SPINE Volume 34, Number 5, pp 479–483

Reduction of Postoperative Spinal Implant Infection Using Gentamicin Microspheres

Alec C. Stall, MD, Ed Becker, MD, Steven C. Ludwig, MD, Daniel Gelb, MD, and Kornelis A. Poelstra, MD, PhD

Study Design. Three noncontiguous spinal implant sites in 1 rabbit were challenged with *Staphylococcus aureus* and local antibiotic prophylaxis was given with gentamicin in controlled-release microspheres (poly(lactic-coglycolic-acid) [PLGA]). Postoperative biomaterial-centered infection on and around the titanium rods was assessed using standard bacterial quantification essays.

Objective. To assess surgical site and biomaterial-centered infection reduction with controlled release gentamicin from microspheres against *S. aureus*.

Summary of Background Data. A postoperative biomaterial-centered infection can be devastating after successful thoracolumbar spinal surgery and puts a high burden on patients, families, surgeons, and hospitals, endangering both our healthcare budget and our ability to perform challenging cases in patients with increasing numbers of comorbidities. Systemic antibiotics often do not reach "dead-space" hematomas where bacteria harbor after surgery, whereas local, controlled release gentamicin prophylaxis through PLGA microspheres showed favorable pharmacokinetics data to achieve local bactericidal concentrations for up to 7 days after surgery.

Methods. A well published rabbit spinal implant model with systemic cephalosporin prophylaxis was challenged to create a baseline infection of ~70% in control sites. We then challenged 3 noncontiguous titanium rods inside the laminectomy defect with 10e6 colony forming units *S. aureus* and randomly treated 2 sites with gentamicin PLGA microspheres and 1 site with PLGA carrier only (control). Standard quantification techniques were used to assess biomaterial centered and soft tissue bacterial growth after 7 days.

Results. After establishing reliable infection rates in control sites, the therapeutic arm of the study was started. Surgical site infections were found in 75% of control sites, whereas gentamicin microspheres reduced the incidence down to 38% in the same rabbits. Biomaterial-centered infection was reduced from 58% to 23% only in all sites challenged with 10e6 *S. aureus*.

Conclusion. Postoperative, biomaterial-centered infection was reduced at least 50% with intraoperative gentamicin microspheres in the face of systemic cephalosporin prophylaxis and high dose *S. aureus* in a laminectomy defect in rabbits. The data are statistically and clinically significant, and further animal testing is planned to confirm these results.

Key words: postoperative infection, biomaterial centered, PLGA, gentamicin, *Staphylococcus aureus*, rabbit model. **Spine 2009;34:479–483**

Surgical site infection (SSI) is the most common, potentially preventable adverse outcome of a major operation. The economic impact alone is enormous and is estimated to cost the US healthcare excess of $1.8 billion per year.[1] The cost of treating a single implant-associated spinal wound infection can run in excess of $900,000 and requires substantial resource allocation on the part of hospitals and physicians who are often poorly reimbursed.[2–4] As such, the burden placed on hospitals and physicians to provide care for these patients is substantial and disproportionately falls on high volume tertiary-care referral centers, where patients with an implant-associated spinal infection are often referred.[5,6] Thus the prevention of SSI (prophylaxis) is a first line defense in the battle against these rising healthcare costs.[7,8]

The cost of orthopedic SSIs to patients, in terms of loss of limb and function, goes beyond the economic impact.[7,9] Infection often results in the need for multiple operations, prolonged antibiotics, and extensive rehabilitation. For patients who develop an SSI, the consequences can be severe as the average length of hospital stay and overall mortality risk are doubled.[9] Interventions that decrease the risk of SSI stand to benefit the patients, their providers, the healthcare system, and society at large.

Despite improvements in surgical technique, systemic antibiotic prophylaxis, and reduced operating time, implant-associated spinal wound infections remain a serious concern.[1,2,7,8] This is especially true in light of the rapid emergence of multidrug resistant pathogen strains and a large immunocompromised patient population. Though under ideal conditions the incidence of infection has been reported to be less than 1% for patients undergoing elective spinal surgery, conditions are rarely ideal. The incidence of deep infection after spinal surgery may be in excess of 10% dependant on patient- and procedure-related factors.[3,7,8,10–13] Geriatric, immunocompromised, diabetic, obese, cognitively impaired, and trauma patients are all known to have greater risks of infection after spinal surgery.[8,11,13,14]

