

Vancomycin-loaded nano-hydroxyapatite pellets to treat MRSA-induced chronic osteomyelitis with bone defect in rabbits

Ji-Le Jiang · Yun-Fei Li · Tao-Lin Fang · Jian Zhou · Xi-Lei Li · Yi-Chao Wang · Jian Dong

Received: 5 April 2011/Revised: 11 October 2011/Accepted: 8 November 2011/Published online: 11 December 2011
© Springer Basel AG 2011

Abstract

Objective To investigate nano-hydroxyapatite (nHA) pellets as carriers for vancomycin in the treatment of chronic osteomyelitis and bone defects due to methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

Methods Chronic osteomyelitis was induced in 45 New Zealand white rabbits. After 3 weeks (chronic infection), all animals were treated with debridement. The rabbits were divided into an experimental group (the bone was filled with vancomycin-loaded nHA pellets), a control group (the bone was filled with nHA pellets alone), and a blank group. The drug release profiles were determined in vitro and in vivo. X-rays, bone specimens, and microorganism cultures were used to evaluate the efficacy of the treatments.

Results Following a rapid initial release into the circulation, the drug concentration remained effective in the osseous and soft tissues for 12 weeks after debridement. Within 3 months, all rabbits in the experimental group recovered from osteomyelitis without a recurrence of the

infection and the bone defects were partially repaired, whereas the infection and bone defects persisted in the control and blank groups.

Conclusions The results demonstrate that vancomycin-loaded nHA pellets successfully repair bone defects and control infection in MRSA-induced chronic osteomyelitis. In addition, nHA is an effective and safe controlled-release vancomycin carrier for chronic osteomyelitis with bone defects that is induced by MRSA.

Keywords Chronic osteomyelitis · Nano-hydroxyapatite pellets · Vancomycin · Methicillin-resistant *Staphylococcus aureus* (MRSA) · Bone defect

Introduction

Controlling chronic osteomyelitis, especially methicillin-resistant *Staphylococcus aureus* (MRSA) osteomyelitis, remains a serious problem for orthopedic surgeons despite the ongoing advances in surgical techniques and the development of antimicrobial agents. Moreover, bone defects due to chronic osteomyelitis remain a challenge for surgeons. At present, the treatment of osteomyelitis primarily involves the surgical debridement of necrotic tissue, irrigation with an antiseptic solution, and the application of a course of systemic antibiotics for 6 weeks [1]. Vancomycin is recognized as an effective antibiotic against MRSA [2]. However, large doses of vancomycin do not penetrate efficiently into local sites due to the development of malformations secondary to the infection and may be associated with nephrotoxicity, ototoxicity, gastrointestinal side effects, increased costs, and low patient compliance.

Local antibiotic delivery systems, such as antibiotic-impregnated polymethyl methacrylate (PMMA), have a

First two authors (Ji-Le Jiang and Yun-Fei Li) contributed equally to this paper as co-first author

Responsible Editor: Michael Parnham.

J.-L. Jiang · T.-L. Fang · J. Zhou · X.-L. Li · Y.-C. Wang (✉) · J. Dong (✉)
Orthopedic Surgery, Zhongshan Hospital of Fudan University, Shanghai, China
e-mail: wang.yichao@zs-hospital.sh.cn

J. Dong
e-mail: dong.jian@zs-hospital.sh.cn

J.-L. Jiang
e-mail: jile.jiang@gmail.com

Y.-F. Li
Orthopedic Surgery, Jiading Centre Hospital, Shanghai, China

superior therapeutic efficacy and a lower toxicity than the intravenous application of antibiotics. However, because PMMA is a non-absorbable material, it is unable to repair bone defects following implantation, and it is associated with a number of serious disadvantages, such as foreign body reaction and secondary infections [3]. Patients often need additional surgical procedures to remove PMMA and the insertion of a bone graft or an endoprosthetic device to rebuild bone [4]. These procedures increase the infection rate and the financial burden on the patient. Recently, attention has focused on the use of various bioabsorbable materials for the local delivery of antibiotics with the purpose of controlling infection and repairing bone defects. Hydroxyapatite (HA) has a high biocompatibility and bioactivity, and there is clinical evidence that it can successfully repair bone defects with appositional bone formation after approximately 3.5 years [5]. HA also demonstrates slow-releasing antibiotic elution characteristics *in vitro*, which suggests that it may provide adequate antibiotic delivery *in vivo* [6].

Although previous studies have demonstrated the slow-releasing antibiotic elution characteristics of HA *in vitro* and *in vivo*, the concentration of vancomycin that was released from HA decreased to levels that were too low to be detected 10 days after implantation [7]. In contrast, slowly increasing concentrations of antibiotics were detected for up to 30 days when nano-HA pellets were used [8]. The drug release profile in *in-vivo* experiments indicates that there is a significant difference between HA and nano-HA. HA releases and maintains a low concentration of antibiotic (up to 0.16 µg/ml) [9]; however, the nano-hydroxyapatite (nHA) method of delivery is a superior alternative because it exhibits a more efficient release profile that is characterized by an initial rapid release of the antibiotic followed by a long-term sustained release [8]. Data from the above study showed that the nHA particles released a surge of antibiotics in the first 72 h, and the antibiotic levels remained above the minimum inhibitory concentration for 42 days, which helps to kill bacteria initially and to control infection over a longer period. Because different antibiotics often show different elution characteristics, the release profile of vancomycin *in vivo* needs further testing.

