

## Cortical allograft as a vehicle for antibiotic delivery

Eivind Witsø<sup>1</sup>, Leif Persen<sup>1</sup>, Pål Benum<sup>1</sup> and Kåre Bergh<sup>2,3</sup>

Departments of <sup>1</sup>Orthopaedic Surgery, <sup>2</sup>Microbiology, University Hospital, <sup>3</sup>Laboratory Medicine, Norwegian University of Science and Technology, NO-7006 Trondheim, Norway  
Correspondence EW: eivind.witso@stolav.no  
Submitted 04-01-02. Accepted 04-11-01

**Background** Infection can be a devastating complication after implantation of a cortical bone allograft. The allograft could act as a vehicle for local antibiotic prophylaxis.

**Material and methods** We studied the release of antibiotics in vitro from cortical bone allografts impregnated with antibiotics for different periods of time. We also studied whether cortical allografts impregnated with antibiotics could eradicate *Staphylococcus aureus* from an experimentally infected graft in vivo. In the in vitro study, pieces of cortical bone were impregnated with netilmicin, vancomycin, ciprofloxacin and rifampicin for 1 h, 10 h and 100 h. The antibiotics were eluted into phosphate-buffered saline (PBS) for 7 days, with daily transfer of the bone into fresh PBS. In the in vivo study, cortical allografts impregnated with antibiotics were placed in rats intramuscularly. 10 µL of an *S. aureus* suspension ( $0.6 \times 10^5$  CFU) was placed in the intramedullary cavity. After 15 days, the allografts were removed and examined for bacterial growth.

**Results** The amount of antibiotics released in vitro was influenced by the time used for antibiotic impregnation of the bone. Allografts impregnated with netilmicin, vancomycin and rifampicin effectively eradicated perioperative contamination with *S. aureus* in vivo.

**Interpretation** This study shows that a cortical bone allograft would be an effective vehicle for local antibiotic delivery.

With cortical allografts, graft-host nonunion, graft fracture, and graft infection occur (Aro and Aho 1993). Infection rates of 4–12% have been reported (Lord et al. 1988, Tomford et al. 1990, Aro and

Aho 1993, Gross et al. 1995, Haddad et al. 2000). Webb et al. (1994) studied antibiotic resistance in *Staphylococcus aureus* adhering to polyethylene, polymethylmetacrylate, and cortical bone allografts in vitro. Compared to bacteria growing on the surface of polyethylene and polymethylmetacrylate, bacteria from the surface of a cortical allograft were associated with the highest degree of antibiotic resistance. A cortical allograft may serve as a dead foreign body that is not protected by the local cellular defense mechanisms. Hence, a prolonged period with prophylactic antibiotics has been recommended after implantation of large allografts (Lord et al. 1988, Aro and Aho 1993). In vitro studies have shown that cortical bone can act as an antibiotic carrier (Winkler et al. 2000). We studied whether increasing the time period used for antibiotic impregnation of cortical bone allograft could result in increased in vitro release of antibiotic from the graft subsequently. We also studied whether cortical allografts impregnated with antibiotics could eradicate perioperative bacterial contamination with *S. aureus* in vivo.

### Material and methods

#### *In vitro* studies

- Human femoral cortical bone was prepared under sterile conditions and cut into pieces of similar size. All marrow tissue was removed. The average surface area of the bone pieces was 645 (552–734) mm<sup>2</sup> and the average weight was 1.46 (1.35–1.55) g. The bone pieces were stored by freezing at –70°C. After thawing, the bone was put into small

