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Research Paper

Effects of Local Application of Nano-silver on Osteomyelitis and Soft Tissue Infections: An Experimental Study in Rats

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Abstract

Purpose: Bone and soft tissue infections are among the least desired complications after orthopaedic surgery. This study analysed the *in vivo* effects of the local application of nano-silver particles (AgNPs) [1nm = 1 billionth of a meter] in soft tissue infections.

Materials-Method: An experimental osteomyelitis model was formed by inoculating both tibias of 24 rats with methicillin-resistant *Staphylococcus aureus*. The rats were followed without treatment for 21 days. Blood samples and tibial x-rays at day 21 confirmed the development of infection. Then, the rats were divided randomly into two groups. One group (12 rats) underwent surgical debridement and received 21 days of teicoplanin therapy. The second group had the same treatment, with the addition of local nano-silver. All of the rats were sacrificed at day 42. Blood and wound swab samples were taken and the culture results were analysed.

Results: No differences were observed between the groups in healing values at pathological examination, or in changes in the number of colonies at days 21 and 42. No differences in white blood cell count (WBC) were observed between the groups before and after the treatment.

Conclusion: Although *in vitro* studies suggest the effectiveness of AgNPs on pathogens, we found that the application of nano-silver did not make any difference when used in addition to the classical osteomyelitis treatment with antibiotics and local surgical debridement. We believe that additional *in vivo* studies using repeated nano-silver application could be beneficial.

Key words: Nano-silver, Osteomyelitis, Soft Tissue Infection

Introduction

The treatment of osteomyelitis is expensive and the increasing prevalence of antibiotic-resistant bacteria is resulting in longer hospital stays. The rate of osteomyelitis has increased with advances in technology due to the increased use of orthopaedic implants in surgical procedures (1). The reported rates

of deep tissue infection after intramedullary nailing and joint replacement surgery are 0.5–17% (2).

Several modalities have been described for the treatment of osteomyelitis, each with different advantages and side effects. The basis of treatment is intravenous antibiotic therapy and surgical debridement, including local antibiotic therapy, antibiotic-containing cement, bone grafts, antibiotic-soaked sponges, polymethylmethacrylate (PMMA) beads, and antibiotic-coated implants (3).

Silver ions exhibit antimicrobial activity, having both bactericidal and bacteriostatic effects. The role of silver in the treatment of burns, urinary tract infections, central venous catheter infections, and chronic osteomyelitis has been demonstrated (4).

The antifungal and antimicrobial effects of both micron- and nano-sized metal silver have been studied for years. Nano-sized silver particles can be used alone or can be coated or precipitated on a surface using various methods, such as biochemical reduction, ultrasonic spray pyrolysis, and anodic oxidation (5–8).

Silver ions protect against bacteria in various ways because they affect bacterial cell membranes and the intracellular transport of essential molecules by integrating into the thiol groups of the enzymes and proteins essential for bacterial survival (9–11). These interactions inactivate the bacteria.

In this study, we investigated whether nano-silver particles (AgNPs) are a potential new alternative for the treatment of osteomyelitis using an *in vivo* rat model to determine the effectiveness of the local application of AgNPs in the treatment of osteomyelitis and infected soft tissue.

Materials and Methods

Ethics approval was obtained from the Bezmialem University Animal Subjects Ethics Committee. The study used 24 male and female Sprague-Dawley rats weighing 200–300 g. All surgical procedures were performed under anaesthesia induced with ketamine and xylazine. Four rats died during the study, so a total of 20 rats were included in the analyses.

Preparation of the inoculum

Bacteria were inoculated on sheep blood agar supplemented with 2% NaCl (sodium chloride), incubated at 35°C for 16 h, suspended in 0.85% NaCl, and centrifuged at 5000 rpm for 15 min. Then, the upper part was discarded and 10 mL of 0.85% NaCl was added to the solution. This was repeated twice. Finally, 0.85% NaCl was added to obtain a bacterial suspension (1×10^7 CFU/mL) equal to 0.5 McFarland.

Preparation of AgNPs

AgNPs with a mean size of 15 nm were prepared from a silver nitrate solution, using sodium borohydride ($NaBH_4$) as the hydrogen donor (reductant), sodium lauryl sulphate as the surface modification agent, and oleic acid (Figure 1).