Furthermore, previous studies have shown that HA is not reabsorbed, and no biodegradation or deep bone ingrowths were observed 3 months after implantation [7]. In contrast, nHA stimulated new bone formation and repaired the bone defects. Some studies have shown that osteoblast adhesion increases while fibroblast adhesion decreases with the application of nHA, and nHA possesses mechanical and surface properties that are desirable and have the potential to achieve orthopedic implant efficacy [10]. The results of *in vivo* experiments also demonstrate that a nHA composite stimulates the growth of new bone, indicating that it is an osteoconductive material [11, 12]. Additionally, recent

studies show that nHA possesses specific properties that may also influence the surface kinetics of the material, which may influence cell activity and induce further bone responses [13, 14]. Several nHA composite scaffolds have been tested for bone tissue engineering, and the results show that nHA composites display excellent mechanical and biological characteristics [15, 16]. Experiments have proven that nHA is a satisfactory bone substitute in the normal physiological environment [17], but few studies have addressed bone defects that are associated with infection, focusing instead on the bone defect caused by chronic osteomyelitis and on the efficacy of reconstructing new bone in an infectious environment to advance clinical practices.

In this study, we used nHA as a vancomycin delivery carrier and bone substitute for the treatment of MRSA-induced chronic osteomyelitis with bone defect in rabbits. The release profile and the efficacy of the treatment were determined *in vivo* and *in vitro*. The aim of this study is to investigate nHA as a drug carrier material and a bone graft substitute to control bacterial infection and to reconstruct osseous defects.

Materials and methods

Preparation of drug delivery system

Powdered nHA (Berkeley Advanced Biomaterials Inc., San Leandro, CA, USA) was mixed with vancomycin hydrochloride (Eli Lilly Japan K.K., Kobe, Japan) at a concentration of 160 mg vancomycin/g nHA, a concentration that has been demonstrated to be a safe and effective dose in previous studies [9, 18]. The resulting mixture was placed into cylinder molds (diameter 3.2 mm, height 10 mm) and was allowed to harden for 20 min and to dry for 24 h to form pellets. Control nHA pellets without vancomycin were produced using the same method. All pellets were prepared under sterile conditions.

Bacterial preparation

The strain of MRSA was isolated from the sinus tract of a patient suffering from chronic osteomyelitis that was induced by open fractures. Clinical tests showed that the isolated bacteria were resistant to cephalosporin, gentamicin and kanamycin and were sensitive to vancomycin and rifampicin. The bacterium was identified as MRSA using a coagulase tube test and an antibiotic resistance test. The bacteria were subcultured in 10 ml of nutrient broth (Oxoid, Basingstoke, Hampshire, UK) and were stored overnight at 37°C the day before inoculation. Each broth culture was diluted in sterile phosphate-buffered saline (PBS) to a density of 1×10^9 colony-forming units/ml of saline before inoculation.

Antibacterial activity in vitro

The release profile of impregnated vancomycin was determined in vitro by measuring inhibition diameters. An agar-dilution technique was applied in the test, and the bacterial strains *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922) and MRSA were used. Each bacterial strain (1×10^8 colony-forming units) was inoculated on Müller–Hinton agar medium (Becton–Dickinson Microbiology Systems, Cockeysville, MD, USA). Vancomycin-loaded nHA pellets (18) were placed on the inoculated plates (6 per plate) and the plates were incubated at 37°C. The pellets were moved to new plates every 3 days and the inhibition diameter was recorded. The end of antibacterial activity was determined by the disappearance of the inhibition diameters.

Animal model

New Zealand white rabbits (NZWR) (provided by the laboratory animal center of Zhongshan Hospital, Fudan University, China), 45 in total, aged 4 months and weighing 1.8–2.2 kg were used for this randomized study. The Institutional Animal Care and Use Committee approved all the animal experimental protocols. The osteomyelitis model was established as follows: briefly, anesthesia was induced using 3% sodium pentobarbital (30 mg/kg) that was injected intravenously into the right hind leg of the rabbit, which had been shaved from the knee to the ankle, cleaned with povidone-iodine skin cleanser, and draped with sterile sheets. A 12-gauge needle was inserted percutaneously through the lateral aspect of the proximal tibial metaphysis and into the medullary cavity. Before injection, 0.3 ml bone marrow was extracted to accommodate the injection. Subsequently, 0.1 ml 5% sodium morrhuate, 0.1 ml MRSA (1×10^9 colony-forming unit/ml) in PBS, and 0.1 ml sterile PBS were injected sequentially. During the experiment, the animals were kept in individual cages, were fed a routine diet and were allowed to be fully active. The animals were physically examined every week.

