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Original Article

Efficacy of tobramycin-loaded coating K-wire in an open-fracture rabbit model contaminated by staphylococcus aureus

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Abstract: The incidence of implant-related infections has recently increased, especially in open fractures. While a prolonged systemic antibiotics application may lead to drug resistance, local or systemic toxicity and potentially compromise bone growth, immune system surveillance, etc. In this study, we developed a rabbit fracture model that contaminated by staphylococcus aureus in order to evaluate the antimicrobial efficacy of the tobramycin-PDLLA-coated titanium Kirschner-wire drug delivery system. A transverse osteotomy of the rabbit's tibia was taken, followed by inoculation of phosphate-buffered saline or S. aureus. Simultaneously Kirschner wires coated with PDLLA or tobramycin-PDLLA were implanted into rabbit tibiae correspondingly. All animals were microbiologically, histologically and radiologically monitored or detected. At the 8 weeks of endpoint, 4 out of 6 animals of group III (PDLLA-coated + S. aureus) demonstrated partially healed fractures or non-union. Whereas all 6 animals of group I (uncoated + PBS) and group II (PDLLA-coated + PBS) and 5 of group IV (tobramycin-PDLLA-coated + S. aureus) showed healed fractures. The animals that were identified to have kidney infections (PDLLA-coated + S. aureus group) corresponded to the animals that showed histological signs of infection. The study showed that tobramycin-PDLLA was effective as a local drug delivery material for application at the fracture site to control bacterial infection in the contaminated osteotomy rabbits. Moreover, the kidney of rabbits without a local tobramycin delivery showed histological signs of infection.

Keywords: Contaminated open fracture, implant-related infection, PDLLA coating, staphylococcus aureus, tobramycin

Introduction

The incidence of implant-related infections has increased over the past decade despite the widespread use of intravenous antibiotic prophylaxis and a focus on aseptic surgical technique [1-3]. Infections of orthopaedic and reconstructive devices occur in approximately 5% of cases and total about 100,000 cases per year in the USA alone [4-6]. Implant-related infections are a substantial cause of morbidity and even mortality, leading to prolonged hospitalization, poor functional outcome, sepsis [7-9], and also are very costly to the patient and society in general [4, 6].

Bacteria, especially Staphylococcus aureus (S. aureus) can colonize the surface of an implant, forming a biofilm of an extracellular glycocalyxon implanted metallic plastic materials. Although biofilm play an important role in technical processes like bioremediation, or bioprocessing [10], it indeed blocks penetration of immune cells and antibiotics, promoting bacterial survival [1, 6, 11, 12], which may lead to persistent infection of the underlying bone and the adjacent soft tissues [4, 13]. Therefore, implant-related infections often require aggressive treatment including removal of the infected implant, multiple revisions with radical debridement, and long-term systemic antibiotic therapy [14, 15]. Thus, killing bacteria locally in the early stage appears extremely important in the prophylaxis or treatment of implant-related infections.

However, prolonged systemic use of antibiotics at higher doses to cure such implant-related

infections may lead to drug resistance, local or systemic toxicity and potentially compromise bone growth, immune system surveillance and implant osseointegration [4]. Such limitations have prompted the development of various systems for the local administration of antibiotics at the tissue-implant interface [14]. Local application of antibiotics is advantageous in that high local antibiotic levels without systemic toxicity [15]. Local antibiotic delivery systems, such as antibiotic impregnated polymethylmethacrylate cements (PMMA), can reduce the risk of infection directly at the site of implant and the surrounding tissue [16]. Unfortunately, PMMA is a non-absorbable material, it is associated with a number of serious disadvantages, such as foreign body reaction and secondary infections [17]. In most recent years, the attention has progressively moved to resorbable materials, which can be left in situ and do not require later surgical removal. A broad variety of these materials are ranging from inorganic to organic, natural to synthetic, such as Poly (D, L-lactide) (PDLLA), Poly (D, L-lactic-co-glycolic acid) (PLGA), and Hydroxyapatite (HA) are of the most widely used [18]. Furthermore, there are quite a lot studies in the literature showing that PDLLA has excellent features with respect to implant coating, with high mechanical stability, good osteoinductive potential and excellent biocompatibility in vivo and in vitro [19].

In this study, we investigated a biodegradable, tobramycin-loaded PDLLA coating, which can be applied on the surface of orthopaedic implants for local antibiotic delivery. The antimicrobial efficacy and the effects on fracture healing of these coatings were evaluated in a contaminated open fracture model on rabbits in vivo. Moreover, the potential systemic and local toxicity were studied as well.

Materials and methods

This study was carried out in strict accordance with the guidelines of the Local Animal Welfare Committee for the Care and Use of Laboratory Animals (Permit Number: 2014KY097). A total of 24 New Zealand White rabbits weighing 2.81±0.35 kg were randomly assigned to four groups: Group I treated with uncoated wire + 1 ml PBS (control, n=6); Group II treated with PDLLA-coated wire + 1 ml PBS (n=6); Group III treated with PDLLA-coated wire + S. Aureus 10^6 CFU/1 ml (CFU, colony forming units, n=6); Group IV treated with tobramycin-PDLLA-coated wire + S. aureus 10^6 CFU/1 ml (n=6).

Implants

Kirschner wires (Shanghai Microport Orthopedics Co., Ltd, China) with a solid Ti6Al4V core were used. The implant had a total diameter of 2 mm and a length of 90 mm. The implants were either uncoated or PDLLA (Boehringer Ingelheim, Germany) coated with a thickness of about 50 μm [20, 21]. The tobramycin-PDLLA-coated Kirschner wires containing 25% w/w tobramycin, the total tobramycin load is 4 mg. All implants were sterilized by gamma irradiation [22].

Bacteria preparation

S. aureus ATCC 25923 (American Type Culture Collection, Manassas, VA) was used to induce infection in the implant bed. This strain had been previously used in several other infection studies and its ability to cause osteomyelitis in rabbits has been proven [23, 24]. S. aureus was streaked onto Mueller-Hinton agar (MHA) and grown at 37°C overnight. Bacterial cells were pelleted, resuspended and washed twice with sterile phosphate-buffered saline (PBS, pH 7.4). The bacterial suspension was diluted with sterile PBS to a concentration of 10^6 CFU/ml.

Surgical procedure

Anaesthesia was performed by using an intravenous infusion of 10% chloral hydrate solution (2 ml/kg body weight). The right hindleg of each rabbit was shaved, depilated and disinfected with 70% alcohol. All surgical procedures were performed under sterile conditions with a different set of surgical instruments for each site.