On the first day of the study, the haemograms of each rat were recorded. Then, both hind limbs were shaved, povidone iodine solution was applied, and an approximately 1.5-cm longitudinal incision was made on the anteromedial side of the anterior tibia under sterile conditions. Anterior to both proximal metaphyseal regions, the tibia was opened with a 2-mm dental burr and a 4–6-mm diameter cortical lid was formed. Using this method, mechanical trauma was created in the metaphyseal region and the intramedullary area was carved using various sized Kirchner wires and a burr. Methicillin-resistant *Staphylococcus aureus* (MRSA; 1×10^7 CFU; ATCC 43300) was inoculated through the cortical lid on the tibia into the intramedullary area.

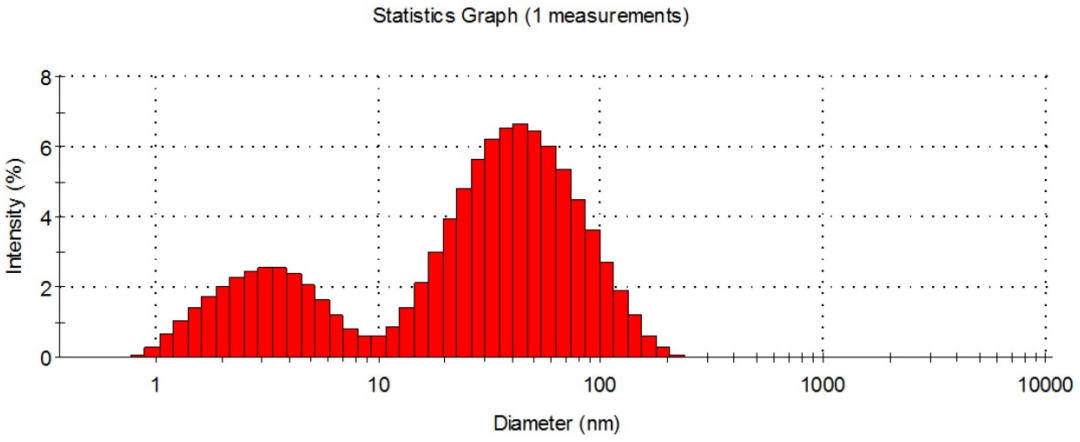

Figure 1: Distribution of particles by size in the 15 nm nano-silver solution. *(Before beginning our study, in vitro tests performed at the Turkish Centre for Disease Control revealed that nano-silver (used in our study) produced at the Istanbul Technical University Faculty of Metallurgy was effective against Escherichia coli (ATCC 259922), Staphylococcus aureus (ATCC 25923), and Salmonella spp. (RS) with no bacterial growth in the cultures.)*

The incision was closed using 4.0 polypropylene, followed by routine wound dressing, and both legs were bandaged with Tensoplast. The mobility of the rats was not restricted. The rats were fed a standard regular pellet diet and water, and food intake was measured for 21 days before any treatment was given.

At day 21 post-surgery, blood samples were obtained from the carotid arteries and haemograms were obtained. Anteroposterior (AP) and lateral x-rays of the tibias were obtained. The x-rays were analysed by four different orthopaedic surgeons and evaluated according to the criteria of Aktekin *et al*. Chronic osteomyelitis formation was observed. The presence of bone deformation, joint effusions, soft tissue swelling, osteolysis, metaphyseal widening, and periosteal reaction were recorded. A diagnosis of osteomyelitis was made when three of these factors were present. Infectious parameters (number of leukocytes) were higher after the operation compared with preoperatively.

The rats were randomised into two groups consisting of 12 rats each. Wound swab and tissue culture samples were obtained from the proximal tibia where the surgery was performed, and MRSA colonies were counted. Soft tissue abscesses and purulent discharge were observed in the majority of rats. Hyperaemia, fistula formation, discharge, and knee joint effusions were observed in some rats. Partial cortical defects and the formation of soft fibrous cortical bone were also observed.

The rats were divided into two groups (A and B). In group A, local wound and infectious bone debridement were performed. The area was then irrigated with 100 mL of 0.09% NaCl isotonic solution, and systemic 6 mg/kg/day teicoplanin was administered for 21 days. In group B, after wound and bone debridement, the area was irrigated with 100 mL 0.09% NaCl isotonic solution. A total of 0.0002 mg AgNPs (20 mL nanoparticle AgNPs) soaked in 3 mm^3 absorbable haemostatic gelatin sponge was placed in the tibial defect. Then, systemic 6 mg/kg/day teicoplanin was administered for 21 days.