Design of animal study

At 3 weeks following infection, the severity of osteomyelitis was determined using radiograph analysis. To ensure that there were signs of osteomyelitis using the criteria described by Norden et al. [19], two radiologists independently evaluated the radiographs in a blinded fashion. All the rabbits showed radiographic evidence of osteomyelitis and were randomly distributed into three groups. The rabbits in group 1 ($n = 5$, blank group) were treated with debridement, whereas the rabbits in group 2 ($n = 20$, control group) were treated with debridement and nHA

pellet implantation. The rabbits in group 3 ($n = 20$, treatment group) were treated with debridement and vancomycin-loaded nHA pellet implantation. X-ray imaging and biopsies of four rabbits from each group were conducted at 1, 2, 3, 6, and 12 weeks after debridement.

Drug release profile in vivo

Circulation: 2 ml blood was taken from the ear vein at 30 min, 2, 8, and 24 h, 8 days, and 4, 8, and 12 weeks. The serum was separated at 725 g for 5 min, and was stored at -70°C before analysis.

Soft tissue: 1 g subcutaneous soft tissue within 5 mm of the implantation site was removed and 1 ml sterile saline was added. After the mixture was homogenized, the suspension was separated at 11600 g for 5 min and was stored at -70°C .

Bone: the distal and proximal ends of the tibias, the implantation-containing bone marrow and other soft tissues were removed. The tibias were broken into fragments and were pulverized into a powder that could pass through a 0.45-mm membrane filter. Saline (6 ml) was mixed with 400 mg of the powder and the mixture was vortexed at 100 rpm at 37°C for 2 h. The mixture was centrifuged at 11600 g for 5 min, and the supernatant was stored at -70°C .

The concentration of the drug in the blood, soft tissue and bone was determined using HPLC. All samples were mixed with perchloric acid, the supernatants were separated at 11600 g for 5 min, and were transferred onto HPLC columns. The mobile phase of the column was composed of methanol:KH₂PO₄ approximately 79:21, the flow rate of the mobile phase was 1.0 l/min, and the detection wavelength was 236 nm.

Microbiological evaluation

Specimens were collected from the site of infection using sterile methods. After collection, all the specimens were placed in tubes and were taken immediately to the laboratory where they were inoculated onto blood agar and were incubated for at least 48 h at 37°C. The identification of *S. aureus* was based on conventional criteria using the coagulase tube test and the Api Staph system (ATB 32 Staph, bioMerieux, Marcy-l'Etoile, France). Resistance to oxacillin was confirmed by the detection of the *mecA* gene using PCR as previously reported [20].

Histological analysis and X-ray

Specimens from bone-defect sites were fixed using 10% paraformaldehyde and were embedded with paraffin. Histological examination (hematoxylin and eosin stain)

was evaluated in non-decalcified cross-sections of bone (5 mm). Each examination was classified using the scoring system suggested by Smeltzer et al. [21], which comprises four categories of investigation (acute and chronic inflammation, periosteal inflammation and bone necrosis). A pathologist determined the scores in a blinded fashion.

When the rabbits were killed, anteroposterior and lateral radiographs of their right tibias were prepared at 50 kV, 100 mA, and 12 ms. The radiographic signs were assessed as described previously and were compared with the postoperative radiographs.

Statistics

The results are expressed as the mean and standard deviation of the mean. A paired *t* test (when the data had equal variance) and a *t* test (when the data had unequal variance) were performed using SPSS software (version 11.0), and the level of significance was set at $P < 0.05$.

Results

The antibacterial activity of vancomycin-loaded nHA pellets is shown in Figure 1. The vancomycin release in vitro decreased gradually and remained effective for 25 days in plates that were inoculated with MRSA and *S. aureus*, whereas the vancomycin remained effective for only 7 days in plates that were inoculated with *E. coli*.

No animals died during this study, and no local or systemic side effects of nHA or vancomycin were observed. At week 3, MRSA was detected in all 45 animals that had evidence of osseous destruction. Due to the induced infection, the body weight of all the rabbits

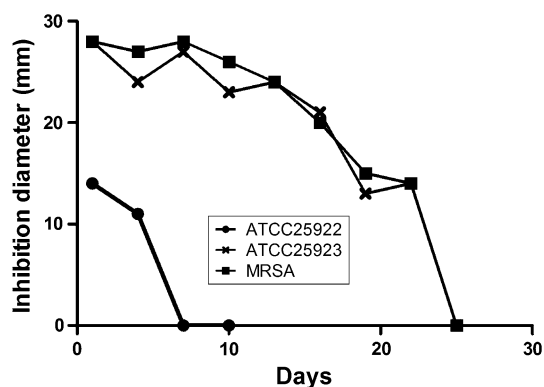


Fig. 1 Inhibition diameter on plates in the presence of vancomycin-loaded nHA pellets. Results show that vancomycin is gradually released, and remains effective for more than 25 days in plates inoculated with MRSA and *Staphylococcus aureus* (ATCC25923), which is much longer than is observed in plates inoculated with *Escherichia coli* (ATCC25922), in which vancomycin only remained effective for 7 days