The skin and fascia was incised longitudinally over the lateral right hindleg. Dissection continued to retract the muscles until the midshaft tibia. A transverse mid shaft osteotomy was made with a wire saw. Another longitudinal incision was made at the proximal tibial metaphysis to expose the tibial tuberosity. With a hand-driven burr a 3 mm diameter hole was drilled over the tibial tuberosity. After that, 1 ml of either PBS (control) or suspensions containing 10^6 CFU/1 ml of S. aureus were inoculated into the medullary cavity of the fracture edges correspondingly. The fractured tibia then was

reduced and held manually. According to study groups, uncoated Kirschner wires, PDLLA-coated or tobramycin-PDLLA-coated wires were inserted into the medullary canal. The wound was then closed in layers using a non-absorbable suture and post-operative X-ray control was performed. Full-limb light splints were used as external fixations for fracture recovery. Each rabbit was kept in a separate cage, and after recovery, activity and weight bearing were not restricted.

General assessment

The animals were monitored daily to evaluate the wound healing, signs of sepsis, weight bearing, activity. On the day of surgery and in weekly intervals throughout the observation period, body weight and body temperature of the animals were measured. During dissection for bone harvest, the soft tissue and the intramedullary cavity was assessed for pus, abscess formation, sinus drainage, cortical lysis, malunion, and non-union.

Radiographic evaluation

Radiographs of the right tibia in posterior-anterior and lateral view were performed on weeks 0, 2, 4, and 8. To assess development and progression of bone infection, the following radiographic appearances were assessed according to a modified score by An et al [25]: 1. Periosteal reaction. 2. Osteolysis. 3. Soft tissue swelling. 4. General impression. 5. Sequestrum formation. The maximum score to be achieved was 13. To evaluate the progression of fracture healing, a radiographic scaling system developed by *Whelan* et al [26], the Radiographic Union Score for tibial fractures (RUST) was implied. The individual RUST with 12 being the maximum and 4 being the minimum score, indicating that the fracture is definitely healed or not.

Laboratory analysis

Blood samples were taken on day 0, 1, 3, 7, 14, 28, 56. Laboratory parameters analyzed included haemoglobin, leukocyte count, C-reactive protein (CRP), kidney function index such as creatinine and urea nitrogen. The levels of parameters mentioned above were determined using commercial assay kits (Kanto Chemical Co., Inc., Tokyo, Japan).

To determine the tobramycin release profile *in vivo*, blood samples were taken at 30 minutes, 2, 8, 24 hours, and on day 3, 7, 28, 56. In the meantime, urine samples were collected on day 1, 2, 3, 7, 28, 56 to analyse the duration of renal excretion of tobramycin. The concentration of the drug in the blood and urine was determined using high-performance liquid chromatography (HPLC)/evaporative light scattering detector (ELSD) method [27]. The HPLC/ELSD analysis was carried out by using a Shimadzu VP Series HPLC (Duisburg, Germany).

Microbiological evaluation

The Kirschner wires which had been explanted under sterile manner were inoculated on the nutrient agar plates using an inoculation loop and then placed in the tryptic soy broth (TSB). Swabs taken from the fracture edges of osteotomy were smeared over the agar plates. 3 ml of blood was collected for culture at the first 48 hours after the inoculation of S. aureus. Besides, 3 ml of blood and urine were also collected for culture on the day of sacrifice. The agar plates and TSB were incubated at 37°C for 24 hours. Infection was determined as positive S. aureus CFUs growth on the agar plates and cloudy appearance in the TSB. The number of CFUs was counted for each positive agar plate.

Histological analysis

The tibiae were cut with a wire saw at 1.5 cm distal and proximal to the original osteotomy site, transverse to the shaft axis. These specimens were fixed in 4% formaldehyde for 24 hours, and subsequently decalcified, processed and embedded in paraffin. The kidneys of each animal were dissected out and embedded in paraffin as well. Longitudinal sections in a sagittal plane of 5 μm thickness were cut and then were stained with hematoxylin-eosin (H&E) for light microscope examination using conventional protocol.

Statistical analysis

Statistical analysis was done using the Mann-Whitney *U* test for comparisons among those four groups and Kruskal-Wallis test for multiple comparisons. All data were expressed as the means ± standard deviation (SD). Values of $P<0.05$ were considered statistically significant. SPSS for Windows (Version 22) was used for all analyses.

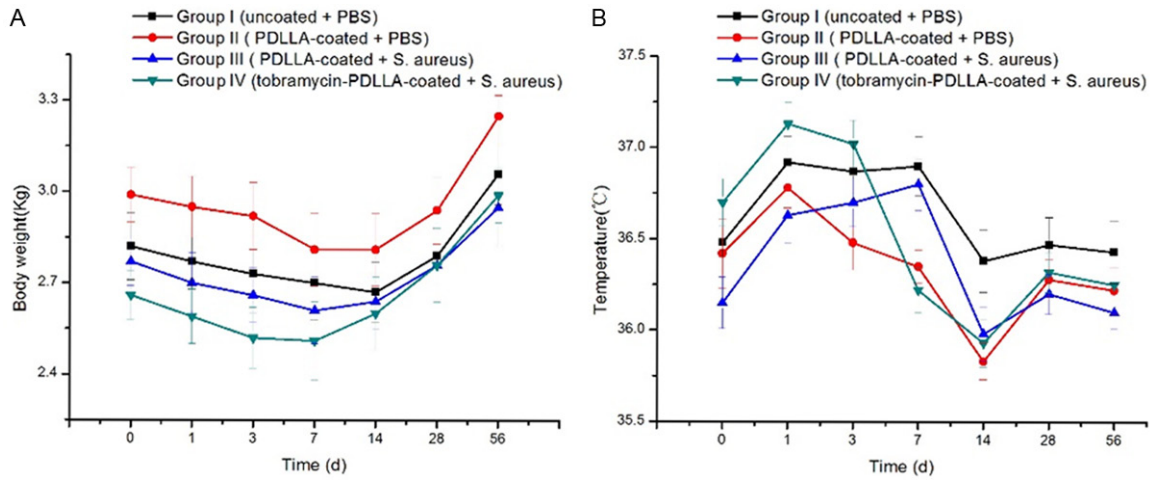

Figure 1. Mean values of body weight and temperature of all groups during the observation period. A. Body weight slightly decreased and reached the minimum on day 7, followed by a continuous gain of weight in all groups. B. Body temperature slightly increased after postoperation and remained within the normal fluctuation margins in all groups.

