* calculation was based on Eq. (4).