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 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/VAN@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.

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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 polymethylmethacrylate 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 attention, 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. Xia 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(dimethylallylammonium chloride) as a crosslinking agent. The drug loading content of vancomycin was up to 36.4 wt.% at pH = 6.8. In
previous work, we have found that poly(acrylic acid) grafted MSNs (PAAMSNs) 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·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 ºC–39.6 ºC with the maximal variation in body temperature lower than 0.4 ºC 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

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Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>Test extracts (mL)</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Saline solution (mL)</td>
<td>2.0</td>
<td>2.1</td>
<td>2.2</td>
<td>2.3</td>
<td>2.4</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>D.I. water (mL)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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<td>2% red blood cell suspension (mL)</td>
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<td>2.5</td>
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<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
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</tbody>
</table>

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Fig. 1. (A) TEM images and (B) XRD pattern of MSNs.
temperature between the laboratory and the feeding cages was not beyond 5 °C. Extracts were prepared by adding the CS/VAN@APS–MSN pellets into 0.9% saline solution with a concentration of 3 mg/mL and then incubated at 38 °C for 24 h. Three rabbits were fixed so that they could not move freely while measuring the normal body temperature by an ordinary way. Then, the extract was slowly injected into the rabbits’ ear vein within 15 min. After injection, the body temperature of the rabbit was totally measured six times, and the measurement interval was 30 min. The body temperature rise value for a tested rabbit was defined as the difference between the highest body temperature and its normal body temperature; the total temperature rise value for one tested rabbit was defined as the sum of the six times measuring differences mentioned earlier.

2.6. Hemolytic test

The fresh blood for hemolytic test was extracted from the rabbit mentioned above. 5 mL of fibrin-removed rabbit blood sample was diluted by addition of 50 mL of 0.9% saline solution. After shaking for 10 min, the red blood cells (RBCs) were isolated from serum by centrifugation. RBCs were further washed three times with 0.9% saline solution, and then 2% red blood cell suspension was obtained by addition of saline solution. A certain amount of CS/VAN@APS–MSN pellets was immersed into 0.9% saline solution with a concentration of 3 mg/mL, and then incubated at 37 °C for 24 h to form test extract. For the test group, a certain amount of test extract was mixed with 2.5 mL of 2% RBC suspension and additional saline solution in a tube. Herein, RBC incubation with 0.9% saline solution and D.I. water were used as the negative and positive controls, respectively. The several substances of each test tube were listed in Table 1. The sixth tube was blank control and the seventh tube was D.I. water control. All of the tubes were kept in static condition at 38 °C, and recorded the sample hemolysis after 0.5 h, 1 h, 2 h, and 3 h.

2.7. Cytotoxicity test

The cytotoxicity of CS/APS–MSN cements to HEK-293 cells was evaluated by CCK-8 kit assays. The cells were seeded in 96-well plates with a density of 1 × 10⁴ cell per well and incubated at 37 °C in an atmosphere containing 5% CO₂ for 24 h to allow cell attachment. Then, the medium was replaced with a fresh medium containing the indicated concentration of CS/APS–MSN. After incubation for 24 and 48 h, the medium was aspirated and replaced by 100 mL of fresh medium containing 10 μL of CCK-8. The cells were incubation for another 2 h at 37 °C in dark. Afterward, the absorbance at 450 nm of each well was measured using a microplate reader.

2.8. Characterizations

Field emission scanning electron microscopy (FE–SEM) was carried out on a Zeiss-Ultra55 scanning electron microscope. Transmission electron microscopy (TEM) images were obtained on a Tecnai G2 20 TWIN transmission electron microscope. The surface analysis was performed by nitrogen sorption isotherms at 77 K with an ASAP2020 sorptometer. The surface areas were calculated by the Brunauer–Emmett–Teller (BET) method, and the pore size distributions were
calculated by the Barrett–Joyner–Halenda (BJH) method. All samples were degassed at 180 °C for 6 h prior to the analysis. The total pore volume was estimated from the amount adsorbed at a relative pressure \( P/P_0 \) of 0.989. Powder X-ray patterns (XRD) were recorded on a Bruker D4 X-ray diffractometer. Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer Pyris 1 TGA instrument at a heating rate of 20 °C/min in a nitrogen flow from 100 °C to 800 °C. Fourier transform infrared (FT-IR) spectra were recorded on Thermo Nicolet 6700. The UV–vis absorbance spectra were measured with a Perkin-Elmer Lambda Nicolet 6700 spectrophotometer. The zeta potential of the nanoparticle was measured with a Malvern Zetasizer Nano ZS90 instrument. The porosity of bone cement pellet was measured by a mercury intrusion method (AutoPore IV 9500, USA) with three pellet measured for each group and took the average. The test temperature was 23 °C and the test pressure was from 0 to 30,000 PSIA.