The purpose of the study was to investigate the use of novel local antibiotic delivery vehicle, as an adjunct to

From the University of Maryland, Shock Trauma Center, Baltimore, MD.
Acknowledgment date: February 14, 2008. Revision date: August 14, 2008. Acceptance date: September 21, 2008.
The device(s)/drug(s) that is/are the subject of this manuscript is/are not FDA-approved for this indication and is/are not commercially available in the United States.
No funds were received in support of this work. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.
Address correspondence and reprint requests to Kornelis A. Poelstra, MD, PhD, University of Maryland, 22 South Greene St-Suite 11B, Baltimore, MD 21212; E-mail: kpoelstra@gmail.com

routine perioperative systemic antibiotic prophylaxis, using a spinal implant animal model. An FDA-approved biodegradable polymer (poly(lactic-co-glycolic-acid) [PLGA]) was used to create resorbable microspheres to facilitate the controlled local delivery of gentamicin to wounds and hematoma. The efficacy of these microspheres in prevention of implant-associated spinal wound infections was evaluated using a well published spinal implant model in New Zealand white Rabbits (NZW).[15]

■ Materials and Methods

Animals

This investigation was approved by the Institutional Animal Care and Use Committee. Twenty-five NZW female rabbits were obtained weighing between 3.0 and 3.5 kg each. Female rabbits were used because, in the experience of the senior author, they are generally more docile and less prone to territorial marking with sprayed urine, which can potentially serve as a source of surgical site contamination.

Experimental Design

The current investigation was a randomized, prospective blinded study of the efficacy of a novel local antibiotic delivery vehicle for the prevention of implant-associated spinal wound infections, using a previously described animal infection model in the NZW rabbit.[15] This multisite biomaterial-centered animal model is time tested and reliably mimics the human condition of posterior spinal surgery with instrumentation. By using 3 noncontiguous implant sites, a single animal may serve as both a treatment and internal control, thereby minimizing the number of animals needed for the study. Using an FDA-approved biodegradable polymer PLGA slurry containing 20% gentamicin, resorbable microspheres (~10 μm, resorption in 3–7 days) were created to facilitate a reliable, controlled release delivery system to wounds and hematoma. The pharmacokinetics of the release were studied *in vitro* and *in vivo* before application in an animal model and have been previously described.[4]

In short, the gentamicin-microspheres or powdered gentamicin was administered into the rabbit spinal defect in the absence of bacteria (500 μg antibiotics per site). Animals were subsequently killed after 2, 4, 10, 24, 48, 72, 144, 168, and 208 hours. Hematoma was harvested from the implant sites and released gentamicin was determined in the supernatant after homogenation and centrifugation. Representation of release for both the microspheres and powdered gentamicin can be seen in Figure 1. Systemic levels never rose above the detection limit of 0.05 μg/mL in serum.

During the initial phase of the current study, 13 NZW rabbits were challenged at each of 3 surgical sites with varying concentrations of *Staphylococcus aureus* bacteria to reliably create a SSI in ~70% of control sites in the absence of antibiotics local. Once an infectious dose (ID-70) was established, the second phase of the study investigated the efficacy of gentamicin microspheres (2.5 mg per site containing 500 μg of gentamicin) for the prevention of implant-associated spinal wound infection. Twelve rabbits were used for the second phase of the study. Three noncontiguous surgical sites were used in each rabbit; 2 treatment sites and 1 control site, which were assigned in a random fashion. After 7 days, postoperative wound infection was assessed using standard tissue sampling and bacterial quantification techniques to study our hypothesis that the incidence of SSI and of implant-associated wound infection can be reduced using controlled, local delivery of gentamicin using microsphere technology.

Bacterial Inoculum

One day before surgery, *S. aureus* (ATCC 25923) was suspended in 5 mL trypticase soy broth and incubated at 37°C. After 18 hours, the culture was centrifuged (10,000 RPM) for 10 minutes, and the pellet was diluted in sterile saline. This washing process was repeated twice. Final concentrations of bacteria were obtained by making different dilutions in sterile saline. The final bacterial concentration (colony forming units (CFU) per milliliter) was estimated by using a densometric apparatus and assay (LaMotte 2020e, LaMotte, Chestertown, MD) and final determination was done by plating on Trypticase Soy Agar plates with 5% sheep blood (Fisher Scientific, Boston, MA).