Debridement was performed only once for each group. Blood was drawn from the carotid arteries and haemograms were obtained on postoperative day 42. Both of the lower extremities were shaved. The proximal tibial metaphysis was reached by dissecting through the previous incision line with a number 15 scalpel. Wound swab and tissue cultures were obtained from the tibial wound areas using sterile swabs. Bacterial colony numbers were studied by inoculating samples on blood agar. After the rats were sacrificed, both of their tibias were fixed in formaldehyde and examined histopathologically. Osteomyelitis and healing scores were calculated separately. The incidence of acute and chronic inflammation, the number of necrotic centres, number of micro-abscess foci, percentage of fibroblastic proliferation areas, and degree of vascularisation (number of lumens forming vessels) were investigated (Figure 2a,b). Fibroblast proliferation and vascularisation were considered as indications of healing, and inflammation, necrosis, and micro-abscess foci were considered as osteomyelitis (Figure 3a,b). The degree of osteomyelitis was scored on a scale of 1–3, ranging from mild to severe, while healing levels were scored as 1–3, ranging from low to high.

Results

Histopathological scoring was performed. White blood cell (WBC) counts were compared using the Mann–Whitney *U*-test. The osteomyelitis scores of groups A and B did not differ statistically ($p = 1$). Moreover, there were no significant differences in the soft tissue healing scores of the pathological specimens from the two groups ($p = 0.244$). At the beginning of the treatment phase, there were no significant differences in the colony numbers on the wound sites between the two groups ($p = 0.693$). However, on day 42 of treatment, the colony counts for groups A (mean, 10.5) and B (mean, 11.6) differed significantly ($p = 0.041$). There were no significant differences in the change of the number of colonies between the two groups from day 21 to 42 ($p = 0.328$) (Tables 1 and 2).

Table 1: Statistical comparison of healing and the number of colonies between the two groups.

	Osteomyelitis	Healing	Colony Day 21	Colony Day 42	Colony Day 42–21
Z	0.000	–1.165	–0.395	–2.042	–0.978
p	1.000	0.244	0.693	0.041	0.328

Table 2: Comparison of the healing scores and number of colonies between the two groups.

Groups	Osteomyelitis	Healing	Colony Day 21	Colony Day 42	Far-Colony Day
AN	20	20	20	20	20
Mean	1.350	2.600	68.500	11.650	–56.8500
Standard deviation	0.4894	0.5026	35.6673	21.7795	37.90747
Median	1.000	3.000	92.500	6.000	–61.0000
BN	20	20	20	20	20
Mean	1.350	2.350	70.650	10.500	–60.1500
Standard deviation	0.4894	0.6708	37.0082	18.4890	42.12172
Median	1.000	2.000	100.000	0.000	–67.5000

At the beginning of the study, the WBC counts of all rats were similar ($p = 0.880$). Moreover, there were no differences in osteomyelitis formation between the groups on treatment day 21 ($p = 0.096$); however, WBC counts were higher compared with the

preoperative values. At the end of the treatment period, no significant differences were found in treated rats between the groups (p = 0.880), and there were no differences in changes to WBC counts between days 21 and 42 (p = 0.076) (Tables 3 and 4).

Discussion

Coagulase (–) staphylococci are frequent etiologic agents of osteomyelitis. *S. aureus* is the organism isolated most commonly, particularly in acute haematogenous osteomyelitis, and it is the most common cause of osteomyelitis (13). Non-collagen components of bone, including osteonectin, osteocalcin, proteoglycan, and sialoprotein, are targets for *S. aureus*. Indeed, a specific connection has been found between sialoprotein and a specific osteomyelitis-causing subspecies of *S. aureus* (14). *S. aureus* is an intracellular microorganism that lives inside osteoblasts (15); it triggers inflammation and inhibits the reproduction of T lymphocytes. This bacterium can invade neutrophils and monocytes drawn into areas of inflammation, continuing its existence intracellularly (16,17).

Despite recent advances in antibiotic treatment, osteomyelitis remains difficult to treat. Low antibiotic concentrations, bone necrosis, and the low vascularity of bone render treatment even more difficult (18). Additionally, extracellular glycocalyx and necrotic bone tissue can lead to the development of biofilms, a major complication of chronic osteomyelitis.