polypropylene tubes with 3 mL of antibiotic solution at room temperature. We performed the experiments in triplicate. 4 antibiotic solutions were used: netilmicin 400 mg/mL (Essex Chemie AG, Toepferstrasse 5; CH-6004 Luzern, Switzerland), vancomycin 100 mg/mL (Vancocin, Lilly, Eli Lilly Co., Indianapolis, IN), ciprofloxacin 2 mg/mL (Ciproxin, Bayer, Bayer AG, Leverkusen, Germany) and rifampicin 50 mg/mL, pH 8.1 (Rimactan, Biochemie, Biochemie BmbH, Kundl, Austria). The pH of the netilmicin, vancomycin and ciprofloxacin solutions was adjusted to 6.6–6.7 by adding 10M NaOH. 3 impregnation periods were used: 1 h, 10 h and 100 h. To remove antibiotics not adsorbed to the bone, a standard washing procedure was performed after the impregnation: the cortical bone pieces were put into test tubes with 5 mL of saline and mixed on a vortex mixer at full speed for 15 sec. The bone was then submerged into another 5 mL of saline before it was put into a test tube with 5 mL of phosphate-buffered saline (PBS) and incubated at 37°C for 24 h. After incubation, the bone piece was removed from the test tube, washed in 5 mL saline and transferred into a test tube with 5 mL PBS. The study was continued for 7 days, with daily transfer of the bone into a test tube with PBS after washing in saline. Each day, 2-mL aliquots of elution fluid were frozen and stored at 70°C. We measured the total amount of antibiotics eluted over the 7 days. In addition, antibiotic concentration analyses were performed for days 1, 3 and 7.

#### Measurement of antibiotic concentration

We determined netilmicin and vancomycin concentrations using fluorescence polarization immunoassay (FPIA) technology (TDx/TDxFLx Vancomycin and Netilmicin Assay System, Abbott Laboratories, Diagnostics Division, Abbott Park, IL). In this assay, 0.09 mg/L and 2.0 mg/L are the lowest detectable concentrations of netilmicin and vancomycin, respectively.

Ciprofloxacin and rifampicin concentrations were determined using agar disk diffusion bioassay with *Escherichia coli* MB-3804 and *Staphylococcus epidermidis* I-1478, respectively, as the indicator strains. The technique is based on the inhibitory activity of disks containing a standardized concentration of antibiotic (PDM Diagnostic

Discs, AB Biodisk, Sweden). In addition, test paper disks were impregnated with 10 µL of the elution fluid. Agar was flooded with 5 mL of the indicator bacterial strain suspension ( $2 \times 10^5$  CFU/mL). Superfluous bacterial suspension was removed and the agar was dried. The standard paper disks and the test paper disks were placed on the seeded agar and incubated overnight at 37°C. The diameter of the zone of inhibition for each standard paper disk was measured and drawn onto semilogarithmic paper to obtain a standard curve for each antibiotic. The unknown concentrations were determined by measuring the zones of inhibition and reading the concentration from the curve.

#### Experimental infection of cortical allograft

The Norwegian Council for Animal Experimentation approved the study. 20 male albino, outbred Wistar rats (M&B A/S, 8680 Ry, Denmark), 8 weeks old and weighing 299 (280–320) g, were divided into 5 groups, with 4 animals in each group. Groups 1–4 received allografts impregnated with netilmicin, vancomycin, ciprofloxacin or rifampicin. Group 5 acted as control and consisted of cortical allografts not impregnated with antibiotics. The study was done blind.

We prepared allografts as follows. Soft tissue was removed from circumferential rat femoral diaphyses. The average allograft length was 20.0 (19.5–20.5) mm, and the average weight was 0.38 (0.30–0.50) g. The bone grafts were stored by freezing at –70 °C. After thawing, the marrow canal was cleaned with a 0.35-mm Kirshner wire and flushed with 10 mL saline. In the mid-part of the diaphyses, a 1.5-mm drill hole was made through the first cortex and into the intramedullary cavity. The cortical allografts were impregnated for 10 h at room temperature with 3 mL antibiotic solution (netilmicin, vancomycin, ciprofloxacin or rifampicin) of the concentrations described previously for the in vitro study. Control bone was impregnated with saline. To remove antibiotics not adsorbed to the bone, the allograft was placed into a test tube with 5 mL saline and mixed on a vortex mixer at full speed for 15 seconds. The bone was then washed with saline in a new test tube before the intramedullary cavity was flushed with 10 mL normal saline.

