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Bone cement based on vancomycin loaded mesoporous silica nanoparticle and calcium sulfate composites

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ABSTRACT

A novel bone cement pellet, with sustained release of vancomycin (VAN), was prepared by mixing VAN loaded mesoporous silica nanoparticle (MSN) and calcium sulfate α-hemihydrate (CS) together. To improve the VAN loading ability, MSN was functionalized with aminopropyltriethoxysilane (APS) to give APS–MSN. The VAN loading content and entrapment efficiency of APS–MSN could reach up to 45.91 ± 0.81% and 84.88 ± 1.52%, respectively, much higher than those of MSN, which were only 3.91% and 4.07%, respectively. The nitrogen adsorption–desorption measurement results demonstrated that most of the VAN were in the pores of APS–MSN. The CS/VAN@APS–MSN composite pellet showed a strongly drug sustained release effect in comparison with CS control pellet. The in vitro cell assays demonstrated that CS/APS–MSN composite was highly biocompatible and suitable to use as bone cement. Furthermore, CS/VAN@APS–MSN pellet showed no pyrogenic effect and meet the clinical requirements on hemolytic reaction. These results imply that CS/VAN@APS–MSN was an ideal candidate to replace CS bone cement in the treatment of open fractures.

1. Introduction

The bone grafting has been widely applied in the treatment of open fractures, to provide mechanical or structural support and improve bone tissue formation [1–3]. However, the local vascularity is compromised, which induced that nonunion and infection are common complications. To avoid wound infection during bone grafting, systemic antibiotic therapy is essential, and commonly high concentration of systemic antibiotics are required, which might lead to antibiotic resistance and some other side effects [4,5]. Thus, an efficient antibiotic delivery system (ADS), which could release the loaded antibiotic at the lesion site, is highly required, so as to reduce the requirement for follow-up care, and improve the patient comfort [6–10].

Up to date, various ADSs embedded in bone cement have been investigated and reported. For example, the antibiotic loaded polymethylmetacrylate bead is traditional and commercial ADS [11–13]. However, it required a 'two-stage' operative procedure for the patients to remove the beads [14]. In contrast, calcium sulfate (CS) impregnated with antibiotics can avoid the secondary surgery for implant removal, due to its biodegradability. Since CS has been introduced to bone grafting by Dreesmann in the 19th century, many attempts have been devoted to develop CS bone cement [15–21]. For instance, Piattelli et al. [22] have investigated the influence of CS cement on curing the bone defects, and found that the CS cement showed a high biocompatibility and advantage to promote new bone formation in rabbit model. Our cooperators [23] have implanted vancomycin loaded CS to treat open fractures of long bones, and found that it was favorable to defend infection and promote bone union. Though CS bone cement has drawn so much attentions, the initial burst release of loaded drug in the first few days greatly hindered its application.

Mesoporous silica nanoparticle (MSN) is an ideal drug carrier, due to its extraordinary chemical and physical properties, e.g. tunable particle and pore size, large specific surface area, high chemical and thermal stability, excellent biocompatibility, and versatile chemistry for further functionalization [24]. Most of the drug was loaded in the pore of MSN, the release behavior of loaded drug was mainly controlled by diffusion. Thus, it could reduce the burst release of loaded drug and prolong drug release period by using MSN as a drug carrier [25–27]. Shen et al. have incorporated PMMA with MSN as a new bone cement to give highly efficient and sustained release of antibiotics [28]. However, while using MSN to load vancomycin, a large antibiotic molecule (3.2 × 2.2 nm) to treat osteomyelitis, both the drug loading content and entrapment efficiency were very low, and vancomycin would be released immediately after administration [29–31]. To resolve these problems, one of the strategies is modification MSN with functional groups. Xiao et al. [32] have reported an efficient pH-responsive drug delivery system using carboxylic acid modified SBA-15 silica rods as drug carriers and poly(dimethyldiallylammonium chloride) as a crosslinking agent. The drug loading content of vancomycin was up to 36.4 wt.% at pH = 6.8. In

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Table 1
The tubes of hemolysis test.

Sample	1	2	3	4	5	6	7
Test extracts (mL)	0.5	0.4	0.3	0.2	0.1	–	–
Saline solution (mL)	2.0	2.1	2.2	2.3	2.4	2.5	–
D.I. water (mL)	–	–	–	–	–	–	2.5
2% red blood cell suspension (mL)	2.5	2.5	2.5	2.5	2.5	2.5	2.5

previous work, we have found that poly(acrylic acid) grafted MSNs (PAA–MSNs) showed a high drug loading efficiency [33,34].

In this work, MSN was functionalized with amino group to improve its drug loading content of vancomycin. The APS–MSN employed as a drug storehouse and formulated with CS-based bone cement for sustained drug release. The in vitro cellular cytotoxicity test was performed to evaluate the biocompatibility of composite bone cement, and pyrogen test and hemolytic test were carried to evaluate biocompatibility of drug loading composite bone cement.

2. Experimental section

2.1. Chemicals

Tetraethyl orthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB, 99%), mesitylene (TMB), and aminopropyltriethoxysilane (APS) were purchased from J&K CHEMICA, Shanghai, China. NaOH (96%) and HCl (37.4%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Calcium sulfate α-hemihydrate (α-CSH) was purchased from Heowns biochem Technologies. Vancomycin hydrochloride was purchased from Eli Lilly Japan K.K. All the reagents were analytical grade and used without further treatment.