Teicoplanin is a broad-spectrum bactericidal antibiotic that is effective against most Gram-positive and anaerobic bacteria, including MRSA and methicillin-resistant coagulase-negative *S. aureus* (19). It is an ideal antibiotic with a long half-life, high absorption capacity from bone, high protein-binding capacity, and low nephrotoxic side effects compared with vancomycin, which makes it an ideal antibiotic for the treatment of MRSA-induced osteomyelitis. However, it is never adequate when used alone.

The current treatment methods for osteomyelitis and infection by highly virulent bacteria such as MRSA remain inadequate, and the search for different treatments continues. A variety of treatment options have been explored, such as local antibiotic administration. The most important reason why local antibiotic administration is effective is that it results in higher tissue concentrations than systemic administration (20). Several studies have investigated this, and the use of antibiotic-loaded hydroxyapatite blocks to increase the concentration of antibiotics in bone tissue has become a treatment for osteomyelitis (18).

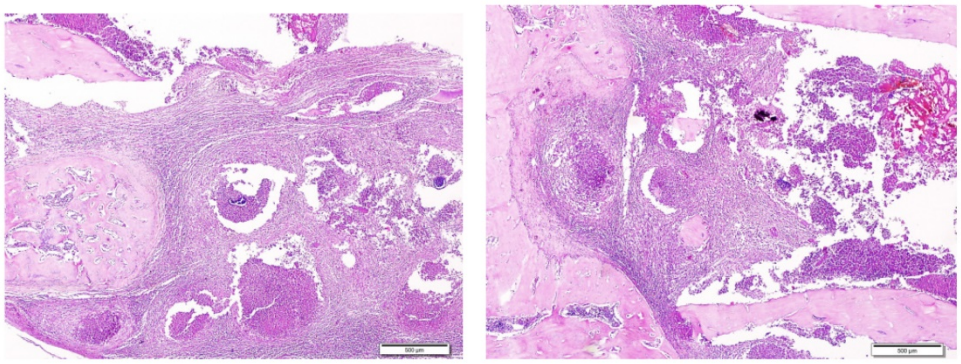

Figure 2: a: Wide osteomyelitis region showing high grade inflammation (grade 3). In the middle, intensive accumulation of polymorphonuclear leucocytes and mononuclear inflammatory cells and also wide necrosis and abscess foci seen. **b:** Wide destruction of bone lamellae in these foci. H&E, x400.

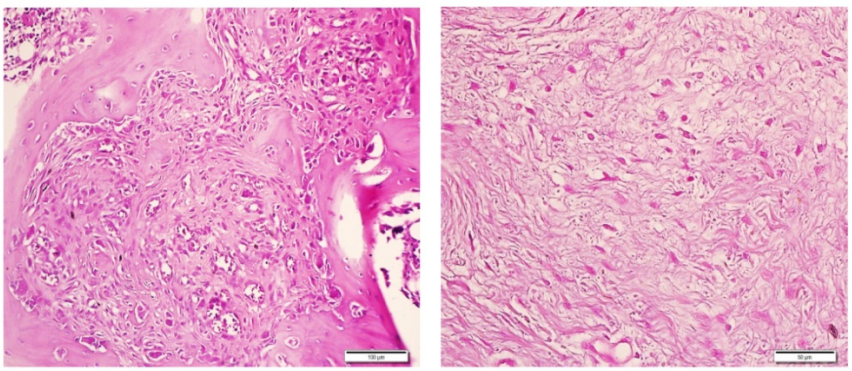

Figure 3: a: Healing signs in the foci of chronic osteomyelitis. **b:** Apparent neovascularization showing lumen formation in these foci. H&E, x2000.

Table 3: WBC counts of rats on days 21 and 42.

Groups	Preoperative WBC	Day 21 WBC	Day 42 WBC	Change Day 21 – Preoperative	Change Day 42 – Day 21	Change Day 42 –Preoperative
AN	10	10	10	10	10	10
Mean	12.9170	13.7010	10.8720	.7840	–2.8290	–2.0450
Standard deviation	1.67203	3.30691	2.92038	3.34590	2.20561	2.30046
Median	12.8400	13.7050	10.4500	–.1750	–3.5550	–1.7250
BN	10	10	10	10	10	10
Mean	13.3260	16.5310	10.9010	3.2050	–5.6300	–2.4250
Standard deviation	3.77413	3.53991	1.87925	3.90455	4.11149	4.91005
Median	12.1700	15.7600	10.3550	2.8000	–5.1950	–2.2100

Table 4: Statistical comparisons of WBC counts by time and between the two groups.