The animals were operated under standard operating theater conditions. They were given general

Triplicates of cortical bone pieces were impregnated with netilmicin (400 mg/mL), vancomycin (100 mg/mL), ciprofloxacin (2 mg/mL) and rifampicin (50 mg/mL). The total amount of antibiotic was the amount eluted into PBS over 7 days. The peak concentration was the amount of antibiotics eluted into 5 mL PBS during the first 24 h

	Bone impregnated for 1 h			Bone impregnated for 10 h			Bone impregnated for 100 h			Jonckheere-Terpstra test
Total amount (mg)										
Netilmicin	1.34	2.30	3.26	6.07	9.24	9.55	6.09	14.5	16.1	p = 0.01
Vancomycin	1.05	1.47	1.65	3.01	5.32	6.76	8.79	9.49	12.4	p < 0.01
Ciprofloxacin	0.10	0.13	0.15	0.22	0.22	0.29	0.28	0.30	0.32	p = 0.01
Rifampicin	0.56	0.56	0.60	2.31	2.31	2.49	4.90	5.95	5.95	p < 0.01
Peak concentrations (mg/L)										
Netilmicin	240	420	540	1020	1640	1740	990	2610	2900	p = 0.02
Vancomycin	179	214	291	550	756	873	1064	1306	2713	p < 0.01
Ciprofloxacin	17	19	20	32	32	50	40	49	49	p = 0.02
Rifampicin	99	115	123	360	390	400	860	860	860	p < 0.01

anesthesia with a subcutaneous injection of 0.7 mL midazolam/fentanyl-fluanisone. One cortical allograft was implanted intramuscularly in each animal, in the interscapular region. Perioperatively, 10  $\mu$ L (0.6 (0.5–0.7)  $\times 10^7$  CFU/mL) of a freshly prepared *S. aureus* ATCC 25923 suspension, was injected through the diaphyseal hole into the intramedullary cavity. The muscular fascia and the skin were closed with sutures. During the operation, the bacterial suspension was kept on ice to ensure that each animal received an identical bacterial inoculum. As postoperative sedation, each animal received a subcutaneous injection of 0.03 mg buprenorphin. Postoperatively, the animals were housed in a 12-hour night-day cycle environment. They were allowed free movement, standard food (“Rat and Mouse diet” BK001E) and water ad libitum. After 15 days, the rats were given general anesthesia with 0.7–0.8 mL midazolam/fentanyl-fluanisone, and the cortical allografts were removed under sterile conditions. A separate set of instruments was used for each animal. The animals were then killed by CO<sub>2</sub> intoxication. The cortical bone was placed in a sterile polypropylene tube and crushed into small pieces with sterile forceps. 5 mL of saline was added to the tube and the tube with its contents was mixed on a vortex mixer at full speed for 60 sec. Quantitative bacterial culture was performed to determine the number of viable bacteria in the washing fluid. Briefly, 10 L of undiluted sample and 10-fold serially diluted samples of the saline were seeded on blood agar and incubated at 37°C for 24 h. The number of colony-forming units (CFU) was counted. Growth of *S.*

*aureus* from the excised allografts was analyzed genotypically with pulsed-field gel electrophoresis as described in detail by Andersen et al. (1999). We determined the Minimum Inhibitory Concentration (MIC) of the antibiotics for *S. aureus* ATCC 25923 in advance by the E-test (AB Biodisk, Solna, Sweden): netilmicin 0.19 mg/L, vancomycin 2.0 mg/L, ciprofloxacin 0.25 mg/L, rifampicin 0.016 mg/L.