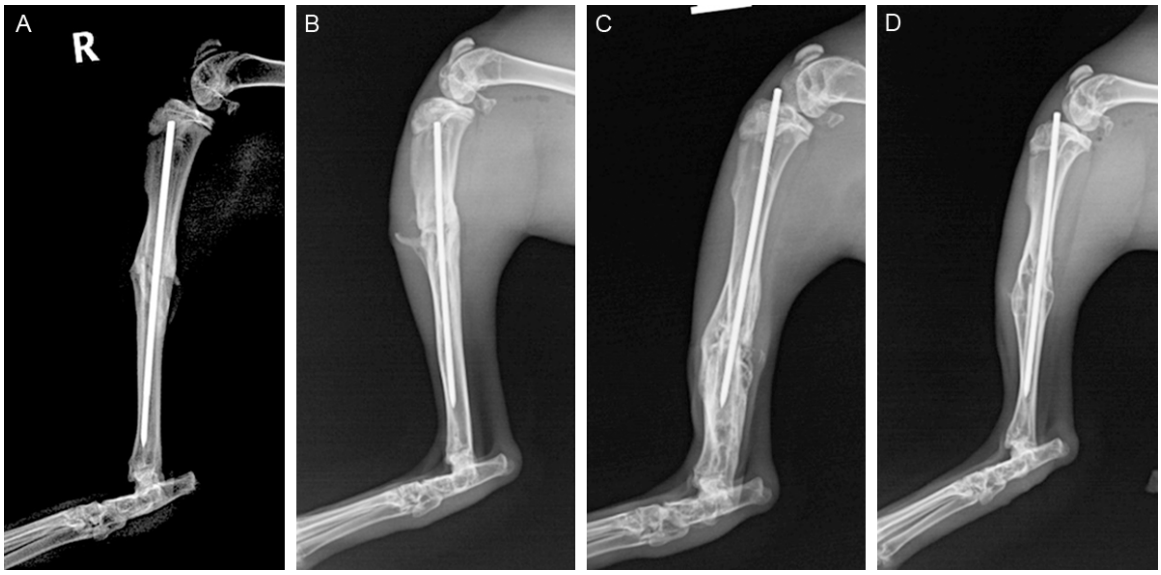

Figure 2. X-rays of right tibiae in lateral view on day 56 post-operative of all groups. A. Group I (uncoated + PBS, control). Healed fracture and physiological callus without any radiographic appearance of infection. B. Group II (PDLLA-coated + PBS). No radiographic signs of infection with healed fracture and physiological callus. C. Group III (PDLLA-coated + S. aureus). A typical chronically osteomyelitis of the tibia, including reactive pathological subperiosteal new bone formation, as well as cortical bone destruction and sequestra. D. Group IV (tobramycin-PDLLA-coated + S. aureus). Healed fracture and mild cortex structure disorders, but without typical radiographic appearance of osteomyelitis.

Results

General assessment results

All 24 rabbits recovered well from an aesthesia and surgeries, then survived to the 8 weeks of endpoint. Body weights and body temperatures remained stable in all groups during the experimental period (**Figure 1**). Postmortem examination, 4 out of 6 animals in group III showed clearly clinical signs of purulent infections, while other rabbits revealed normal soft tissue and callus. All infected rabbits did not show any signs of systemic septic disease during the entire observation period of 8 weeks.

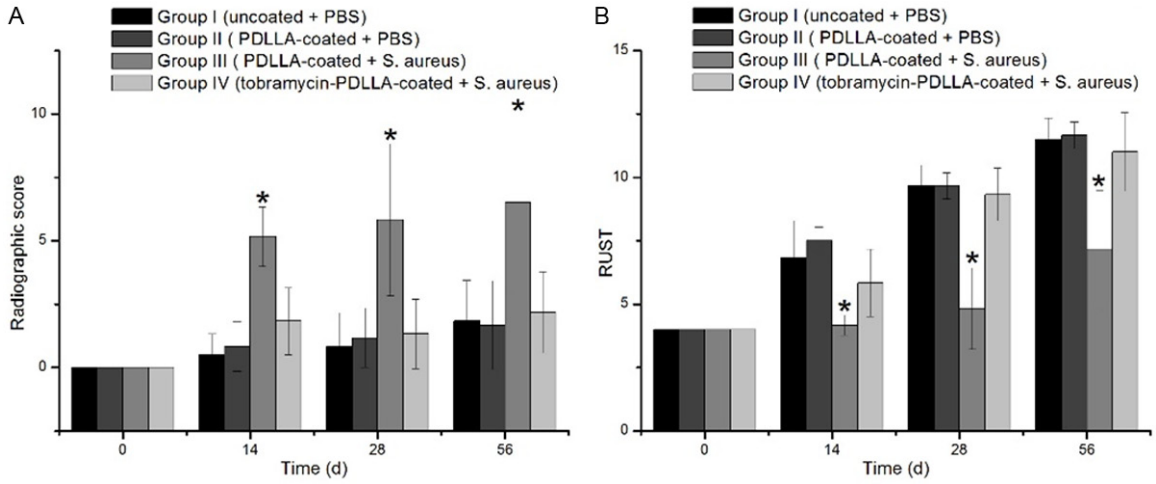

Figure 3. Mean radiographic score values and RUST of all groups on day 0, 14, 28, 56 post-operative. *represents significant to the other 3 groups at the same time point, P<0.05.