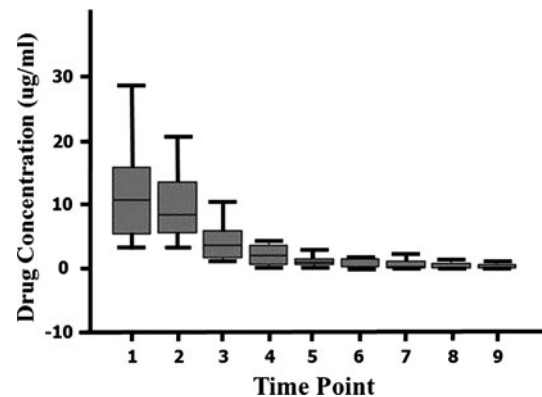


Fig. 2 Vancomycin concentration in the circulation. The time points are defined as 0.5, 2, 8 and 24 h; 4 and 8 days; 4, 8 and 12 weeks. The curve shows that vancomycin concentrations in the circulation peaked 2 h after implantation, a burst of the drug was released in the first 4 days, and a low level of the drug remained in the circulation until 56 days after implantation. All results are expressed as mean \pm standard deviation

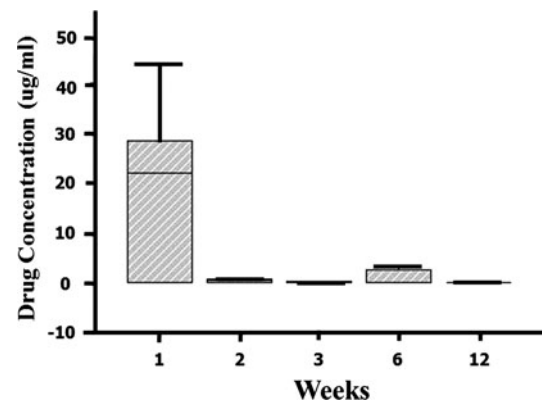


Fig. 3 Vancomycin concentration in soft tissue. The curve shows that a burst of release occurred in the first week and the concentration of the drug remained at a low level until week 12; however, a second peak in the drug concentration was observed in week 6, which may correlate with the reabsorption of nHA pellets and bone reconstruction

decreased slightly. The average implanted weight was 71.27 mg per rabbit, containing 9.83 mg vancomycin.

The drug concentration in the circulation peaked at 2 h following implantation, declined quickly until day 4, and was too low to detect at week 8 (Fig. 2). The results were similar in the soft tissue samples (Fig. 3) with a significant decrease from 18.81 $\mu\text{g/ml}$ at week 1 to nothing at week 12. However, a second peak in vancomycin concentration appeared at week 6 (1.07 $\mu\text{g/ml}$). The vancomycin concentration in the bone also decreased from 412.12 $\mu\text{g/ml}$ at week 1 to 2.53 $\mu\text{g/ml}$ at week 12 (Table 1).

The clearest evidence of the efficacy of the treatment was observed in the microbiological investigations that were performed after treatment. With the exception of one

Table 1 Vancomycin concentration in bone

Time (weeks)	Vancomycin concentration ($\mu\text{g/ml}$)	
	Average	Standard deviation
1	412.12	196.67
2	8.60	5.59
3	6.87	1.65
6	6.42	3.55
12	2.53	3.09

Table 2 Bacterial account in control group

Time (weeks)	Average (colony-forming units/g)	Standard deviation
1	22,400	13,576.45
2	2.1×10^8	1.5×10^7
3	1.7×10^8	7.3×10^7
6	2.3×10^8	4.9×10^7
12	3.4×10^8	0.7×10^7

specimen that was contaminated with a mold fungus, all specimens in the experimental group exhibited negative results for the presence of bacteria after 72 h incubation. The results of bacterial culture in the control group are shown in Table 2. The bacterial counts were low in the first week, but grew rapidly in the absence of local antibiotics. Significant differences were observed between week 1 and the other weeks ($P < 0.05$).

Typical radiographs from each of the four groups are shown in Figure 4. At the time of killing, most of the implants in the treatment group had been reabsorbed and

were replaced with large areas of newly formed bone, whereas the control group exhibited new abscesses, intensified bone malformation, and profound cortical reactions. Few implants in the control group were absorbed because of a worsening of the infection and a poor blood supply.

The macroscopic and microscopic manifestations are described in Tables 3 and 4. Significant differences between the histopathological scores of two groups were observed (Fig. 5). For the rabbits in the control group, the typical histopathological findings consisted of a marked infiltration of polymorphonuclear leukocytes, necrotic debris-containing pus cells in the center, sequestra formation, destruction of bone, and obvious osteomyelitis. For the majority of the rabbits in the treatment group, the typical histological findings revealed newly formed bone around the implanted pellets. There were no signs of large or isolated nHA at 3 weeks after implantation; the pellets took part in the formation of trabecular bone, or they were replaced by medullary cavity tissues (Fig. 6). The nHA was not absorbed completely until 12 weeks after implantation, and this was confirmed by X-ray analysis.