### Statistics

If not otherwise stated, the results are presented as arithmetic mean (dispersion as total range). For statistical analysis, we used a non-parametric statistical test (Jonckheere-Terpstra test). Statistical significance was considered at p-values less than 0.05.

## Results

### Elution of antibiotics from cortical allografts *in vitro*

The duration of time used for antibiotic impregnation of cortical bone had a profound influence on the subsequent release of antibiotics (Table). The total amount of netilmicin, vancomycin and rifampicin released increased 3–4 fold when the time used for impregnation was increased from 1 h to 10 h, and 6–10 fold when the time was increased from 1 h to 100 h. The total amount of ciprofloxacin released increased 2–3 fold. The increase in peak antibiotic concentration showed the same trend. The elution profile showed an exponential decay of antibiotic

eluted from the cortical bone pieces throughout the study period, irrespective of the time used for impregnation of the bone. The portion of antibiotics released during the first 24 h (expressed as percentage of the total amount of antibiotics released over 7 days) was 88% (83–91) for netilmicin, 78% (60–100) for vancomycin, 75% (63–86) for ciprofloxacin, and 84% (72–100) for rifampicin. For bone pieces impregnated for 100 h, the antibiotic concentration in the elution fluid after 7 days was 4 (4–5) mg/L for netilmicin, 3 (2–4) mg/L for vancomycin, 0 mg/L for ciprofloxacin, and 2 (1–4) mg/L for rifampicin.

#### **Eradication of experimental allograftinfection**

One of the rats died immediately after the operation, probably due to respiratory failure. The implanted bone was removed under sterile conditions and implanted into another rat of similar weight. Otherwise, the rats were in good health after the operation. They all gained weight during the study period; the average weight increase was 57 (33–85) g. At the final operation, all the cortical allografts were still positioned intramuscularly. There were various degrees of local reaction around the allograft, ranging from tissue necrosis with pus to no signs of local infection. Experimental infection with *S. aureus* ATCC 25923 was established in all four allografts that had not been impregnated with antibiotics. In the washing fluid of the extirpated bones, the number of CFU of *S. aureus* ATCC 25923 was  $12 (8–21) \times 10^6$  /mL. In bone impregnated with netilmicin, vancomycin and rifampicin, *S. aureus* ATCC 25923 infection was eradicated. In 2 of the 4 allografts impregnated with ciprofloxacin, *S. aureus* ATCC 25923 at  $4 \times 10^3$  and  $4 \times 10^5$  CFU/mL, respectively, grew from the washing fluid. The *S. aureus* isolates recovered were found by pulsed field electrophoresis to be genotypically identical to the infecting *S. aureus* ATCC 25923 strain (results not shown). The MIC of ciprofloxacin for the *S. aureus* isolates recovered was identical to that recorded preoperatively.

#### **Discussion**

We found that peak concentrations and the total amount of netilmicin, vancomycin, ciprofloxacin

and rifampicin eluted from cortical allografts in vitro for 7 days were dependent on the time used for antibiotic impregnation of the graft. In some of the groups studied, we observed a considerable disperse between the three parallels. The bone pieces were of similar weight and had similar surface area. They were all taken from the same cortical diaphysis and they all appeared macroscopically similar. An uneven distribution of the amount of Haversian and Volkmann's canals may explain the disparity between the three parallels.

An accurate comparison of the elution profiles of the different antibiotics was not possible, due to the different antibiotic concentrations used for impregnation of the graft and the different methods used for determination of antibiotic concentration in the elution fluid. Furthermore, the antibiotics have different molecular weight, ranging from 331 Da (ciprofloxacin) to 1 449 Da (vancomycin), and they have different polarity. However, the patterns of release were similar with high early release over a few days. For the in vitro and in vivo studies, we chose antibiotics that are often used for local prophylaxis and treatment in orthopedic surgery: aminoglycoside (netilmicin) and glycopeptide (vancomycin). Rifampicin is mainly used as a tuberculostaticum. However, several reports on the use of rifampicin when treating orthopedic infections have been published (Drancourt et al. 1993, Zimmerli et al. 1998). To avoid the emergence of rifampicin-resistant bacterial strains, rifampicin has been combined with a glycopeptide or a fluoroquinolone (ciprofloxacin). We also chose water-soluble compounds (netilmicin and vancomycin) and compounds that were not water-soluble (rifampicin and ciprofloxacin).