3. Results and discussions

The template-removed MSNs were uniform spherical nanoparticles with a mean diameter of approximately 150 nm, as shown in the TEM image (Fig. 1A) and in the FESEM image (Fig. 1B, inset). A highly ordered mesoporous network with a hexagonal array could also be clearly seen, which was the characteristic of MCM-41 type MSN. This result was further confirmed by XRD measurement (Fig. 1B), four well-resolved diffraction peaks, assigned as (100), (110), (200), and (210) planes, were also consistent with the characteristic diffraction pattern of MCM-41 type MSN [35,36]. As shown in Fig. 2A, the nitrogen adsorption–desorption analysis of MSN exhibited a type IV isotherm with a BET surface area of 1181 m²/g and a pore volume of 2.11 cm³/g. After modified with APS, the adsorbed nitrogen amount of APS–MSN was reduced slightly with a BET surface area of 1053 m²/g and a pore volume of 1.62 cm³/g, but the shape of the hysteresis loop remained unchanged, which indicated that the pore shape was not significantly changed after functionalized with APS. Because that TMB, a widely used pore expanding agent, was added along with CTAB, the pore size of template-removed MSN was as large as 4.5 nm, as shown in Fig. 2B. After modifying with APS, the average pore size was decreased from 4.5 nm to 4.26 nm (Fig. 2B), which suggested that the pore of MSN was also modified by APS.

As seen in the FT-IR spectra (Fig. 3), after removal of CTAB and TMB, the peaks at 2930 cm⁻¹ and 2855 cm⁻¹, assigned to the stretching vibrations of \( \text{C–H} \), were almost disappeared, which suggested that the CTAB and TMB were clearly removed. The broad absorption peak in the range of 3730–3000 cm⁻¹ was assigned to the stretching vibration of silanol groups in the surface of MSN. After reacting with APS, the broad peak was decreased and became sharp at 3430 cm⁻¹, due to the forming of Si–O–Si and functionalization with amino group. Furthermore, the peaks at 2930 cm⁻¹ and 2875 cm⁻¹ were intensified, ascribed to the stretching vibrations of \( \text{C–H} \), and a new peak appeared at 1556 cm⁻¹, attributed to N–H asymmetric bending vibration. All these indicated that the MSN was successfully functionalized by APS. This was also confirmed by the zeta potential characterization. Due to the presence of silanol group, the zeta potential of MSN was −26.6 mV. After reacting with APS, the zeta potential was increased to 24.1 mV, indicated the existence of amino groups.

The grafted amount of APS on MSN was evaluated by thermogravimetry analysis (TGA). As seen in Fig. 3B, after heating to 800 °C, the weight loss of MSN was less than 6 wt.%, due to the dehydration reaction between residual CTAB and silanol groups. And APS–MSN showed

### Table 2

<table>
<thead>
<tr>
<th>Carrier</th>
<th>VAN/carrier</th>
<th>Loading content (%)</th>
<th>Entrapment efficiency %</th>
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<tr>
<td>MSN</td>
<td>1</td>
<td>3.95 ± 0.11</td>
<td>4.11 ± 0.17</td>
</tr>
<tr>
<td>APS–MSN</td>
<td>1</td>
<td>45.91 ± 0.81</td>
<td>84.88 ± 1.52</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>25.22 ± 0.26</td>
<td>82.57 ± 0.76</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7.43 ± 1.16</td>
<td>80.26 ± 1.43</td>
</tr>
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</table>