Discussion

Chronic osteomyelitis remains a challenge in the clinic because it is difficult for antibiotics to penetrate into local sites due to malformations that are secondary to chronic infection [22]. In our studies, we have observed that the number of bacteria decreases significantly following debridement; however, without a high concentration of local antibiotics, the bacteria proliferate again and develop

Fig. 4 Change in representative X-ray images before debridement and after treatment. **a** X-ray of control group before debridement showing osteomyelitis manifestation, **b** X-ray of control group 6 weeks after debridement with a pure HA implant showing an increase in malformations of diaphysis and new intramedullary abscesses, **c** X-ray of treatment group before debridement, **d** X-ray of treatment group 6 weeks after debridement showing that the architecture is normal, periosteal action has disappeared, and the swelling of soft tissue is reduced. (Arrow, nHA cement)

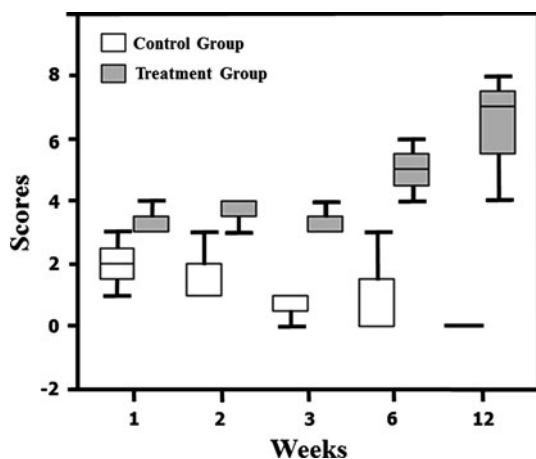


Table 3 Macroscopic manifestation of tibia transection

	Week 3	Week 6
Experimental group	No pus under skin	Start to reconstruct red bone marrow-like tissues
Control group	Pus and implantation were seen under skin	Pus, yellow fibrous tissue and new abscess in bone

Table 4 Appearance of sections under microscope

Week	Treatment group	Control group
1	Implantations were surrounded by few fibrous tissues; hyperplasia of osteoblasts was also observed	Large area of inflamed tissues
2	Implantation tightly bound with bone, surrounded by bone-like matrix	Inflammation cells and swollen tissues in medullary cavity
3	Trabecular bone-like tissues found	Still full of inflammation cells without signs of reconstruction
6	New bone and good shape of trabecular bone structure were observed, and normal red bone marrow appeared in center of medullary cavity	Few bindings between implantation and bone in margin of cortical bone, and large area of inflammation and necrotic tissues in medullary cavity
12	Normal trabecular bone and bone marrow were reconstructed. Few implantations bound tightly to trabecular bone	New abscess appeared, with sequestra

**Fig. 5** Smeltzer histopathology scores of control and treatment groups. The results show significant differences ($P < 0.05$, ANOVA) in the histopathology scores between the two groups from week 1 to week 12

into chronic osteomyelitis, which is difficult to cure. MRSA has been a common infection pathogen in the clinic and most MRSA are resistant to gentamicin. As a result, research has concentrated on other antibiotics [23, 24]. Other studies have focused on the effect of administering vancomycin systemically or using a local drug carrier method [2]. Moreover, studies show that MRSA is sensitive to high local concentrations of vancomycin, and the high success rate that was achieved is encouraging for the clinical use of vancomycin for MRSA-induced bone infections.

The use of implantations, such as PMMA, is an effective way to control the infection; however, because PMMA is not absorbable, its use has some obvious disadvantages including the induction of a foreign body reaction and a predisposition towards secondary infections. Because it is more porous than PMMA, HA pellets, which are an absorbable biomaterial, deliver higher drug concentrations and maintain sufficient levels of the antibiotic drug locally over a longer period. Compared with HA, nHA pellets exhibit a higher porosity and a larger surface area and therefore display a better release profile (fast initial release followed by a long-term sustained release) [8].

Following a review of the literature including a report from Joosten and Shirtliff [9, 18], we chose a vancomycin concentration of 160 mg vancomycin/g nHA. The average weight of the implanted nHA was 61.44 mg per rabbit, including 9.83 mg vancomycin. In addition, the highest drug concentration in the circulation was shown to be 11.8 $\mu\text{g/ml}$, which is considerably less than what is considered toxic. Throughout the experiment, no local or systemic toxicity was observed. A concentration of 160 mg vancomycin/g nHA proved to be an effective and safe choice. Furthermore, the local antibiotic delivery system effectively inhibited the proliferation of the infection.