When cortical bone was impregnated with netilmicin and vancomycin for 100 h, the total amount of antibiotic released per gram of cortical bone was approximately 7 mg. This is less than one-tenth of the amount of netilmicin or vancomycin released from 1g of cancellous human bone impregnated with antibiotics for 24 h (Witsø et al. 2002). However, a circumferential human femur cortical allograft of 10 cm length weighs approximately 100 g (unpublished data). Thus, it could contain a large reservoir of antibiotics.

In our animal model, *S. aureus* was efficiently eradicated from an experimentally infected cortical

allograft impregnated with netilmicin, vancomycin or rifampicin. The inoculum used in this study ( $0.6 \times 10^5$  CFU) is comparable to that used in other surface-colonizing infection models (Gristina et al. 1989, Zimmerli et al. 1994). According to our in vitro study, the peak ciprofloxacin concentration was approximately 150 times the MIC for *S. aureus* ATCC 25923 when ciprofloxacin was eluted from bone impregnated for 10 h. By comparison, the peak concentrations of netilmicin, vancomycin and rifampicin were 7,700, 360 and 24,000 times the respective MICs for *S. aureus* ATCC 25923, respectively. These observations probably explain the reduced antibacterial effect of ciprofloxacin-impregnated bone in vivo. We did not observe any development of increased resistance against any of the antibacterial agents tested. To date, there is no proof that local use of antibiotics in orthopedic surgery in general has been associated with the emergence of resistant strains. However, Hope et al. (1989) reported an increased prevalence of gentamicin-resistant *S. epidermidis* in patients who had been operated on previously using gentamicin-containing bone cement. The prolonged release of antibiotics from antibiotic-containing bone cement may possibly explain this observation.

There have been many reports on contamination of bone allografts during the process of procurement (Deijkers et al. 1997, Journeaux et al. 1999, Hirn et al. 2001). Rinsing of a large bone allograft with an antibiotic solution after removal from the donor does not eradicate microorganisms efficiently (Deijkers et al. 1997, Hirn et al. 2001). The exposure time may be too short for the antibiotics to be effective (Deijkers et al. 1997). However, due to long and extensive surgery, multiple operations, problems with wound healing and hematogenous spread of bacteria, large bone allografts are probably contaminated more often during and after the operation (Lord et al. 1988, Tomford et al. 1990). Moreover, many of these patients have malignant tumors and are treated concomitantly with adjuvant radiation or immunosuppressive chemotherapy. Although not statistically proven, Ozaki et al. (1997) observed a reduction in late infections when the intramedullary cavity of bone allograft had been filled with gentamicin- and vancomycin-containing bone cement. The reduced risk of infection may be due to a reduced amount of dead

space and sustained release of antibiotics. Thus, antibiotic impregnation of cortical allograft with high early release and an exponential decay over a few days might be an option when choosing an effective method for infection prophylaxis. However, antibiotic impregnation of cortical allografts may have adverse effects. In vitro studies have shown that a high level of tobramycin (400 mg/L) impairs cell replication (Miclau et al. 1995). However, no systemic adverse effects were observed in patients operated with impaction of netilmicin-impregnated cancellous bone (Witsø et al. 2004). In an experimental study in rats, Gray and Elves (1981) observed that topically applied antibiotic powder was detrimental to the osteogenesis in fresh corticocancellous isograft. Subcutaneous injection of ciprofloxacin in an experimental rat model impaired fracture healing (Huddleston et al. 2000). However, Petri (1984) showed that the osteogenic activity of cephalotin- and tobramycin-supplemented morselized cortical mineralized and demineralized bone was comparable to bone not supplemented with antibiotics. Furthermore, high local levels of tobramycin and vancomycin used in bone graft do not appear to affect bone healing in vivo (Lindsey et al. 1993, Buttarò et al. 2003). To our knowledge, no clinical studies on graft-host nonunion when using antibiotic-impregnated cortical allografts have been performed.