### Table 3

The samples of CS/VAN@APS–MSN pellets with different VAN loading content.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Loading content (%)</th>
<th>CaSO₄·H₂O (g)</th>
<th>VAM@APS–MSN (mg) (loading content: 45.91%)</th>
<th>VAM (mg)</th>
<th>H₂O (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2</td>
<td>1</td>
<td>46.0</td>
<td>0</td>
<td>1.046</td>
</tr>
<tr>
<td>b</td>
<td>5</td>
<td>1</td>
<td>123.6</td>
<td>0</td>
<td>1.123</td>
</tr>
<tr>
<td>c</td>
<td>8</td>
<td>1</td>
<td>213.6</td>
<td>0</td>
<td>1.688</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>1.000</td>
</tr>
</tbody>
</table>

![Fig. 4. XRD pattern of CS/VAN@APS–MSN and inset image of the composite bone cement pellets with a diameter of 4.5 mm and a height of 3.5 mm.](image)

![Fig. 5. SEM images of (A) CS and (B) CS/VAN@APS–MSN.](image)
a weight loss of 16 wt.%. Thus, the grafted ratio of APS could be calculated to 10 wt.%.

In the dispersion experiment, 5 mg of APS–MSN and MSN was dispersed into 5 mL of deionized water under ultrasonication. After standing for 5 days, most of MSN was precipitated from the solution, however, there was no obvious precipitation in the APS–MSN solution, suggested that the dispersibility of APS–MSN was significantly enhanced due to the grafted of APS, which was propitious to the biomedicine application.

Conventionally, while loading drug, vancomycin was just added into the MSN dispersion, and the concentration of vancomycin was higher outside the MSNs than that inside the MSNs. Thus, the vancomycin would diffuse from the outside to the channels of MSNs driven by the diffusion effect and adsorb in the channels by physical adsorptions and hydrogen bond interactions. However, the uptake of vancomycin hydrochloride was poor, due to its good water solubility and its large dimension (2.2 × 3.2 nm), which is comparable to the pore size of MSN. Herein, the VAN loading experiments to MSN and APS–MSN were both conducted. Considering that the density of vancomycin was 1.106 g/cm³ and the pore volume of MSN was 2.11 cm³/g, the theoretical loading content of vancomycin could reach up to about 83%. However, as listed in Table 2, the loading content of VAN@MSN was less than 4%, and the entrapment efficiency was only about 4%, at VAN/MSN = 1. This might be due to the excellent water solubility of VAN and the poor interactions between VAN and MSN. After modified with APS, the loading content and VAN@APS–MSN entrapment efficiency was 45.91 ± 0.81% and 84.88 ± 1.52%, respectively, at VAN/APS–MSN = 1. The loading content of VAN was also confirmed by TGA, as shown in Fig. 3B. The weight loss of VAN@APS–MSN was 45 wt.%, corresponding that the loading content was 29 wt.%. This result indicated that after functionalized with APS, the interactions between VAN and APS–MSN were enhanced.

The drug encapsulation process was also characterized by nitrogen adsorption–desorption measurement. After loading VAN (45.91 ± 0.81%), the BET surface area decreased from 1053 to 709 m²/g and the pore volume reduced from 1.62 to 0.54 cm³/g (Fig. 2A), and the hysteresis loop of BET isotherm curve of VAN@APS–MSN can be barely seen, in the meantime, the average pore size was decreased from 4.26 nm to less than 3 nm (Fig. 2B, inset). These results suggested that VAN was successfully encapsulated into the channels of APS–MSN.

Calcium sulfate hemihydrate could transform into calcium sulfate dihydrate by reacting with water below 42 °C, and calcium sulfate dihydrate was a stable phase under standard conditions [37]. The calcium sulfate dihydrate pellets and calcium sulfate/VAN@APS–MSN composite (CS/VAN@APS–MSN) pellets were formed with the same mold. The size of the cylindrical pellets was 4.5 mm in diameter and 3.5 mm in height (Fig. 4, inset). As it could be seen in Fig. 4, compared with the X-ray database (PDF 33-31) of pure calcium sulfate dihydrate, CS/VAN@APS–MSN showed a standard XRD pattern of calcium sulfate dihydrate, with a very low residual amount of bassanite.