Previous in-vitro studies have shown that powdered HA does not decrease the efficacy of vancomycin after compaction [25], and an in-vitro antibiotic activity test showed that vancomycin carried by nHA pellets maintains its antibacterial activity. In the agar plates, the diameters of antibacterial activity measured more than 10 mm for more than 3 weeks, which demonstrates a satisfactory in-vitro

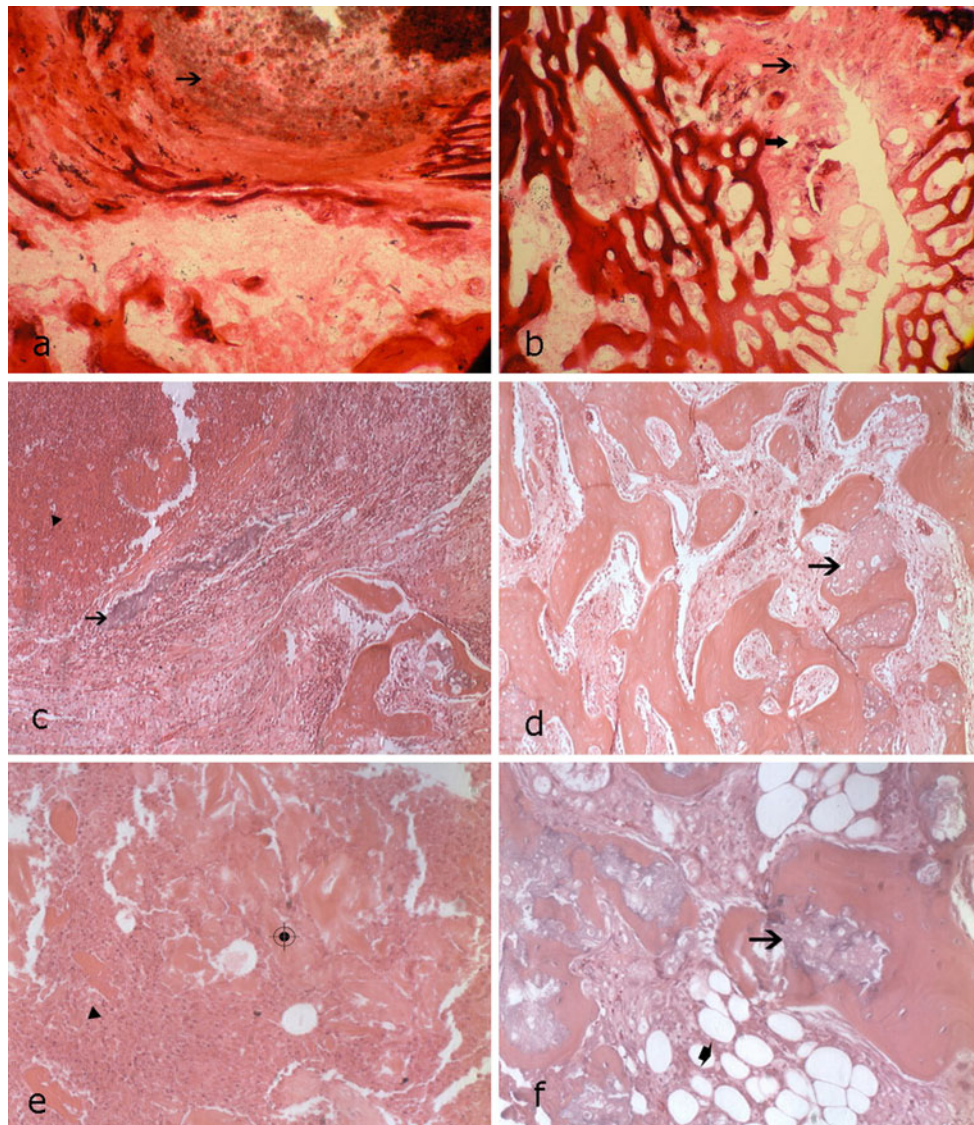


Fig. 6 Comparison of histological examinations (hematoxylin and eosin stain). Non-decalcified cross-section ($\times 100$) in the control group (a) and treatment group (b) at 3 weeks; decalcified cross-section ($\times 100$) in the control group (c) and treatment group (d) at 6 weeks; decalcified cross-section ($\times 200$) in the control group (e) and

treatment group (f) at 12 weeks. *Long arrow*, nHA; *thick arrow*, nHA architecture transformed like cancellous bone at 3 weeks; *short arrow*, recovering normal bone marrow tissue; *arrowhead*, inflammatory cell; *bull's eye*, bone necrosis

antibacterial activity and a controlled release profile. This controlled release profile was significantly improved compared to previous studies in which the concentration of vancomycin was undetectable 10 days after implantation.

We chose nHA as the local delivery system, and the results of bacterial cultivation experiments show that the nHA pellets successfully cured chronic osteomyelitis within a short period, prevented a recurrence of the infection, and maintained this effect for a relatively long period. In experimental tests, the X-ray and microscopic pathology indicate that the infected bone becomes normal bone after 6 weeks, exhibits a reduced periosteal action and a well-shaped trabecular bone structure, and contains

newly-produced bone. Moreover, yellow fibrous tissue and pus were observed in the control group, whereas red bone marrow was observed in the experimental group. In addition, at the end of 12 weeks, normal trabecular bone and bone marrow were present in the experimental group, and few implantations remained.