Surgical Procedure

Induction of general anesthesia was performed using a combination of ketamine and xylazine, and subsequently maintained using isoflurane inhalation *via* nose-cone mask. All rabbits were given intravenous prophylactic ceftriaxone (20 mg/kg) before surgery to mimic preoperative prophylaxis in humans. After induction of anesthesia, the rabbits were positioned prone and each back was shaved, prepared, and draped in a sterile fashion. Three noncontiguous sites were created in each rabbit overlying the T13, L3, and L6 vertebrae. The surgical approach was identical for each site, though separate instruments and drapes were used for each surgical site to prevent cross contamination.

A 1.5-cm dorsal skin incision was made longitudinally in the midline, followed by a single incision in the fascia to expose the spinous process. Using a small rongeur, the entire spinous process with surrounding musculature and ligaments was excised from the base, creating a self-contained defect, approximating a partial laminectomy defect. The ligamentum flavum was not violated, and the dura was not exposed. A 1-cm Ti90/Al6/V4 rod (2-mm diameter, Item: TI017905, Goodfellow corporation, Oakdale, PA) was implanted into the defect. Wound hemostasis was achieved with a flowable hemostatic agent (Surgifoam, Johnson and Johnson Wound Management, Somerville, NJ), mixed with either a nonantibiotic PLGA resomer (control group) or gentamicin PLGA microspheres. Bacterial inoculum (100 μL) was placed into the defect using a sterile syringe needle (30 G). The fascia was closed using running sutures with biodegradable Vicryl 2/0 suture (Ethicon Inc. Piscataway, NJ). The skin was closed using a running subcutaneous suture with Vicryl 3/0 (Ethicon Inc.). During the initial phase of the study only the nonantibiotic PLGA Resomer was used and each of the 3 surgical sites was challenged with a randomly assigned bacterial load between 10^4 and 10^6 CFU to establish the ID-70. The second phase of the study started once the infectious dose was established. In this phase, 1 control site and 2 treatment sites were assigned randomly to each rabbit (using a random number generator). All wounds were challenged with 10^6 CFU.

After the procedure, analgesia was provided using a standard protocol, and all rabbits were permitted to drink, eat, and weight bear *ad libitum*. They were monitored daily, especially in regard to their wound healing, body weight, and signs of systemic infection.

Evaluation

After 7 days, postoperative wounds infections were assessed using standard tissue sampling and bacterial quantification

Table 1. Incidence of Surgical Site Infection (SSI) in Phase 1

Inoculum (CFU *Staphylococcus aureus*)	Total Sites Challenged	Infected	Noninfected	Infected (%)
10^3	6	0	6	0
10^4	6	0	6	0
10^5	17	3	14	18
10^6	7	5	2	71
Total sites	36			

To determine a reliable ID-70 where the desired infection incidence would be ~70% (achieved in 10^6 CFU (shaded row). Twelve rabbits challenged with 36 surgical sites and only placebo treatment (PLGA resin).

techniques. Rabbits were killed using an intravenous injection of phenobarbital (10 mg/kg). After the skin was removed off the entire back using sterile technique, samples of the fascia, the hematoma, and the vertebral lamina were taken and the implanted metal rods were removed from all sites. A piece of the right liver lobe and an intravenous blood sample were obtained to monitor for systemic infection. Harvested tissues weighed, then immediately homogenized (PowerGen 35, Fisher Scientific, Pittsburgh, PA), and implants were sonicated (UBATH, World Precision Instruments, Sarasota, FL) for 15 minutes in cold saline to detach bacteria. Serial dilutions of all samples were created and plated on blood agar plates for 24 hours of incubation at 37°C. The final CFU was determined per gram of tissue sample and per centimeter of titanium rod. Biomaterial-centered infection was defined to occur where *S. aureus* was present on the implanted rods and at least 1 other tissue sample from the same site. All samples were collected by and evaluated by a member of the team blind to the treatment type at each site.

χ^2 calculations (SigmaStat 3.5; Systat Inc. San Jose, CA) were used to determine if differences in infection incidence were statistically significant. Student *t* tests were performed to identify statistical differences in severity of bacterial burden, both with a *P* value set at 5% for significance.

■ Results

Two rabbits did not survive to the 7-day endpoint. One rabbit could not be resuscitated after induction of anesthesia before surgical intervention in phase 1 and another animal died unexpectedly during recovery in the postanesthesia incubator after surgery for phase 2. None of the remaining 23 animals suffered from any systemic infection and all started to gain weight again after postoperative day 2. Phase 1 was completed with 12 animals, and results from the increasing bacterial inoculum to achieve an approximate infection incidence of 70% are listed in Table 1.