	Preoperative WBC	WBC Day 21	WBC Day 42	Change Day 21 – Preoperative	Change Day 42–21	Change Day 42 – Preoperative
Z	–0.151	–1.663	–0.151	–1.209	–1.777	–0.227
p	0.880	0.096	0.880	0.226	0.076	0.821

Local antibiotic treatment has also been used to treat chronic osteomyelitis and infected prostheses, and has been described as an adjunct treatment method. Cements containing antibiotics have been shown to be effective; they continue to release antibiotics for up to 6 weeks and maintain the antibiotic concentrations. Buchholz and Engelbrecht first reported that antibiotics could be incorporated into bone cements to provide local, long-term, and high-dose treatment (21). However, Sener *et al*. demonstrated that the cement remaining at the end of the antibiotic release period can act as a foreign body and result in increased rates of infection (22). They also showed that combining surgical debridement with such cements is more effective than debridement alone. Garcia *et al*. used an antibiotic powder to reduce soft tissue infection and abscess development, but the results were not significant histologically, microbiologically, or radiologically (23).

Another way to administer local antibiotics is via implants. Schmidmaier *et al*. studied the use of antibiotic-covered implants for the treatment of osteomyelitis and observed significant results (24). PMMA containing antibiotics has been presented as an alternative treatment modality; successful results have been reported with aggressive debridement, repeated irrigations, and PMMA soaked with 500 mg gentamicin and 2.4 g tobramycin (25). Keeling *et al*. showed that PMMA soaked with vancomycin and daptomycin decreased biofilm formation (26).

The administration of antibiotics in biologically degradable materials has been used for the same purpose. This is an important procedure because the material does not require removal. The use of collagen sponges containing gentamicin, polymyxin B, and amikacin has been reported. Lactic acid polymers have been used with ampicillin, gentamicin, polymyxin B, chloramphenicol, tobramycin, clindamycin, vancomycin, fusidic acid, fluoroquinolones, moxifloxacin and rifampicin. Isefuku *et al*. found that the administration of gentamicin and rifampicin prevented fracture healing (27).

The local application of antibacterials has also been used to treat osteomyelitis effectively. However, due to the increasing prevalence of antibiotic resistance, it is necessary to develop other treatment modalities.

Biofilms can render the treatment of osteomyelitis very difficult. The potential for new technological developments to revolutionise the treatment of infections can be investigated by examining the direct effects of AgNPs on biofilms. AgNPs help treat biofilms by affecting the expression of genes (*icaA* and *icaR* for *Staphylococcus epidermidis*; *fnbA* and *fnbB* for MRSA) that decrease bacterial adhesion and inhibit biofilm formation (28).

Simone *et al*. found that using AgNP-covered silk sutures decreases the incidence of surgical site infections and bacterial colonisation and reduces hospitalisation costs and morbidity (29). Shameli *et al*. found that AgNPs in zeolite exhibits antibacterial activity against Gram-negative (*Escherichia coli* and *Shigella dysenteriae*) and Gram-positive bacteria (*S. aureus* and MRSA) (30).

Gopinath *et al*. found that biogenic AgNPs are effective against endospores of *Bacillus* and *Clostridium* species (31). Wang *et al*. studied the antimicrobial effects of AgNP-coated titanium surfaces, which resulted in decreased expression of biofilm-related genes (*icaA* and *icaR* for *S. epidermidis*; *fnbA* and *fnbB* for MRSA), prevented bacterial adhesion, and inhibited biofilm formation (28). Albers *et al*. found that AgNPs have antimicrobial effects against *S. epidermidis*, but questioned their

biocompatibility with implants that are directly exposed to bone, and recommended future *in vivo* studies (32).

Consistent with previous findings, the AgNPs applied in our study were effective against *E. coli* (ATCC 259922), *S. aureus* (ATCC 25923), and *Salmonella* spp. *in vitro*.

The inhibitory effects of AgNPs are greater than those of silver micro-particles (33). Accordingly, AgNPs have been shown to have cytotoxic effects against both osteoblasts and osteoclasts. Cytotoxicity decreases with increasing particle counts and surface area, and osteoblasts are more susceptible to AgNPs than are osteoclasts (32).