Preservation of the grafts for up to 100 h in an antibiotic solution might influence the mechanical stability of the bone. 18% fractures of structural allografts not impregnated with antibiotics have been reported (Sorger et al. 2001). Parrish (1973) operated on 21 patients with tumors at the end of a long bone, with replacement by a massive osteochondral allograft. The grafts were submerged in a solution of 1% neomycin, to which 500,000 units of bacitracin was added, for 24 h before the operation. None of the patients had a wound infection. However, fracture of the graft occurred in 8 patients (38%). Although the study of Parrish was too small to make any definite conclusions, mechanical testing of osteochondral and structural allografts impregnated with antibiotics should be performed before this option is taken into clinical use.

In conclusion, antibiotic-impregnated cortical allografts may act as an efficient carrier of antibiotics. The amount of antibiotics subsequently

released in vitro is dependent on the time used for impregnation. Cortical allografts impregnated with netilmicin, vancomycin or rifampicin efficiently eradicated *S. aureus* from perioperatively contaminated grafts in vivo.

We thank Schering-Plough A/S, Norway, for supplying us with netilmicin sulfate powder and Bayer AS, Norway, for supplying us with ciprofloxacin powder.

No competing interests declared.

- Andersen B M, Bergh K, Steinbakk M, Syvertsen G, Mag-naes B, Dalen H, Bruun J N. A Norwegian nosocomial outbreak of methicillin-resistant *Staphylococcus aureus* resistant to fusidic acid and susceptible to other antistaphylococcal agents. *J Hosp Infect* 1999; 41 (2): 123-32.
- Aro H T, Aho A J. Clinical use of bone allografts. *Ann Med* 1993; 25: 403-12.
- Buttaro M A, Della Valle A M G, Piñero L, Mocetti E, Moranidi A A, Piccaluga F. Incorporation of vancomycin-supplemented bone allografts. Radiographical, histopathological and immunohistochemical study in pgs. *Acta Orthop Scand* 2003; 74: 505-13
- Deijkers R L M, Bloem R M, Petit P L C, Brand R, Vehmyer S B W, Veen M R. Contamination of bone allograft. Analysis of incidence and predisposing factors. *J Bone Joint Surg (Br)* 1997; 79 (1): 161-6.
- Drancourt M, Stein A, Argenson J N, Zannier A, Curvale G, Raoult D. Oral rifampicin plus oxofloxacin for treatment of *Staphylococcus*-infected orthopaedic implants. *Antimicrob Agents Chemother* 1993; 37 (6): 1214-8.
- Gray J C, Elves M W. Osteogenesis in bone grafts after short-term storage and topical antibiotic treatment. An experimental study in rats. *J Bone Joint Surg (Br)* 1981; 63 (3): 441-5.
- Gristina A G, Jennings R A, Naylor P T, Myrvik Q N, Webb L. Comparative in vitro antibiotic resistance of surface-colonizing coagulase-negative staphylococci. *Antimicrob Agents Chemother* 1989; 33: 813-6.
- Gross A E, Hutchison C R, Alexeff M, Mahomed N, Leitch K, Morsi E. Proximal femoral allografts for reconstruction of bone stock in revision arthroplasty of the hip. *Clin Orthop* 1995; (319): 151-8.
- Haddad F S, Spangehl M J, Masri B A, Garbutz D S, Duncan C P. Circumferential allograft replacement of the proximal femur. *Clin Orthop* 2000; (371): 98-107.