Based on the results of phase 1, all spinal implant sites in phase 2 were inoculated with 10^6 CFU *S. aureus*. Eleven rabbits were evaluated after 7 days and final results for infection incidence are listed in Table 2. Both for SSI as well as implant-associated infection, incidence of infection was significantly reduced using gentamicin microspheres compared with control sites ($P < 0.01$: χ^2 test; SigmaStat 3.5, Systat Software, Inc.). Severity of infection was assessed using serial plating techniques with average bacterial counts for infected samples shown in Table 3. There was no significance between the control- and treatment group for severity of bacterial growth once a site became infected.

■ Discussion

Despite meticulous technique, bacteria end up inside the surgical wound after long procedures.[16,17] Though the routine use of systemic antibiotic prophylaxis has revolutionized the care of surgical patients, this modality alone is insufficient for high-risk patients.[17] Local hematoma harboring bacteria at the end of the procedure, combined with systemic malnutrition, tissue hypoxia, compromised skin under a stabilizing brace, and poor wound healing while patients are bedridden are important factors for the progression of these initial bacterial burdens into clinically significant infection. Even the implants themselves conspire against the surgeon physician to decrease the body's ability to eliminate bacteria. The use of implants enhances the formation of a surface adherent and protective "biofilm" that is difficult to eradicate despite the use of antibiotics that are highly effective in standard *in vitro* susceptibility tests.[18–21]

Local delivery of antibiotics to spinal surgical wounds is intuitively attractive as an adjunct to systemic perioperative antibiotics. Thus, it allows for the local environment, where intravenous antibiotics cannot reach (hypoxic, devitalized tissue, dead space, pooled hematoma without vascular supply), to be sterilized. The ability to delivery antibiotics locally to wounds, primarily in the form of antibiotic powder impregnated in bone cement, is well established in the treatment of musculoskeletal infections.[22–25] However, the use of bone cement for the local delivery of powdered antibiotics has many drawbacks. Foremost, the pharmokinetics of antibiotic delivery with bone cement are unpredictable and vary depending on the porosity of the cement used, the type of antibiotic, and the mixing conditions.[26–31] In general,

Table 2. Postoperative SSI and Implant-Associated Infection Incidence for Surgical Sites Treated Locally With Placebo (Control PLGA Resin) or Gentamicin Microspheres

Treatment	Total Sites Challenged	SSI	Implant Infection	Noninfected	Percentage SSI (Implant Infection)
Control	12	9	7	3	75 (58)
Gentamicin microspheres	21	8	5	13	38* (23)*
Total sites	33				

Eleven rabbits total with 33 sites challenged with 10^6 CFU *Staphylococcus aureus*. Results statistically and clinically significant (* indicates $P < 0.01$) for both outcomes compared with controls.

Table 3. Average Bacterial Count as Measured for Severity of Infection per Sample (With Standard Deviation of the Mean) in Log_{10} Values

Treatment	Fascia	Hematoma	Implant	Bone	Totals (Log_{10} CFU)
Control	6.0 (1.6)	6.7 (2.0)	4.8 (1.0)	6.1 (1.5)	6.0
Gentamicin microspheres	6.4 (1.3)	6.7 (1.5)	5.3 (0.9)	6.0 (1.5)	6.1

No significant differences were found between control and treatment groups for severity of colonization once a site became infected, despite their treatment.

the pharmokinetics are characterized by initial burst levels of antibiotics, which may be cytotoxic, and which rapidly decline often below therapeutic levels.[23,32–35] Because bone cement is not bio-absorbable, the cement itself may serve as a nidus for infection once the antibiotics have been delivered.[36,37] Bone cement also allows bacterial adhesion and growth even in the presence of antibiotics and sustained exposure to subtherapeutic antibiotic levels contributes to the further development of drug-resistant bacteria.[38–43] Furthermore, the bulk of the bone cement may compromise a surgeon's ability to close the surgical wound and, because the bone cement is nonabsorbable, additional surgery is often required for its explantation.