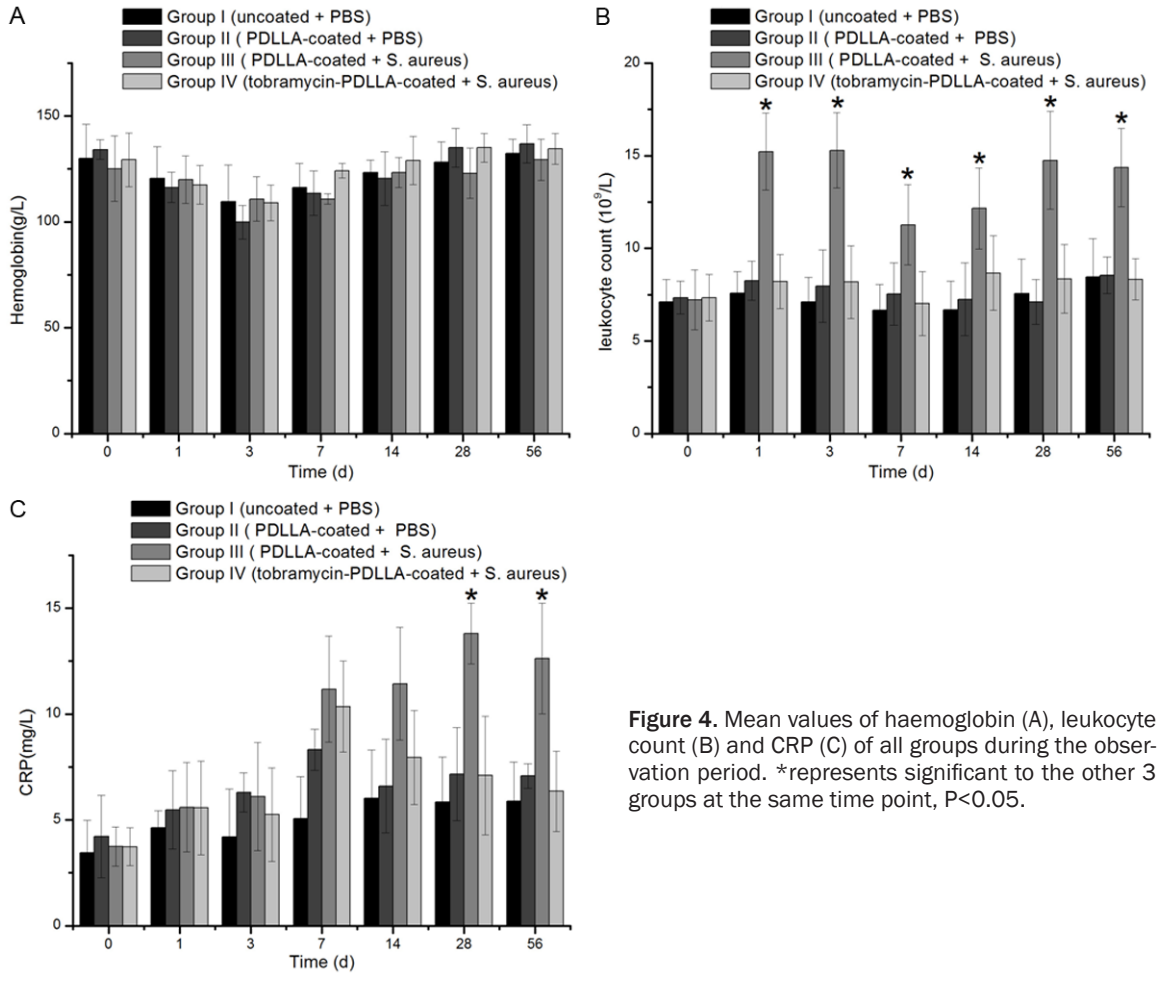

Figure 4. Mean values of haemoglobin (A), leukocyte count (B) and CRP (C) of all groups during the observation period. *represents significant to the other 3 groups at the same time point, P<0.05.

Radiographic results

X-rays of group III (PDLLA-coated + S. aureus) revealed radiographic signs of infection in all 6 animals, soft tissue swelling and osseous destruction could be clearly detectable after 2 weeks. At the 8 weeks of endpoint, 4 out of 6 animals of group III demonstrated partially

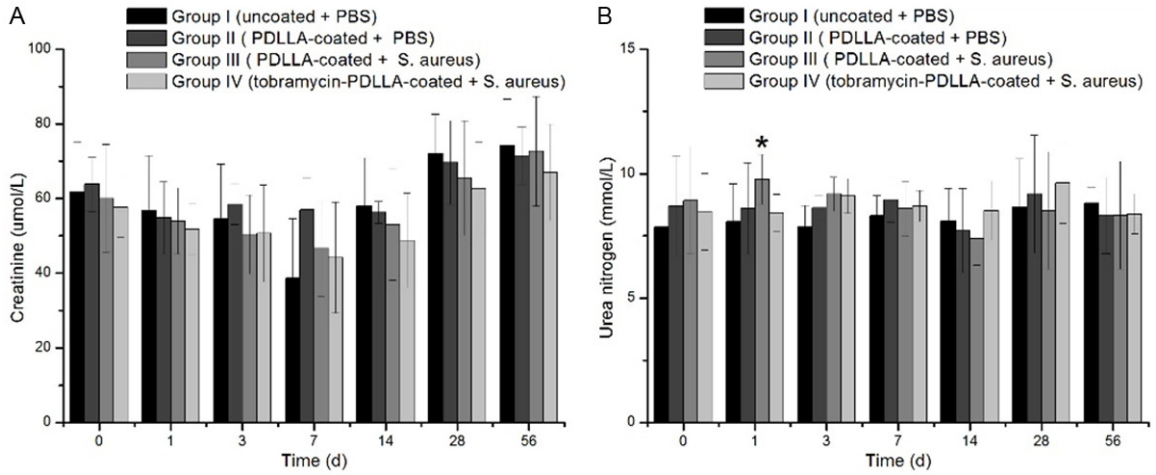

Figure 5. Mean values of creatinine (A) and urea nitrogen (B) of all groups during the observation period. A statistical significance difference of urea nitrogen was observed at the first day after surgery. *represents significant to the other 3 groups at the same time point, P<0.05.

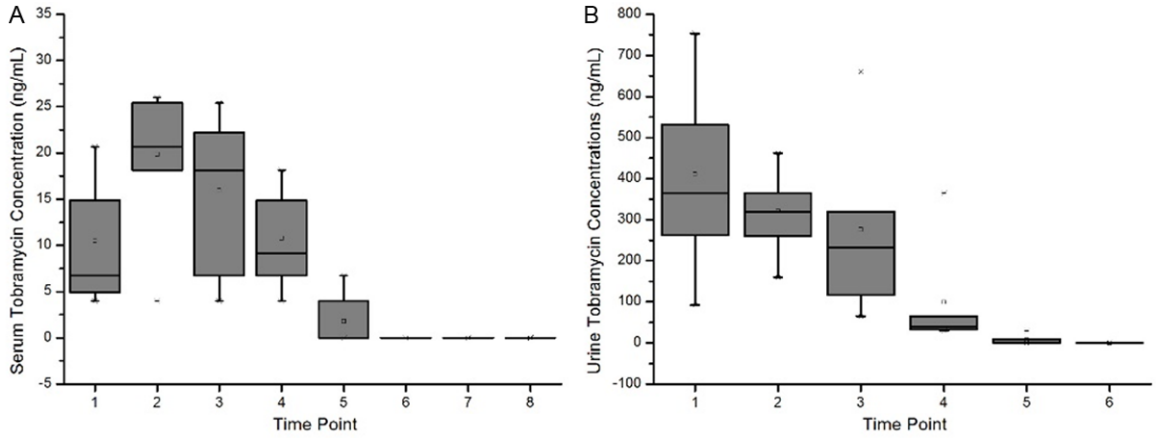

Figure 6. The serum and urine tobramycin concentration of group IV (tobramycin-PDLLA-coated). A. The serum tobramycin concentration. The time point was respectively defined as 0.5 h, 2 h, 8 h, 24 h and 3, 7, 28, 56 days. B. The urine tobramycin concentration. The time point was respectively defined as 1, 2, 3, 7, 28, 56 days.

healed fractures (one or two bridged cortices) or non-union. Whereas all 6 rabbits in group I (uncoated + PBS) or group II (PDLLA-coated + PBS), 5 of group IV (tobramycin-PDLLA-coated + S. aureus) showed healed fractures (three or four bridged cortices) and did not present any of radiographic appearance of infection on X-rays. 1 out of 6 rabbits from group IV appeared to be less severe radiographic signs of infection with healed fractures (**Figure 2**).

