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Treatment of Osteomyelitis in Rats by Injection of Degradable Polymer Releasing Gentamicin

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Abstract

We evaluated the potential of an injectable degradable polymer-Poly(sebacic-co-ricinoleic-ester-anhydride) containing gentamicin for the treatment of osteomyelitis.

Osteomyelitis of both tibiae was induced in 13 female Fischer rats by injecting a suspension containing approximately 105 (CFU)/ml of *S. Aureus* into the tibial medullar canal. Three weeks later both tibiae were X rayed, drilled down the medullar canal, washed with 50μl gentamicin solution (80mg/2ml) and then injected with 50μl P(SARA) + gentamycin 20% w/v to the right tibia and 50μl P(SA-RA) without gentamicin to the left tibia. After an additional 3 weeks, the rats were sacrificed, and radiographs of the tibiae were taken. Histopathological evaluation of the tibiae was done in a blinded manner.

X ray radiographs showed that all tibiae developed changes compatible with osteomyelitis in 3 weeks.

Histological evaluation revealed significant differences between right and left tibiae in 10 rats. In the left tibia moderate intramedullary abscess formation occurred. In most treated tibiae typical changes included the absence (or minimal grade only) of abscesses. The treated group developed significantly less intramedullary abscesses; the p-value was 0.028.

Locally injected degradable polymer releasing gentamicin proved to be efficient histologically in the treatment of osteomyelitis

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Committee of the Hebrew University of Jerusalem (Reg. No. 10581-4) approved the study protocol.

Introduction

Staphylococcal infections pose an emerging problem with major economic impact [1,2]. Osteomyelitis, a deep bone infection, mostly staphylococcal, can result from trauma, nosocomial infection, or an orthopaedic operation [3,4]. This complicated infection has proven difficult to treat [5]. The difficulty can be attributed to the short half-life of the antibiotic, poor blood circulation in the infected area, and systemic toxicity of the antibiotic. Alleviating osteomyelitis presents an additional challenge because the infecting bacteria form a biofilm mode of growth, which, on devascularized surfaces, shields them from antibiotics [6].

Because of the ischemia and slow healing seen in infected tissue and the relatively low blood flow in bone, antibiotics are administered for extended periods. Soft tissue infections and septic joints usually are treated with parenteral antibiotics for two to four weeks, whereas chronic osteomyelitis is treated for six weeks or longer [7].

The two methods currently available for the administration of antibiotics are intravenous injection with a long-term indwelling catheter [6] and a local implant of antibiotic containing polymethylmethacrylate (PMMA) beads or calcium phosphate bone cements [8,9]. Although the intravenous route produces adequate blood concentrations, it requires an invasive procedure for catheter placement and multiple daily antibiotic doses and achieves low concentration of antibiotics at the site of the infection. Other complications and difficulties associated with this method include infection, catheter failure, and the high cost of the antibiotics and supplies [10]. Antibiotic containing PMMA beads has shown an advantage over intravenous delivery by administering high concentrations of antibiotics locally with low systemic levels, thereby producing minimal systemic complications or allergic reactions. PMMA beads alone, however, usually provide locally bactericidal levels of antibiotics for only two to four weeks, so that supplemental parenteral antibiotics are often needed. In addition, the beads require a second intervention for their removal, after which the cavity may require reconstruction. Moreover, resistant bacteria protected within the beads may be a cause for treatment failure or late recurrence, and finally, polymerization process could cause a thermal damage and neutralize the antibiotic [11].

In recent years, various biodegradable systems have been evaluated for local delivery of antibiotics [12]. Polyanhydrides, one of several types of biodegradable polymeric carriers, are considered a highly effective treatment modality [13,14]. In addition, biodegradable polymers such as poly(lactic acid) [12], poly(glycolic acid) [15], poly(lactide-co-glycolide) [16], collagen [17], chitosan [18], and polyhydroxyalkanoates [19] have also been tested for their effectiveness as antibiotic carriers in local treatment; however, none of these polymers enables a trivial use as an injectable slow-release device.

In recent studies, we explored the use of injectable biodegradable poly(sebacic-coricinoleic-ester-anhydride)s, designated as p(SA:RA)s, for the treatment of solid tumors [20]. The p(SA:RA)s containing more than 65% ricinoleic acid are pasty at room temperature. According to our previous studies, once these polymers encounter an aqueous environment, they increase in viscosity and become semisolid gels. This semisolid polymeric matrix may release antibiotic until the polymer disintegrates. The purpose of this study is to evaluate the potential of p(SA:RA) for the treatment of osteomyelitis induced by *Staphylococcus aureus* (*S. aureus*).

Materials and Methods

Bacteria

S. aureus strain ATCC 29213 was selected for the bacterial inhibition study due to its presence in the majority of orthopedic infections [21]. It was grown in tryptic soy broth (TSB, Becton, Dickinson and Company) at 37°C.