Gentamicin microspheres described herein offer many advantages over antibiotic impregnated bone cement. The reduction of postoperative infection was statistically and clinically significant, although the spheres did not protect against the severity of the infection in cases where infections occurred. Once a site became "overcolonized" despite the presence of the gentamicin microspheres, bacterial burdens were similar as seen in infected control sites. The explanation for that could be the "all-or-nothing" phenomenon. Once *S. aureus* CFUs overcame the local challenge and started surviving more frequently, overcolonization of the sites occurred. There is most likely a ceiling effect, above which CFUs become nutrient deprived and therefore, severity cannot "worsen" in control sites over the sites treated prophylactically with the microspheres. The pharmokinetics of the gentamicin microspheres, which provide a controlled and sustained release of therapeutic levels of antibiotics, are clinically superior to those of powdered antibiotics.[4] Additionally, because the microspheres are bioabsorbable, there is never a need for patients to undergo an additional surgical procedure for their removal nor do they serve as a nidus for infection once their antibiotics are delivered. The small size of

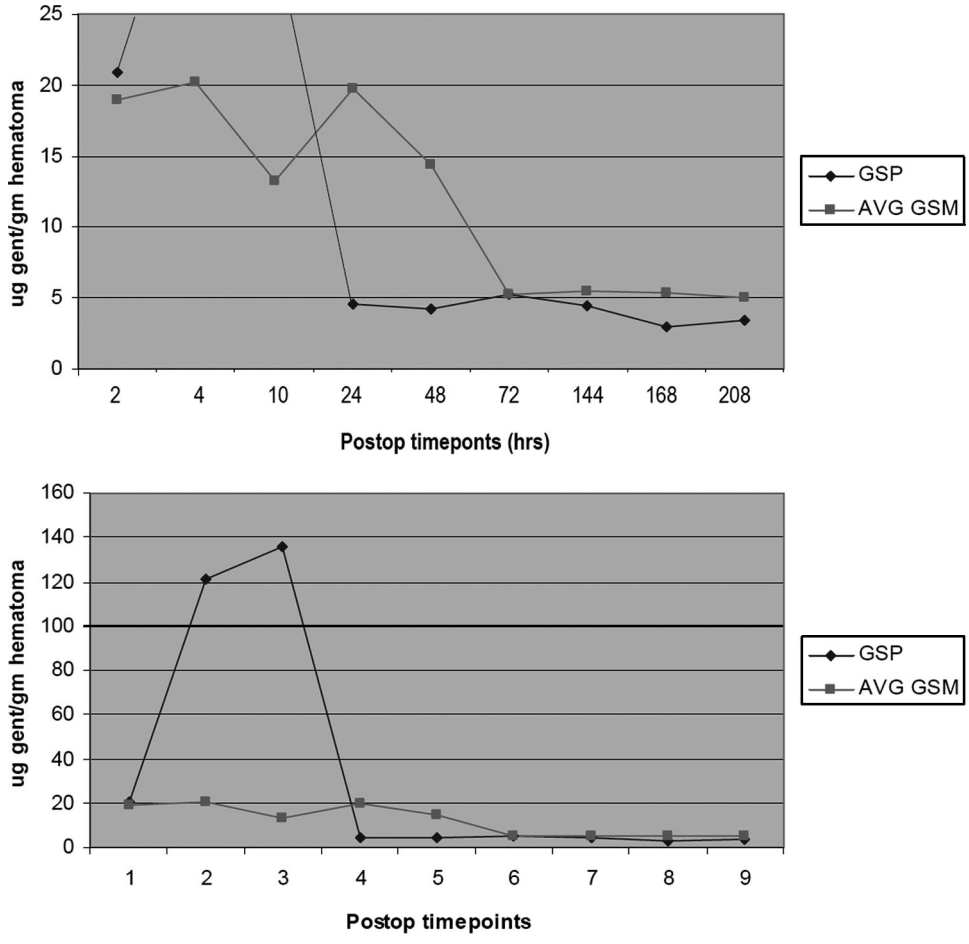

Figure 1. Local release of gentamicin into spinal wound hematoma surrounding the implant. Local antibiotic levels after release from Gentamicin Sulphate Microspheres (GSM) rose quickly to bactericidal concentrations (typically >5ug/ml) for at least 72 hrs, while Gentamicin Sulphate Powder (GSP) delivery did not deliver this level of bacterial protection for any longer than 24 hrs. Gentamicin released from powder (GSP) also overshot the 100ug/ml concentration (bottom figure), known to be toxic for osteoblasts activity, which could impede bone healing and arthrodeses.[32]

the microspheres allows them to accommodate any existing surgical defect, even allowing them to be injected by syringe after primary fascial closure, without ever compromising the surgical wound.