2.2. Preparation of APS–MSN

The APS–MSN was synthesized as our previous work [33]. Typically, 0.5 g of CTAB was dissolved into a solution containing 240 mL of deionized water and 1.75 mL of 2 mol/L NaOH (aq), then 3.5 mL of TMB was added to the solution. After vigorously stirring at 80 °C for 4 h, 2.5 mL of TEOS was quickly added into the mixture. Then, the reaction was kept stirring at 80 °C for another 2 h. The resultant white precipitate was separated by filtration, washing with copious ethanol, and drying overnight in a vacuum at 35 °C. 0.1 g of the as-synthesized white powder was refluxed for 6 h in 20 mL of methanol solution containing 1 mL of HCl to remove the structure-template, CTAB and TMB. The resultant MSN was collected by centrifugation, washing with copious water, and drying in a vacuum at 35 °C for 6 h. Finally, 0.1 g of MSN was refluxed for 12 h in 20 mL of ethanol containing 1 mL of APS to yield the aminopropyl-functionalized MSN (APS–MSN).

2.3. Preparation of VAN@APS–MSN/calcium sulfate composite cements

Typically, APS–MSN and vancomycin were dispersed in deionized water to form 20 $mg \cdot mL^{-1}$ solutions, respectively. Then, 5 mL of vancomycin solution was mixed with 5 mL of APS–MSN solution. The mixture was stirred for 24 h to reach the equilibrium state. The vancomycin loaded APS-MSN (VAN@APS–MSN) was collected by centrifugation at 12000 rpm for 5 min. The VAN@MSN was prepared by a similar procedure. Both VAN@APS–MSN and VAN@MSN were lyophilized to obtain white solids. The loading amount of vancomycin was determined by a UV–vis spectrophotometer at 280 nm.

For preparing calcium sulfate/VAN@APS–MSN (CS/VAN@APS–MSN) and CS/VAN pellets, a certain amount of VAN@APS–MSN or VAN was mixed with 1 g of α-CSH powder. Then, a certain amount of water was added quickly and after mixed thoroughly for 30–45 s, the slurry was injected into a mold and kept there until hardened. The white columniform pellets were collected with bending and extruding the mold.

2.4. In vitro drug release

Typically, a certain amount of VAN@APS–MSN powder was dispersed into deionized water. The solution was transferred into a dialysis bag (MWCO = 14,000), and then the bag was immersed into 30 mL of PBS solution (pH = 7.4) with gentle shaking. At predetermined time intervals, 5.0 mL of solution outside the bag was withdrawn and replaced with the same volume of fresh PBS solution. For CS/VAN@APS–MSN cement, the in vitro drug release behavior was evaluated over a time period of 10 days. Three CS/VAN@APS–MSN cement pellets were placed in 5 mL of PBS solution (pH = 7.4). The dispersion was transferred into a dialysis bag (MWCO = 3500), and then the bag was immersed into 30 mL of PBS solution (pH = 7.4) at 37 °C with gentle shaking. 5.0 mL of the solution outside the bag was collected at a given time interval, followed by supplying the same volume of PBS solution. The released amount of vancomycin was determined by a UV–vis spectrophotometer at 280 nm.

2.5. Pyrogen test

The New Zealand male house rabbits, purchased from Experimental Animal Centre of the Second Military Medical University with body weights in a range of 2–2.5 kg and medicinal animal No. 12-25-5, were selected for pyrogen test. Before the experiment, all of the rabbits had been conformed to the selection criteria of pyrogen test where the body temperature of rabbits should be in the range of 38 °C–39.6 °C with the maximal variation in body temperature lower than 0.4 °C in the measurement of one time per 30 min within 4 h. All the rabbits had been fed under the same circumstances for 1–2 days. The variation in

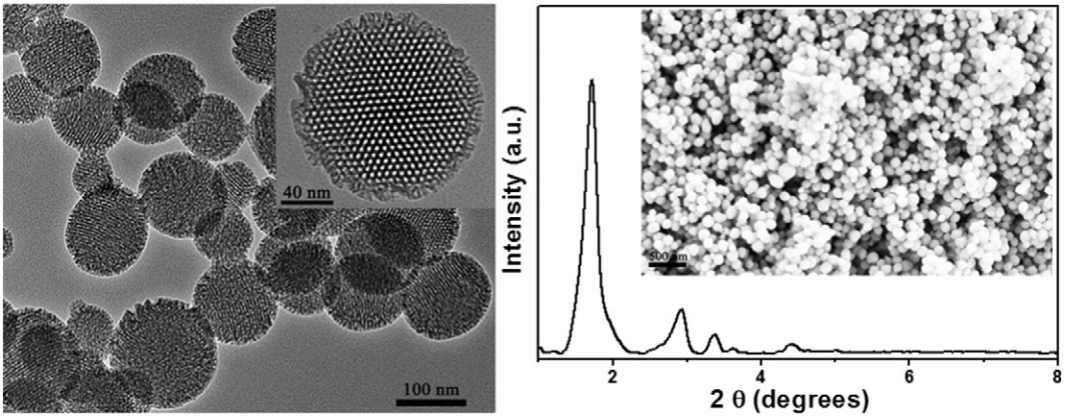

Fig. 1. (A) TEM images and (B) XRD pattern of MSNs.