- Him M Y J, Salmela M, Vuento R E. High-pressure saline washing of allografts reduces bacterial contamination. *Acta Orthop Scand* 2001; 72 (1): 83-5.
- Hope P G, Kristinsson K G, Norman P, Elson R A. Deep infection of cemented total hip arthroplasties caused by coagulase-negative staphylococci. *J Bone Joint Surg (Br)* 1989; 71 (5): 851-5.
- Huddleston P M, Steckelberg J M, Hanssen A D, Rouse M S, Bolander M E, Patel R. Ciprofloxacin inhibition of experimental fracture-healing. *J Bone Joint Surg (Am)* 2000; 82 (2): 161-73.
- Journeaux S F, Johnson N, Bryce S L, Friedman S J, Somerville S M M, Morgan D A F. Bacterial contamination rates during bone allograft retrieval. *J Arthroplasty* 1999; 14 (6): 677-81.
- Lindsey R W, Probe R, Miclau T, Alexander J W, Perren M P. The effects of antibiotic-impregnated autogenic cancellous bone graft on bone healing. *Clin Orthop* 1993; (291): 303-12.
- Lord C F, Gebhardt M C, Tomford W W, Mankin H J. Infection in bone allografts. *J Bone Joint Surg (Am)* 1988; 70: 369-76.
- Miclau T, Edin M L, Lester G E, Lindsey R W, Dahners L E. Bone toxicity of locally applied aminoglycosides. *J Orthop Trauma* 1995; 9 (5): 401-6.
- Ozaki T, Hillmann A, Bettin D, Wuisman P, Winkelmann W. Intramedullary, antibiotic-loaded cemented, massive allografts for skeletal reconstruction. *Acta Orthop Scand* 1997; 68 (4): 387-91.
- Parrish F F. Allograft replacement of all or part of the end of a long bone following excision of a tumor. Report of twenty-one cases. *J Bone Joint Surg (Am)* 1973; 55 (1): 1-22.
- Petri W H. Osteogenic activity of antibiotic-supplemented bone allografts in the Guinera pig. *J Oral Maxillofac Surg* 1984; 42 (10): 631-6.
- Sorger J I, Hornicek F J, Zavatta M, Menzner J P, Gebhardt M C, Tomford W W, Mankin H J. Allograft fractures revisited. *Clin Orthop* 2001; (382): 66-74.
- Tomford W W, Thongphasuk J, Mankin H J, Ferraro M J. Frozen musculoskeletal allografts. A study of the clinical incidence and causes of infection associated with their use. *J Bone Joint Surg (Am)* 1990; 72: 1137-43.
- Webb L X, Holman J, Araujo B de, Zaccaro D J, Gordon E S. Antibiotic resistance in staphylococci adherent to cortical bone. *J Orthop Trauma* 1994; 8 (1): 28-33.
- Winkler H, Janata O, Berger C, Wein W, Georgopoulos A. In vitro release of vancomycin and tobramycin from impregnated human and bovine bone grafts. *J Antimicrob Chemot* 2000; 46: 423-8.
- Witsø E, Persen L, Benum P, Bergh K. Release of netilmicin and vancomycin from cancellous bone. *Acta Orthop Scand* 2002; 73 (2): 199-205.
- Witsø E, Persen L, Benum P, Aamodt A, Schnell Husby O, Bergh K. High local concentrations without systemic adverse effects after impaction of netilmicin-impregnated bone. *Acta Orthop Scand* 2004; 75 (3): 339-46.
- Zimmerli W, Frei R, Widmer A F, Rajacic Z. Microbiological tests to predict treatment outcome in experimental device-related infections due to *Staphylococcus aureus*. *J Antimicrob Chemother* 1994; 33: 959-67.
- Zimmerli W, Widmer A F, Blatter M, Frei R, Ochsner P E. Role of rifampicin for treatment of orthopaedic implant-related staphylococcal infections. *JAMA* 1998; 279 (19): 1537-41.