Throughout the observation period, radiographic score of infection and RUST of group III (PDLLA-coated + S. aureus) were assessed, which seemed statistically significant (P<0.05) as compared to the other 3 groups (**Figure 3**).

Laboratory results

There was no significantly statistical difference of the hemoglobin level among all groups at each time point during the experiment (**Figure 4**). Leukocyte count showed a significant increase postoperation in group III (PDLLA-coated + S. aureus) and reached the highest values 3 days after surgery. At the 56 day of endpoint, leukocyte count and C-reactive protein (CRP) of group III statistically increased (P<0.05) compared to that in other 3 groups (**Figure 4**).

Although there were some variations of renal function values, creatinine and urea nitrogen

Figure 7. The quadrant streaking cultures of explanted Kirschner wires on agar plates after 24 hours' incubation of all groups. A. Group I (uncoated/PBS): No culture growth. B. Group II (PDLLA/PBS): No culture growth. C. Group III (PDLLA/S. aureus): Massive bacterial growth on all of the four quarters. D. Group IV (tobramycin-PDLLA/S. aureus): Only a few colonies developed on the first quarter.

remained in the normal reference range throughout the experimental period (**Figure 5**).

Systemic tobramycin concentrations in the tobramycin-PDLLA-coated group (group IV) reached the highest values within the first 2 hours after surgery, a burst of the drug was released in the first 3 days, the peak concentrations measured in serum were 26.03 ng/mL and 753.48 ng/mL in urine (**Figure 6**). The serum tobramycin concentrations were below detectable levels in the majority of samples tested after 7 days. The excretion of tobramycin in the urine also reduced after 7 days, and detectable up to 28 days.

Microbiological results

All inoculated cultures of the Kirschner wires of group III (PDLLA-coated + S. aureus) showed positive culture growth of S. aureus on the agar plates, with a confirmed positive cultures (cloudy appearance) of the Kirschner wires immersed in TSB (**Figure 7**). All 6 cultures of

Table 1. Microbiological culture results

	Group I (uncoated/PBS)	Group II (PDLLA/PBS)	Group III (PDLLA/S. aureus)	Group IV (tobramycin-PDLLA/S. aureus)
Cultures of implants/agar	0/6	0/6	6/6 (>10^4 CFU)*	1/6 (<10^3 CFU)*
Cultures of implants/TSB	0/6	0/6	6/6#	1/6#
Cultures of smears	0/6	0/6	6/6*	1/6*
Culture of blood	0/6; 0/6	0/6; 0/6	0/6; 0/6	0/6; 0/6
Culture of urine	0/6	0/6	0/6	0/6

a). Row heading of culture of blood contains results of first 48 hours after the inoculation of S. aureus and the day of sacrifice, b). n=6 animals in each group, c). *represents tested positive on S. aureus, d). #represents cloudy broth.

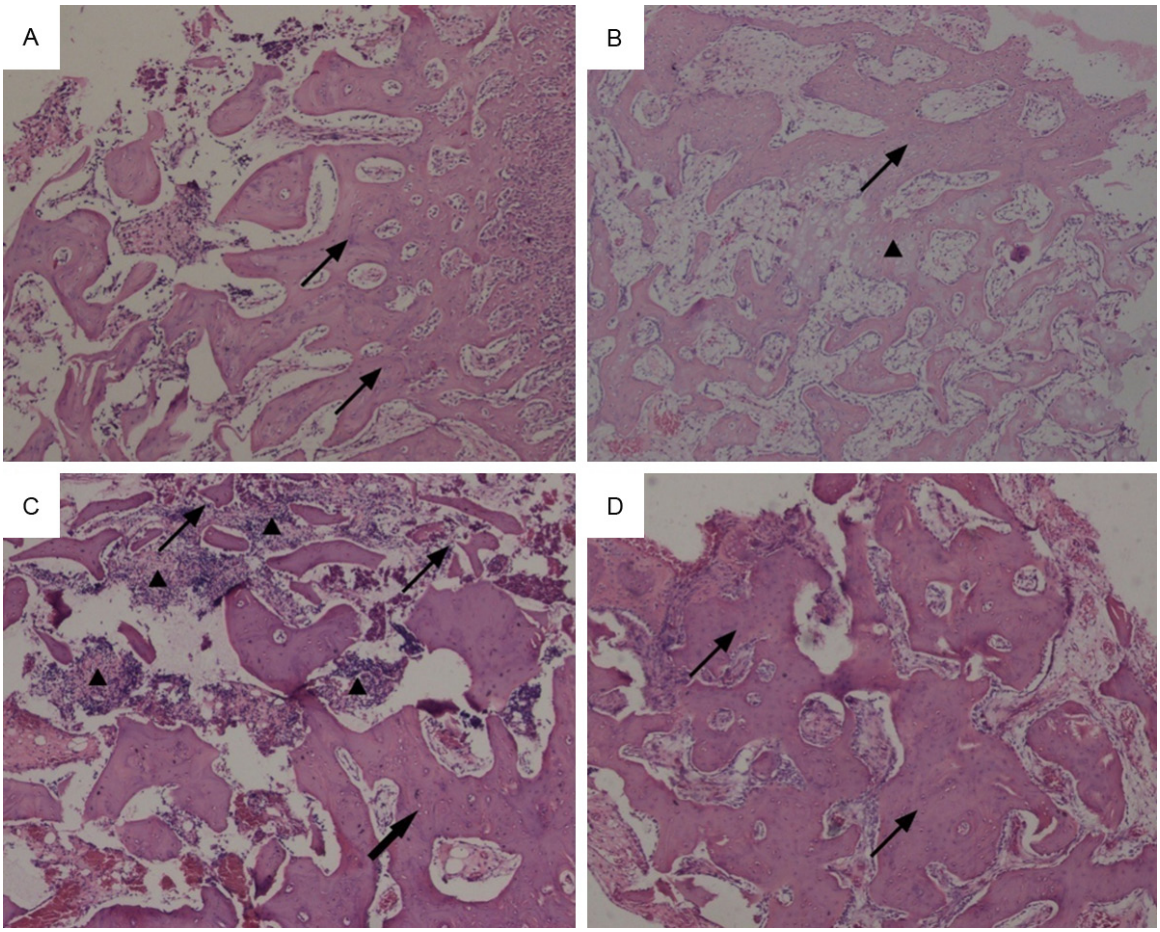

Figure 8. Histological slices in a sagittal plane performed from right tibia of all groups. A. Group I (uncoated + PBS): Vigorous ossification activity of new trabecular bone (black arrow), with no histopathological signs of bone infection. B. Group II (PDLLA-coated + PBS): Vigorous endochondral ossification activity of healing bone (triangle) with new trabecular bone (black arrow). No histopathological signs of bone infection. C. Group III (PDLLA-coated + S. aureus): Disorganized trabecular bones (black arrow) with a mass of cells infiltration (triangle) and reactive new bone formation (black block arrow) are clearly visible. D. Group IV (tobramycin-PDLLA-coated + S. aureus): Reactive trabecular bone hyperplasia (black arrow) without signs of bone infection are evident.