These results were verified in vivo using a vancomycin concentration test. Although the drug concentration decreased significantly starting at day 4, a high concentration remained in the bone and the soft tissues. Even after 12 weeks, the drug concentration in the bone remained above $2.53 \mu\text{g/ml}$, and sufficient antibacterial activity was maintained during the entire 3-month period. Although

conventional HA released vancomycin for 6 weeks, the drug concentration was between 0.16 and 0.02 µg/ml, which is far less than the concentration achieved in our experimental group. Furthermore, the initial burst and controlled release profile observed in our study successfully controlled infection, maintained the antibacterial effect, and prevented recurrence, which demonstrates that nHA pellets are more suitable as drug delivery carriers than conventional HA for which the drug-release curve was shown to decrease smoothly. Notably, second peaks representing vancomycin release were observed in the bone and the soft tissue, which may reflect the induction of bone reconstruction.

Although osseous defects can be repaired using various methods such as bone grafting, bone infection and osseous defects are the two main problems associated with chronic osteomyelitis [26]. However, a substantial morbidity is associated with the harvesting of bone grafts and with bone transportation [27]. Consequently, a drug delivery system that releases antibiotics and reconstructs bone defects would fulfill the requirements for the treatment of chronic osteomyelitis. Previous studies have shown that although conventional HA [28] is biocompatible and is a good osteoconductive osseous defect-filling material, nHA has a higher solubility and a higher calcium release profile.

Considering these advantages, we choose nHA pellets as the drug delivery carrier for these experiments. We observed that in the treatment group, 3 weeks after implantation, the structure of the nHA pellets changed in that a portion of the pellets were absorbed, and a portion were packed to reconstruct trabecular bone. We believe that the structural change in the nHA pellets indicates that nHA has good absorbability in a pathological environment, which is in sharp contrast to the control groups where nHA pellets remained intact until week 12. The residual nHA that was observed in week 12 in the treatment groups may need to be transferred during bone reconstruction.

The bone reconstruction period is lengthy and it can take 3–4 months to reconstruct an adult bone. Only 25% of trabecular bone and 2–3% of cortical bone is renewed in 1 year and every reconstruction only uses 10% of the bone surface, which might explain why we did not observe the complete absorption of the nHA pellets over the course of our experiments.

Our results showed that nHA could control-release vancomycin both in vivo and in vitro. It could still reconstruct bone defects in the environment of antibiotic release and chronic inflammation. nHA successfully cured chronic osteomyelitis and repair bone defects at the same time in rabbits. However, not all the nHA was absorbed and transformed into bone tissues. Further studies are needed to examine the transformation rate and the

mechanical force of vancomycin-loaded nHA pellets and the long-term outcome of their implantation.

Conclusion

We investigated the therapeutic effect of the local administration of vancomycin-loaded nHA pellets in an animal model of chronic osteomyelitis. The results demonstrate that the vancomycin-loaded nHA pellets release high levels of antibiotics over a prolonged period, stimulate the reconstruction of new bone and provide effective local antimicrobial activity. Our research suggests that the vancomycin-loaded nHA pellets are an efficient treatment for MRSA-induced chronic osteomyelitis, especially when associated with bone defects.

Acknowledgments This work was supported by the National Basic Research Program of China (973 program) (2009CB930002); Program of Shanghai Subject Chief Scientist (A type) (07XD14006); National Natural Science Foundation of China (30970718) and the National High Tech Research and Development Program (863 program) (2007AA03Z313).

References

- Lazzarini L, Mader JT, Calhoun JH. Osteomyelitis in long bones. *J Bone Joint Surg Am.* 2004;86(10):2305–18.
- Kinik H, Karaduman M. Cierny-Mader Type III chronic osteomyelitis: the results of patients treated with debridement, irrigation, vancomycin beads and systemic antibiotics. *Int Orthop.* 2008;32(4):551–8.
- Roeder B, Van Gils CC, Maling S. Antibiotic beads in the treatment of diabetic pedal osteomyelitis. *J Foot Ankle Surg.* 2000;39(2):124–30.
- Liu SJ, Wen-Neng SU, Lin SS, Chan EC. In vivo release of vancomycin from biodegradable beads. *J Biomed Mater Res.* 2002;63(6):807–13.
- Yamamoto T, Onga T, Marui T, Mizuno K. Use of hydroxyapatite to fill cavities after excision of benign bone tumours. Clinical results. *J Bone Joint Surg Br.* 2000;82(8):1117–20.
- Queiroz AC, Santos JD, Monteiro FJ. Porous HA scaffolds for drug releasing key engineering materials. *Bioceramics.* 2005;17:407–10. (Volumes 284–286).
- Hasegawa M, Sudo A, Komlev VS, Barinov SM, Uchida A. High release of antibiotic from a novel hydroxyapatite with bimodal pore size distribution. *J Biomed Mater Res B Appl Biomater.* 2004;70(2):332–9.
- Ferraz MP, Mateus AY, Sousa JC, Monteiro FJ. Nanohydroxyapatite microspheres as delivery system for antibiotics: release kinetics, antimicrobial activity, and interaction with osteoblasts. *J Biomed Mater Res A.* 2007;81(4):994–1004.
- Joosten U, Joist A, Gosheger G, Liljenqvist U, Brandt B, von Eiff C. Effectiveness of hydroxyapatite-vancomycin bone cement in the treatment of *Staphylococcus aureus* induced chronic osteomyelitis. *Biomaterials.* 2005;26(25):5251–8.
- Webster TJ, Siegel RW, Bizios R. Enhanced surface and mechanical properties of nanophase ceramics to achieve orthopaedic/dental implant efficacy. *Bioceramics.* 2000;13:321–4.