Gentamicin microspheres are not just intuitively attractive; they have proven to be effective both *in vitro* and *in vivo*. The results of the current study in a well established animal model are promising and have demonstrated the ability of these microspheres to significantly decrease the incidence of implant-associated postoperative wound infections. This is in agreement with prior efficacy data for the spheres against nonimplant-associated infection.[4] Most importantly, the use of gentamicin microspheres demonstrated a protective effect against SSI, in addition to that provided by systemic perioperative antibiotics, mimicking the current clinical standards. Clinical investigation of these gentamicin microspheres in postoperative spine wounds in high-risk patients is eminent.

■ Key Points

- Reliable biomaterial-centered infections were established in 3 spinal implant in NZW.
- Gentamicin microsphere treatments locally inside the hematoma of a laminectomy defect significantly reduced the infection incidence over control treatment in the same animal and will be investigated as an adjunct antibacterial prophylaxis in humans.

References

1. Bratzler DW, Houck PM, Richards C, et al. Use of antimicrobial prophylaxis for major surgery: baseline results from the National Surgical Infection Prevention Project. *Arch Surg* 2005;140:174–82.
2. Gold HS, Moellering RC Jr. Antimicrobial-drug resistance. *N Engl J Med* 1996;335:1445–53.
3. Levi AD, Dickman CA, Sonntag VK. Management of postoperative infections after spinal instrumentation. *J Neurosurg* 1997;86:975–80.
4. Poelstra KA, Vaccaro AR, Albert TA, et al. Gentamicin microspheres for infection prophylaxis in the rabbit spine. In: 53rd Orthopaedic Research Society (ORS) Meeting; February 10–14, 2007; San Diego, CA.
5. Bozic KJ, Ries MD. The impact of infection after total hip arthroplasty on hospital and surgeon resource utilization. *J Bone Joint Surg Am* 2005;87:1746–51.
6. Sculco TP. The economic impact of infected total joint arthroplasty. *Instr Course Lect* 1993;42:349–51.
7. Calderone RR, Garland DE, Capen DA, et al. Cost of medical care for postoperative spinal infections. *Orthop Clin North Am* 1996;27:171–82.
8. Glassman SD, Dimar JR, Puno RM, et al. Salvage of instrumental lumbar fusions complicated by surgical wound infection. *Spine* 1996;21:2163–9.
9. Kirkland KB, Briggs JP, Trivette SL, et al. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. *Infect Control Hosp Epidemiol* 1999;20:725–30.
10. Griffiths HJ. Orthopedic complications. *Radiol Clin North Am* 1995;33:401–10.
11. Massie JB, Heller JG, Abitbol JJ, et al. Postoperative posterior spinal wound infections. *Clin Orthop Relat Res* 1992;99–108.
12. Thalgott JS, Cotler HB, Sasso RC, et al. Postoperative infections in spinal implants. Classification and analysis–a multicenter study. *Spine* 1991;16:981–4.
13. Theiss SM, Lonstein JE, Winter RB. Wound infections in reconstructive spine surgery. *Orthop Clin North Am* 1996;27:105–10.
14. Andreshak TG, An HS, Hall J, et al. Lumbar spine surgery in the obese patient. *J Spinal Disord* 1997;10:376–9.
15. Poelstra KA, Barekzi NA, Grainger DW, et al. A novel spinal implant infection model in rabbits. *Spine* 2000;25:406–10.
16. Maathuis PG, Neut D, Busscher HJ, et al. Perioperative contamination in primary total hip arthroplasty. *Clin Orthop Relat Res* 2005;136–9.
17. Sheridan RL, Tompkins RG, Burke JF. Prophylactic antibiotics and their role in the prevention of surgical wound infection. *Adv Surg* 1994;27:43–65.
18. Gristina AG. Biomaterial-centered infection: microbial adhesion *versus* tissue integration. *Science* 1987;237:1588–95.
19. Gristina AG, Costerton JW. Bacterial adherence to biomaterials and tissue. The significance of its role in clinical sepsis. *J Bone Joint Surg Am* 1985;67:264–73.