group I (uncoated + PBS) and group II (PDLLA-coated + PBS) and 5 of group IV (tobramycin-PDLLA-coated + S. aureus) remained no viable colonies (**Figure 7**). Only one plate of group IV revealed a few S. aureus colony formations (**Figure 7**). As a result, infection rates were 0 (0 of 6) for group I and group II, 100% (6 of 6) for group III, and 16.7% (1 of 6) for group IV. There

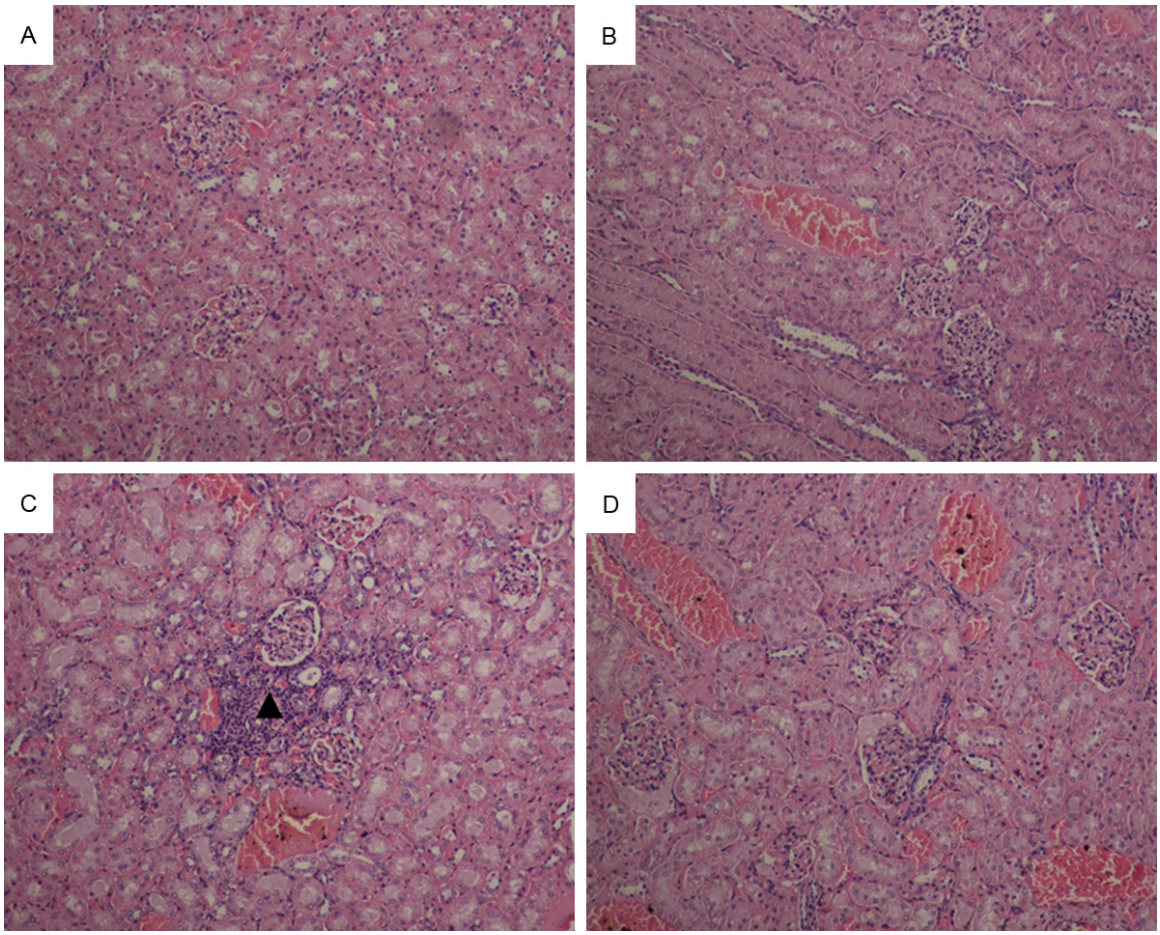

Figure 9. Histological slices in a sagittal plane performed from kidney of all groups. A. Group I (uncoated + PBS): Normal glomerular and tubular structures. B. Group II (PDLLA-coated + PBS): Normal glomerular and tubular structures. C. Group III (PDLLA-coated + S. aureus): Kidney infection with inflammatory cells infiltration in the tubules and interstitium (triangle). D. Group IV (tobramycin-PDLLA-coated + S. aureus): Normal glomerular and tubular structures.

was a statistically highly significant reduction of infection rates by group IV compared to group III ($P<0.05$).

All smears taken from the fracture edges of group III were found to be positive on S. aureus, in contrast none for group I and group II, and 16.7% for group IV were positive (**Table 1**).

None of the blood and urine cultures obtained at the first 48 hours after the inoculation of S. aureus and the day of sacrifice tested positive on any microorganism in all groups (**Table 1**).

Histological results

The majority histological slices of tibia in group III (PDLLA-coated + S. aureus) showed typical histopathological findings of bone infection as destruction of cortical and cancellous bone, periosteal thickening and reactive pathological subperiosteal new bone formation, and abscess formation (**Figure 8**).

Infection-free animals of group I (uncoated + PBS), group II (PDLLA-coated + PBS), and group IV (tobramycin-PDLLA-coated + S. aureus) revealed vigorous osteoblastic activity of healing bone with varying degrees of fusion between the original cortex and the new outer cortex (**Figure 8**). Signs of infection as osteomyelitis or abscess formation were not observed. Whereas in the other 2 animals of group IV, periosteal thickening and micro-abscesses could be seen, which appeared to be less severe compared to group III.

All the histological slices of kidney in group I (uncoated + PBS), group II (PDLLA-coated + PBS), and group IV (tobramycin-PDLLA-coated

+ S. aureus) revealed normal nephron structure, typical histopathological performances of renal toxicity were not observed [28]. 3 of 6 histological slices in group III (PDLLA-coated + S. aureus) showed mild or moderate inflammations of the renal interstitial and nephron (**Figure 9**).