11. Liao J, Zhang L, Zuo Y, Wang H, Li J, Zuo Q, Li Y. Development of nanohydroxyapatite/polycarbonate composite for bone repair. *J Biomater Appl.* 2009;24(1):31–45.
12. Zhu W, Xiao J, Wang D, Liu J, Xiong J, Liu L, et al. Experimental study of nano-HA artificial bone with different pore sizes for repairing the radial defect. *Int Orthop.* 2009;33(2):567–71.
13. Zhu XL, Eibl O, Berthold C, Scheideler L, Geis-Gerstorfer J. Structural characterization of nanocrystalline hydroxyapatite and adhesion of pre-osteoblast cells. *Nanotechnology.* 2006;17(11):2711–21.
14. Meirelles L, Albrektsson T, Kjellin P, Arvidsson A, Franke-Stenport V, Andersson M, et al. Bone reaction to nano hydroxyapatite modified titanium implants placed in a gap-healing model. *J Biomed Mater Res A.* 2008;87(3):624–31.
15. Boissard CI, Bourban PE, Tami AE, Alini M, Eglin D. Nano-hydroxyapatite/poly(ester urethane) scaffold for bone tissue engineering. *Acta Biomater.* 2009;5(9):3316–27.
16. Cunniffe GM, Dickson GR, Partap S, Stanton KT, O'Brien FJ. Development and characterisation of a collagen nano-hydroxyapatite composite scaffold for bone tissue engineering. *J Mater Sci Mater Med.* 2010;21(8):2293–8.
17. Appleford MR, Oh S, Oh N, Ong JL. In vivo study on hydroxyapatite scaffolds with trabecular architecture for bone repair. *J Biomed Mater Res A.* 2009;89(4):1019–27.
18. Shirliff ME, Calhoun JH, Mader JT. Experimental osteomyelitis treatment with antibiotic-impregnated hydroxyapatite. *Clin Orthop Relat Res.* 2002;401:239–47.
19. Norden CW. Experimental chronic staphylococcal osteomyelitis in rabbits: treatment with rifampin alone and in combination with other antimicrobial agents. *Rev Infect Dis.* 1983;5(3):S491–4.
20. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol.* 1991;29(10):2240–4.
21. Smeltzer MS, Thomas JR, Hickmon SG, Skinner AR, Nelson CL, Griffith D, Parr TR Jr, Evans RP. Characterization of a rabbit model of staphylococcal osteomyelitis. *J Orthop Res.* 1997;15(3):414–21.
22. Lew DP, Waldvogel FA. Osteomyelitis. *Lancet.* 2004;364(9431):369–79.
23. Lamp KC, Friedrich LV, Mendez-Vigo L, Russo R. Clinical experience with daptomycin for the treatment of patients with osteomyelitis. *Am J Med.* 2007;120(10):S13–20.
24. Perloth JK, Kuo M, Tan J, Bayer AS, Miller LG. Adjunctive use of rifampin for the treatment of *Staphylococcus aureus* infections: a systematic review of the literature. *Arch Intern Med.* 2008;168(8):805–19.
25. Obadia AGL, Daculsi G, Bouler JM. Calcium-deficient apatite: influence of granule size and consolidation mode on release and in vitro activity of vancomycin. *Biomaterials.* 2003;24:1265–70.
26. Sun Y, Zhang C, Jin D, Sheng J, Cheng X, Liu X, et al. Free vascularised fibular grafting in the treatment of large skeletal defects due to osteomyelitis. *Int Orthop.* 2010;34(3):425–30.
27. Ueng SW, Lee MS, Lin SS, Chan EC, Liu SJ. Development of a biodegradable alginate carrier system for antibiotics and bone cells. *J Orthop Res.* 2007;25(1):62–72.
28. Murugan R, Ramakrishna S. Aqueous mediated synthesis of bioresorbable nanocrystalline Hydroxyapatite. *J Cryst Growth.* 2005;274(1–2):209–13.