Gosheger *et al*. discovered that the incidence of deep tissue infections and need for revision surgery decreased with the use of silver-coated implants. In their study, the infection rate in operations performed with silver-coated endoprostheses was 7%, while it was 47% with titanium prostheses. Moreover, the C-reactive protein levels and WBC counts were significantly lower in a silver-coated implant group than in a titanium group (34). Qin *et al*. reported that AgNP-coated titanium implants decreased the transcription of biofilm-forming genes of *S. epidermidis* both *in vitro* and *in vivo* (35).

Another study reported that silver-coated endoprostheses decreased the rate of deep tissue infections to a greater degree than conventional endoprostheses (36). The short-term results of these studies revealed neither hepatotoxicity nor nephrotoxicity (37).

Although our results were not the same, we found that it was consistent with the literature. We found no significant differences between the groups according to the infectious parameters studied. No significant changes in WBC counts were observed with the application of AgNPs. In Gosheger *et al*., a silver-coated implant group had lower WBC counts than a titanium-coated implant group (34).

In our study, MRSA was grown in cultures obtained from the wound sites on postoperative day 21. No significant differences were observed between the two groups in terms of the bacterial colony count or decrease in bacterial load. The number of bacterial colonies in both groups decreased compared with pretreatment levels. Our histopathological examinations revealed no significant differences between the two groups in terms of osteomyelitis and healing scores. When performed in conjunction with debridement and systemic antibiotic administration, local AgNP application was found to have neither positive nor negative effects.

Conclusion

Although several *in vitro* studies have demonstrated the effects of AgNPs in combination with intravenous antibiotic therapy and local surgical debridement, this combination did not have an effect on osteomyelitis in our *in vivo* setting. Future *in vivo* studies of AgNPs are warranted.

Competing Interests

The authors have declared that no competing interest exists.

References

1. Odekerken Jim CE, Arts JJ, Surtel DA, et al. A rabbit osteomyelitis model for the longitudinal assessment of early post-operative implant infections. Journal of orthopaedic surgery and research. 2013; 8(1): 38-51.
2. Engesaeter LB, Lie SA, Espehaug B, et al. Antibiotic prophylaxis in total hip arthroplasty: effects of antibiotic prophylaxis systemically and in bone cement on the revision rate of 22,170 primary hip replacements followed 0–14 years in the Norwegian Arthroplasty Register. Acta Orthop Scand. 2003;74: 644-51.
3. Garcia EJ, Sieg RN, Abdelgawad AA. Local application of free antibiotic powder in the treatment of osteomyelitis in a rat model. Orthopedics. 2013;36(8):986-9.
4. Feng QL, Wu J, Chen GQ, et al. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J Biomed Mater Res. 2000;52(4):662-8.
5. Chia GJ, Yao SW, Fan J, et al. Antibacterial activity of anodized aluminum with deposited silver. Surface and Coatings Technology. 2002; 157:162-5.
6. Xu J, Han X, Liu H, et al. Synthesis and optical properties of silver nanoparticles stabilized by gemini surfactant. Colloids and Surfaces A: Physicochem. Eng. 2006;273:179-83.
7. Dubas L, Kulsiriwiwat T, Samneingjam K, et al. Formation of silver-nanoparticles composite thin films. Acta Metallurgica Slovaca. 2007;13(2):147-9.
8. Guo G, Gan W, Luo J, et al. Preparation and dispersive mechanism of highly dispersive ultrafine silver powder. Applied Surface Science. 2010;256:6683-7.
9. Cho KH, Park JE, Osaka T, et al. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochimica Acta. 2005;51:956-60.
10. Ivan S, Branka SS. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gramnegative bacteria. Journal of Colloid and Interface Science. 2004;275:177-82.
11. Percival SL, Bowler PG, Russell D. Bacterial resistance to silver in wound care. Journal of Hospital Infection. 2005;60:1-7.
12. Aktekin CN, Ozturk AM, Tabak AY. A different perspective for radiological evaluation of experimental osteomyelitis. Skeletal Radiol. 2007; 36(10):945-50.
13 . Lazzarini L, Mader JT, Calhoun JH. Osteomyelitis in long bones. J Bone Joint Surg. 2004; 86-A(10):2305-18.
14. Ryden C, Yacoub AI, Maxe I, et al. Specific binding of bone sialoprotein to Staphylococcus aureus isolated from patients with osteomyelitis. Eur J Biochem .1989;184(2):331-6.
15. Hudson MC, Ramp WK, Nicholson NC, et al. Internalization of Staphylococcus aureus by cultured osteoblasts. Microb Pathog .1995;19(6):409-19.
16. Yoon KS, Fitzgerald RH, Sud Jr S, et al. Experimental acute hematogenous osteomyelitis in mice. Influence of staphylococcus aureus infection on T-cell immunity. J Orthop Res. 1999;17(3):382-91.
17. Gresham HD, Lowrance JH, Caver TE, et al. Survival of Staphylococcus aureus inside neutrophils contributes to infection. J Immunol. 2000;164(7):3713-22.
18. Itokazu M, Ohno T, Tanemori T, et al. Antibiotic-loaded hydroxyapatite blocks in the treatment of experimental osteomyelitis in rats. J Med Microbiol. 1997;46(9):779-83.
19. Jia WT, Luo SH, Zhang CQ, et al. In vitro and in vivo efficacies of teicoplanin-loaded calcium sulfate for treatment of chronic methicillin-resistant Staphylococcus aureus osteomyelitis. Antimicrob Agents Chemother. 2010;54(1):170-6.
20. Hanssen AD. Local antibiotic delivery vehicles in the treatment of musculoskeletal infection. Clin. Orthop. Relat. Res. 2005;437:91-6.
21. Buchholz HW, Engelbrecht H. Depot effects of various antibiotics mixed with Palacos resins. Chirurg. 1970;41:511-5.
22. Sener M, Kazimoglu C, Karapinar H, et al. Comparison of various surgical methods in the treatment of implant-related infection. Int Orthop. 2010; 34(3):419-23.
23. Garcia EJ, Sieg RN, Abdelgawad AA. Local application of free antibiotic powder in the treatment of osteomyelitis in a rat model. Orthopedics. 2013;36(8):986-9.