Discussion

Open fractures are fractures in which the bone has violated the skin and soft tissue. Microbes are known to complicate open fracture healing through infections and biofilms which are complex communities of bacteria that create extracellular polymers that allow them to adhere to each other, as well as to implanted devices, potentially playing roles innonunion/malunion cases [29]. Gustilo and Anderson found that, when culturing wound infections, staphylococci (specifically coagulase positive staphylococci such as Staphylococcus aureus) were the most commonly isolated organisms [30].

Tobramycin belongs to aminoglycoside antibiotics like gentamicin, was chosen for its broad anti-bacterial spectra, lower resistance rates, long-lasting postantibiotic effect, chemical stability under various conditions, higher thermal stabilities and less possibility of kidney damage compared to gentamicin [31], it has been widely used both in antibiotic loaded PMMA cements and antibiotic loaded coatings on titanium implants. Tobramycin was liberated from the PDLLA coating with an initial burst, the serum tobramycin concentration reached the highest values within the first 2 hours after surgery, and maintained at a high level within the first 3 days, followed by a sustained release, which could be sufficient for infection prophylaxis initially and bacteria inhibition over a longer period subsequently. Local application of tobramycin is advantageous in that high local antibiotic levels without systemic toxicity can be achieved. What's more, the purpose of our study is whether tobramycin-PDLLA-coated Kirschner wire can reduce the open fracture infection rates or not. It is infection prophylaxis, not treatments to implant-related infections, which includes indispensable bacterial drug sensitive test to choose antibiotics accurately. We investigated a contaminated by staphylococcus aureus osteotomy model of rabbit which recapitulated the clinical scenario of open fracture in this study. At the 8 weeks of endpoint, the combination influence of fracture trauma, implant colonization, bone and surrounding soft tissue infection resulting in an altered bone healing response that eventually leads to delayed union or nonunion in Group III (PDLLA-coated + S. aureus). Radiographic evaluation showed a highly significant reduction of bony destruction and an evident formation of physiological callus throughout the follow-up period in animals that received tobramycin-PDLLA coated Kirschner wires in group IV. Besides, bacterial colony formation of S. aureus in animals that received tobramycin-PDLLA coated Kirschner wires in group IV was significantly reduced, 83.3% animals were free of infection. Compared to the noncontaminated control rabbits of group I (uncoated + PBS) and group II (PDLLA-coated + PBS), no significant difference of radiographic scores, leucocyte count, ESR, body temperature and weight were observed, indicates that the previously inoculated bacteria were obviously killed or significantly reduced. Histopathological investigations confirmed these results, vigorous physiological osteoblastic activity of healing bone could be seen.

As indicated form results of the study, PDLLA was effective as a local drug delivery material for tobramycin application from titanium Kirschner wires at the fracture site to control bacterial infection. PDLLA coatings, originally designed to enhance osseointegration of implants, have shown effectiveness as a matrix for controlled release of bioactive substances [15, 32, 33]. The release of the antibiotics from the PDLLA coatings is slower than that from HA coatings, which more than 80-90% of the antibiotics are released within the first 60 min [34]. However, the elution kinetics of antibiotics from the PDLLA coating is still not sustainable enough and currently this method has not been translated to the clinic easily. Recently, there is increasing interest in the concepts such as multiple functionalities for surface coating of implants [35, 36] and immobilization of antibacterials to the implant surface [37]. However, those approaches are still in the initial stage of development. The future of implant coating technology relates to the prevention and treatment of deep infections still has a long way to go.

In addition, it is important to note that half animals in group III (PDLLA-coated + S. aureus) revealed infection of the renal parenchyma at

sacrifice, because inflammatory cells, including polymorphonuclear and lymphocyte, infiltration in the tubules and interstitium, were observed from histological slices in group III. Furthermore, necrosis of renal tubular and glomerulus, which is pathological sign of renal toxicity associated with aminoglycoside antibiotics, was not detected from all groups. While none of the blood and urine cultures obtained at the first 48 hours after the inoculation and sacrifice tested positive on any microorganism. Although there were fluctuations during the study period, no significant differences in the measurement of creatinine and urea nitrogen were observed compared to the pre-operative levels. In view of this result, we deduced that it is corresponding to the subclinical pyelonephritis, which occurs most often in debilitated, chronically ill patients. Under these circumstances metastatic staphylococcal infections may spread to the kidney from distant foci in the bone or skin [38]. In this case, we attribute the subclinical pyelonephritis in group III (PDLLA-coated + S. aureus) to the chronic osteomyelitis in the tibia. In spite of the normal renal function parameters, extended renal parenchyma infection may result in chronic renal scarring and permanent renal damage, which can subsequently lead to diverse complications [39, 40]. As a matter of fact, systemic perioperative application of antibiotics is essential in the treatment of open fractures, not only for the resistance of bacterial contamination around the fracture, but also for the prevention of secondary infection in other organs. Even though the validity of local antibiotic administration system is demonstrated, intravenous perioperative antibiotic prophylaxis is recommended as well in clinic.

In summary, we confirmed that tobramycin-loaded PDLLA coating K-wire was effective and safe at the fracture site to control bacterial infection in this osteotomy model of rabbits contaminated by staphylococcus aureus. Moreover, systemic perioperative application of antibiotics is indispensable despite the effectivity of local antibiotic delivery system.

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Disclosure of conflict of interest

None.

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References

[1] Bernthal NM, Stavrakis AI, Billi F, Cho JS, Kremen TJ, Simon SI, Cheung AL, Finerman GA, Lieberman JR, Adams JS. A mouse model of post-arthroplasty Staphylococcus aureus joint infection to evaluate in vivo the efficacy of antimicrobial implant coatings. PLoS One 2010; 5: e12580.

[2] Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K, Parvizi J. Infection burden for hip and knee arthroplasty in the United States. J Arthroplasty 2008; 23: 984-991.

[3] Bozic KJ, Ries MD. The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. J Bone Joint Surg Am 2005; 87: 1746-1751.

[4] Goodman SB, Yao Z, Keeney M, Yang F. The future of biologic coatings for orthopaedic implants. Biomaterials 2013; 34: 3174-3183.

[5] Moriarty TF, Schlegel U, Perren S, Richards RG. Infection in fracture fixation: can we influence infection rates through implant design? J Mater Sci Med 2010; 21: 1031-1035.

[6] Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med 2004; 350: 1422-1429.

[7] Schmidmaier G, Lucke M, Wildemann B, Haas NP, Raschke M. Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review. Injury 2006; 37: S105-S112.

[8] Young E, Sugarman B. Infections in prosthetic devices. Surg Clin North Am 1988; 68: 167-180.

[9] Bengtson S, Borgquist L, Lidgren L. Cost analysis of prophylaxis with antibiotics to prevent infected knee arthroplasty. BMJ 1989; 299: 719-720.

[10] Buchholz F, Harms H, Maskow T. Biofilm reserch calorimetry-a marriage made in heaven? Biotechnol J 2010; 5: 1339-1350.