The CS pellets were constructed by disordered laminar calcium sulfate dihydrate crystallites and contained many inter-connective macro-pores formed by in-situ volatilization of water (Fig. 5A). The aggregation of numerous crystallites was the intrinsic factor to the high compressive strength of the bone cement pellets. After mixed with VAN@APS–MSN, many VAN@APS–MSN particles were stacked on the surface of calcium sulfate crystallites (Fig. 5B). The stack of MSNs reduces macro-pores of CS bone formed by in-situ volatilization of water to a certain extent. It could be proved by Hg intrusion experiments, compared with CS/VAN pellets, the porosity of CS/VAN@APS–MSN pellets was accordingly decreased to 53.53 and 0.625, respectively, when the drug loading content improved to 8%.
Because of the fact that most of the VAN@APS–MSN particles were stacked in the macro-pores, the aggregation structure of lamellar calcium sulfate dihydrate crystallites was not destroyed. CS/VAN@APS–MSN pellets showed similar mechanical strength with CS pellets, which is important to its application as bone cement.

To evaluate the drug release behavior of CS/VAN@APS–MSN pellets, the in vitro drug release experiment was carried out in PBS solution of pH = 7.4 at 37 °C. The drug loading contents of test samples, a, b, and c and CS/VAN control were 2%, 5%, 8% and 5%, respectively (Table 3). As seen in Fig. 6A, the VAN release behaviors of a, b, and c were acted as a similar pattern over the entire time period of 10 days. Though the total released amount of VAN increased slightly from 83.59% to 89.06% with the increase of drug loading content from 2% to 8%. The positive sustained release effect of CS/VAN@APS–MSN pellets (a, b, and c) could be observed in comparison with CS/VAN (control), especially at the first 24 h (Fig. 6A, inset). To confirm and elucidate the influence of MSN on prolonging the drug release period of calcium sulfate cement, the drug release behavior of VAN@APS–MSN was investigated. The result showed a diffusion-controlled release mechanism, similar to traditional controlled drug delivery system with MSN as the drug carrier [29]. The introduction of MSN to CS cement as VAN carriers could efficiently prolong the drug release period. Thus, CS/VAN@APS–MSN pellet was an ideal candidate to replace CS cement pellet in bone grafting therapy.

The in vitro cytotoxicity of CS/VAN–MSN against HEK-293 with different concentrations and incubation times was estimated by CCK-8 kit assays. As it could be seen from Fig. 7A, at a concentration ranged from 0.01 to 0.1 mg/mL, CS/VAN–MSN showed no obvious cytotoxicity. Even at the concentration up to 1 mg/mL, the cell viability was above 70% after incubation for 24 h. Furthermore, when the incubation time increased to 48 h, the cell viability was increased to 85%. These results indicated that CS/VAN–MSN was nontoxicity at low concentrations and slightly toxicity at high concentration.

CS/VAN@APS–MSN pellet with a VAN loading content of 5% was chosen for pyrogen test and hemolytic test. As shown in Fig. 7B, the pyrogen test results showed that the temperature rise value of each tested rabbit was uniformly, lower than 0.6 °C, compared with its initial temperature. The total temperature rise value was lower than 1.4 °C. The mean temperature rise value for every measurement time was all less than 0.2 °C, and there was no obvious no upward trend. These results indicated that the clinical requirements on pyrogen test, indicating that the CS/VAN@APS–MSN pellet has no pyrogenic effect.

As seen in Fig. 8. There was no obviously hemolytic reaction occurring in the tested samples. The supernatants were clear and colorless, and the precipitation layers were composed of CS/VAN@APS–MSN and red blood cells. These results indicate that the CS/VAN@APS–MSN meets the clinical requirements on hemolytic reaction. Thus, CS/VAN–MSN possessed a good biocompatibility and was suitable to use as bone cement in the treatment of open fractures.

4. Conclusion

In summary, a novel VAN loaded composite bone cement, CS/VAN@APS–MSN, was prepared successfully. The VAN loading content was extremely improved by grafting amino groups onto the internal and external surfaces of MSN. The in vitro drug release experiments demonstrated that CS/VAN@APS–MSN solved the problem of drug burst release of traditional CS bone cement, and could efficiently prolong the drug release period. Furthermore, a series of biocompatible tests suggested that the CS/VAN@APS–MSN had no pyrogenic effect and met the clinical requirements on hemolytic reaction. The in vitro CCK-8 assays indicated that CS/VAN@APS–MSN showed a low cytotoxicity against HEK-293. CS/VAN@APS–MSN represented a new candidate in the treatment of open fractures.

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References