20. Gristina AG, Naylor P, Myrvik Q. Infections from biomaterials and implants: a race for the surface. *Med Prog Technol* 1988;14:205–24.
21. Schwank S, Rajacic Z, Zimmerli W, et al. Impact of bacterial biofilm formation on in vitro and in vivo activities of antibiotics. *Antimicrob Agents Chemother* 1998;42:895–8.
22. Buchholz HW, Elson RA, Engelbrecht E, et al. Management of deep infection of total hip replacement. *J Bone Joint Surg Br* 1981;63-B:342–53.
23. Duncan CP, Masri BA. The role of antibiotic-loaded cement in the treatment of an infection after a hip replacement. *Instr Course Lect* 1995;44:305–13.
24. Hanssen AD, Rand JA, Osmon DR. Treatment of the infected total knee arthroplasty with insertion of another prosthesis. The effect of antibiotic-impregnated bone cement. *Clin Orthop Relat Res* 1994;44–55.
25. Springer BD, Lee GC, Osmon D, et al. Systemic safety of high-dose antibiotic-loaded cement spacers after resection of an infected total knee arthroplasty. *Clin Orthop Relat Res* 2004;47–51.
26. DeLuise M, Scott CP. Addition of hand-blended generic tobramycin in bone cement: effect on mechanical strength. *Orthopedics* 2004;27:1289–91.
27. Lawson KJ, Marks KE, Brems J, et al. Vancomycin vs tobramycin elution from polymethylmethacrylate: an in vitro study. *Orthopedics* 1990;13:521–4.
28. Lewis G, Janna S, Bhattaram A. Influence of the method of blending an antibiotic powder with an acrylic bone cement powder on physical, mechanical, and thermal properties of the cured cement. *Biomaterials* 2005;26:4317–25.
29. Neut D, van de Belt H, van Horn JR, et al. The effect of mixing on gentamicin release from polymethylmethacrylate bone cements. *Acta Orthop Scand* 2003;74:670–6.
30. Penner MJ, Duncan CP, Masri BA. The *in vitro* elution characteristics of antibiotic-loaded CMW and Palacos-R bone cements. *J Arthroplasty* 1999;14:209–14.
31. Penner MJ, Masri BA, Duncan CP. Elution characteristics of vancomycin and tobramycin combined in acrylic bone-cement. *J Arthroplasty* 1996;11:939–44.
32. Isefuku S, Joyner CJ, Simpson AH. Gentamicin may have an adverse effect on osteogenesis. *J Orthop Trauma* 2003;17:212–6.
33. McLaren AC. Alternative materials to acrylic bone cement for delivery of depot antibiotics in orthopaedic infections. *Clin Orthop Relat Res* 2004;101–6.
34. Miclau T, Edin ML, Lester GE, et al. Bone toxicity of locally applied aminoglycosides. *J Orthop Trauma* 1995;9:401–6.
35. Sterling GJ, Crawford S, Potter JH, et al. The pharmacokinetics of simplex-tobramycin bone cement. *J Bone Joint Surg Br* 2003;85:646–9.
36. Oga M, Arizono T, Sugioka Y. Bacterial adherence to bioinert and bioactive materials studied in vitro. *Acta Orthop Scand* 1993;64:273–6.
37. Oga M, Sugioka Y, Hobgood CD, et al. Surgical biomaterials and differential colonization by *Staphylococcus epidermidis*. *Biomaterials* 1988;9:285–9.
38. Hope PG, Kristinsson KG, Norman P, et al. Deep infection of cemented total hip arthroplasties caused by coagulase-negative staphylococci. *J Bone Joint Surg Br* 1989;71:851–5.
39. Kendall RW, Duncan CP, Beauchamp CP. Bacterial growth on antibiotic-loaded acrylic cement. A prospective in vivo retrieval study. *J Arthroplasty* 1995;10:817–22.
40. Kendall RW, Duncan CP, Smith JA, et al. Persistence of bacteria on antibiotic loaded acrylic depots. A reason for caution. *Clin Orthop Relat Res* 1996;273–80.
41. Thomes B, Murray P, Bouchier-Hayes D. Development of resistant strains of *Staphylococcus epidermidis* on gentamicin-loaded bone cement *in vivo*. *J Bone Joint Surg Br* 2002;84:758–60.
42. Tunney MM, Patrick S, Gorman SP, et al. Improved detection of infection in hip replacements. A currently underestimated problem. *J Bone Joint Surg Br* 1998;80:568–72.
43. van de Belt H, Neut D, Schenk W, et al. Gentamicin release from polymethylmethacrylate bone cements and *Staphylococcus aureus* biofilm formation. *Acta Orthop Scand* 2000;71:625–9.