24. Schmidmaier G, Lucke M, Wildemann B, et al. Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review. Injury. 2006;37(suppl 2):105-12.
25. Schade VL, Roukis TS. The role of polymethylmethacrylate antibiotic-loaded cement in addition to debridement for the treatment of soft tissue and osseous infections of the foot and ankle. J Foot Ankle Surg. 2010;49:55-62.
26. Keeling WB, Myers AR, Stone PA, et al. Regional antibiotic delivery for the treatment of experimental prosthetic graft infections. J Surg Res 2009;157:223-6.
27. Isefuku S, Joyner CJ, Simpson AH. Gentamicin may have an adverse effect on osteogenesis. J Orthop Trauma.2003;17:212-6.
28. Wang J, Li J, Guo G, et al. Silver-nanoparticles-modified biomaterial surface resistant to staphylococcus: new insight into the antimicrobial action of silver. Sci Rep. 2016;32699(6):1-16.
29. De Simone S, Gallo AL, Paladini F, et al. Development of silver nano-coatings on silk sutures as a novel approach against surgical infections. J Mater Sci Mater Med. 2014;25(9):2205-14.
30. Shameli K, Ahmad MB, Zargar M, et al. Fabrication of silver nanoparticles doped in the zeolite framework and antibacterial activity. Int J Nanomedicine. 2011;6:331-41.
31. Gopinath PM, Ranjani A, Dhanasekaran D, et al. Multi-functional nano silver: A novel disruptive and theranostic agent for pathogenic organisms in real-time. Sci Rep. 2016;34058(6):1-16.
32. Albers CE, Hofstetter W, Siebenrock KA, et al. In vitro cytotoxicity of silver nanoparticles on osteoblasts and osteoclasts at antibacterial concentrations. Nanotoxicology. 2013;1:30-6.
33. Liu W, Wu Y, Wang C, et al. Impact of silver nanoparticles on human cells: effect of particle size. Nanotoxicology. 2010;4:319-30.
34. Gosheger G, Hardes J, Ahrens H, et al. Silver-coated megaendoprostheses in a rabbit model--an analysis of the infection rate and toxicological side effects. Biomaterials. 2004;25(24):5547-56.
35. Qin H, Cao H, Zhao Y, et al. In vitro and in vivo anti-biofilm effects of silver nanoparticles immobilized on titanium. Biomaterials.2014;35(33):9114-25.
36. Hardes J, Eiff C, Streitbuerger A, et al. Reduction of periprosthetic infection with silver-coated megaprostheses in patients with bone sarcoma. J Surg Oncol. 2010;101:389-95.
37. Hardes J, Ahrens H, Gebert C, et al. Lack of toxicological side-effects in silver-coated megaprostheses in humans. Biomaterials 2007;28:2869-75.