[11] Del Pozo JL, Patel R. Infection associated with prosthetic joints. N Engl J Med 2009; 361: 787-794.
[12] Trampuz A, Widmer AF. Infections associated with orthopedic implants. Curr Opin Infec Dis 2006; 19: 349-356.
[13] Gristina AG. Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 1987; 237: 1588-1595.
[14] Fuchs T, Stange R, Schmidmaier G, Raschke MJ. The use of gentamicin-coated nails in the tibia: preliminary results of a prospective study. Arc Orthop Trauma Surg 2011; 131: 1419-1425.
[15] Lucke M, Schmidmaier G, Sadoni S, Wildemann B, Schiller R, Haas N, Raschke M. Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. Bone 2003; 32: 521-531.
[16] Diefenbeck M, Mückley T, Hofmann GO. Prophylaxis and treatment of implant-related infections by local application of antibiotics. Injury 2006; 37: S95-S104.
[17] Jiang JL, Li YF, Fang TL, Zhou J, Li XL, Wang YC, Dong J. Vancomycin-loaded nano-hydroxyapatite pellets to treat MRSA-induced chronic osteomyelitis with bone defect in rabbits. Inflamm Res 2012; 61: 207-215.
[18] Campoccia D, Montanaro L, Speziale P, Arciola CR. Antibiotic-loaded biomaterials and the risks for the spread of antibiotic resistance following their prophylactic and therapeutic clinical use. Biomaterials 2010; 31: 6363-6377.
[19] Tang L, Zhao C, Xiong Y, Wang A. Preparation, antibacterial properties and biocompatibility studies on vancomycin-poly(D,L)-lactic loaded-plates. Int Orthop 2010; 34: 755-759.
[20] Radin S, Ducheyne P. Controlled release of vancomycin from thin sol-gel films on titanium alloy fracture plate material. Biomaterials 2007; 28: 1721-1729.
[21] Adams CS, Antoci V, Harrison G, Patal P, Freeman TA, Shapiro IM, Parvizi J, Hickok NJ, Radin S, Ducheyne P. Controlled release of vancomycin from thin sol-gel films on implant surfaces successfully controls osteomyelitis. J Orthop Res 2009; 27: 701-709.
[22] Moojen DJ, Vogely HC, Fleer A, Nikkels PG, Higham PA, Verbout AJ, Castelein RM, Dhert WJ. Prophylaxis of infection and effects on osseointegration using a tobramycin-periapatite coating on titanium implants-An experimental study in the rabbit. J Orthop Res 2009; 27: 710-716.
[23] Koort JK, Suokas E, Veiranto M, Mäkinen TJ, Jalava J, Törmälä P, Aro HT. In vitro and in vivo testing of bioabsorbable antibiotic containing bone filler for osteomyelitis treatment. J Biomed Mater Res A 2006; 78: 532-540.
[24] Jia WT, Zhang CQ, Wang JQ, Feng Y, Ai ZS. The prophylactic effects of platelet-leucocyte gel in osteomyelitis: an experimental study in a rabbit model. J Bone Joint Surg Br 2010; 92: 304-310.
[25] An YH, Friedman RJ. Animal models of orthopedic implant infection. J Invest Surg 1998; 11: 139-146.
[26] Whelan DB, Bhandari M, Stephen D, Kreder H, McKee MD, Zdero R, Schemitsch EH. Development of the radiographic union score for tibial fractures for the assessment of tibial fracture healing after intramedullary fixation. J Trauma 2010; 68: 629-632.
[27] Megoulas NC, Koupparis MA. Development and validation of a novel HPLC/ELSD method for the direct determination of tobramycin in pharmaceuticals, plasma, and urine. Anal Bioanal Chem 2005; 382: 290-296.
[28] Baradaran A, Rafieian-kopaei M. Histopathological study of the combination of metformin and garlic juice for the attenuation of gentamicin renal toxicity in rats. J Renal Inj Prev 2013; 2: 15-21.
[29] Wallstabe S, Schulz AP, Paech A, Gerlach U. Is non-union of tibial shaft fractures due to non-culturable nonculturable bacterial pathogens? A clinical investigation using PCR and culture techniques. J Orthop Surg Res 2012; 7: 20.
[30] Gustilo RB, Anderson JT. Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. J Bone Joint Surg Am 1976; 58: 453-458.
[31] Vázquez-Espinosa E, Girón RM, Gómez-Punter RM, García-Castillo E, Valenzuela C, Cisneros C, Zamora E, García-Pérez FJ, Ancochea J. Long-term safety and efficacy of tobramycin in the management of cystic fibrosis. Ther Clin Risk Manag 2015, 11: 407-415.
[32] Lucke M, Schmidmaier G, Sadoni S, Wildemann B, Schiller R, Stemberger A, Haas N, Raschke M. A new model of implant-related osteomyelitis in rats. J Biomed Mater Res B Appl Biomater 2003; 67: 593-602.
[33] Schmidmaier G, Wildemann B, Stemberger A, Haas N, Raschke M. Biodegradable poly (D, L-lactide) coating of implants for continuous release of growth factors. J Biomedical Mater Res 2001; 58: 449-455.
[34] Radin S, Campbell JT, Ducheyne P, Cuckler JM. Calcium phosphate ceramic coatings as carriers of vancomycin. Biomaterials 1997; 18: 777-782.
[35] Muszanska AK, Busscher HJ, Herrmann A, van der Mei HC, Norde W. Pluronic-lysozyme conjugates as anti-adhesive and antibacterial bifunctional polymers for surface coating. Biomaterials 2011; 32: 6333-6341.

[36] Brohede U, Forsgren J, Roos S, Mihranyan A, Engqvist H, Stromme M. Multifunctional implant coatings providing possibilities for fast antibiotics loading with subsequent slow release. J Mater Sci Mater Med 2009; 20: 1859-1867.

[37] Hickok NJ, Shapiro IM. Immobilized antibiotics to prevent orthopaedic implant infections. Adv Drug Deliv Rev 2012; 64: 1165-1176.

[38] Ramakrishnan K, Scheid DC. Diagnosis and management of acute pyelonephritis in adults. Am Fam Physician 2005; 71: 933-942.

[39] Orellana P, Baquedano P, Rangarajan V, Zhao JH, Eng ND, Fettich J, Chaiwatanarat T, Sonmezoglu K, Kumar D, Park YH. Relationship between acute pyelonephritis, renal scarring, and vesicoureteral reflux. Pediatr Nephro 2004; 19: 1122-1126.

[40] Czaja CA, Scholes D, Hooton TM, Stamm WE. Population-based epidemiologic analysis of acute pyelonephritis. Clin Infect Dis 2007; 45: 273-280.