From an overnight culture of *S. aureus* portions of 100 µl were transferred into sterile tubes containing 10 ml of TSB. These tubes were incubated for 8 h at 37°C in order to obtain log-phase growth. After incubation, tubes with a final optical density of 0.22 (shown by measurement of the absorbance at 600 nm using a spectrophotometer from VERSAmax, Molecular Devices Corporation, CA, USA) were used. Based on the optical density and plating in TBS agar plates, a suspension containing approximately 10⁵ colony-forming units (CFU)/ml of *S. aureus* was used as inoculum.

Polymers

Ricinoleic acid (RA) 85% pure (Fluka, Buch, Switzerland), sebacic acid (SA) 99% pure (Aldrich, Milwaukee, WI), and acetic anhydride (Merck, Darmstadt, Germany) were used.

All solvents were of analytical grade from BioLAB (Jerusalem, Israel) or Frutarom (Haifa, Israel) and were used without further purification.

Polymer containing 20% w/v gentamicin was used for the local treatment of osteomyelitis. Poly(sebacic-co-ricinoleic-ester-anhydride) 2:8 w/w ratio (P(SA-RA)) was synthesized as described elsewhere [22,23]. The polymer structure is shown in fig. 1.

Preparation of formulations and *in vitro* drug release

Formulations were prepared by mixing all components at room temperature. Gentamicin was mixed by trituration with the polymer to get a homogenous smooth polymer paste.

The gentamicin release from polymer formulation was conducted by placing each of the polymers (20% w/w gentamicin) into a vial prior to adding the releasing solution (0.1 M, pH 7.4) at 37°C with constant shaking (100 rpm). The solution was periodically replaced with fresh buffer solution, and gentamicin concentration was determined by using a UVvisible spectrophotometer (Ultrospec 2100 Pro, Biochrom, Cambridge, UK) at the maximum absorbance of gentamicin-o-phthaldialdehyde complex at $\lambda_{\text{max}} = 332 \text{ nm}$ [24]. A calibration curve of gentamicin in buffer was plotted before each determination.

Animals

Thirteen female Fischer rats (Harlan, Israel) approximately 3 months old, weighing about 200 g (range 190–210g) were used. Rats were fed a standard laboratory diet. The animals were housed with a constant room temperature. All experiments were carried out in accordance with the guidelines of the local Animal Welfare Committee. The Ethical Committee of the Hebrew University of Jerusalem (Reg. No. 10581-4) approved the study protocol.

Study design

Osteomyelitis of the tibia was induced according to the method described by Zak et al. [25]. Rats were anesthetized by intra peritoneal injection of a mixture of xylazine and ketamine. After shaving of right and left hind legs and sterilization of the shaved area with ethanol, the skin was incised over a length of approximately 1.0 cm at the medial aspect of the proximal tibia. A cortical bone defect was produced by means of a twist drill (diameter 1 mm) with a 0.8 mm kw. The entry point was between the tibial plateau and the tibial tuberosity to a depth

of approximately 1cm down the medullar canal. 100 μ l of defined *S. aureus* suspension was injected into the medullary cavity. The skin was closed by stitches and disinfected. Osteomyelitis of the right and left tibia was induced and proved by X rays 21 days following inoculation.

21 days following inoculation—The entry region was re exposed in each tibia, and it was debrided and re drilled as described above. Both tibiae were washed with 50 μ l gentamicin solution (40mg/ml) first. Then, the right tibia was injected intramedullary with 50 μ l polymer including gentamicin 20% w/v, and the left tibia with 50 μ l plain polymer. The skin was closed by stitches and disinfected.

42 days following inoculation—The rats were anesthetized and sacrificed. The tibiae were evaluated macropathologically, radiographically, and histologically.

Histopathologic evaluation

After scarifying the rats, tibiae with attached distal femurs were stored in 4% neutral buffered formalin. The segments were sent for histological processing (Patho-lab, Ness-Ziona, Israel). The tibiae were trimmed longitudinally, and both halves were embedded in paraffin, and routinely processed for light microscopy. The trimming was done between the distal femur and the distal tibia (Fig. 2). Sections were stained with hematoxylin and eosin (H&E). For each sample, one histological section was generated. A board-certified toxicological pathologist (A.N.) performed the histopathological evaluation. Each sample was evaluated and graded for histopathological changes. The reactive and inflammatory changes were assigned severity grades of 0–4 representing unremarkable, minimal, mild, moderate, and marked changes, respectively. The relevant evaluated parameters included evidence for infection (abscess formation), destruction of cortical and cancellous bone, periosteal new bone formation, and intramedullary fibrosis. Evaluated parameters were based on the study of Lucke et al. (27). Gram staining was applied on all histological sections to identify the presence of the *S. aureus* colonies within the medullary cavities.

Radiographic evaluation

X ray evaluation was done twice during the study by orthopaedic surgeons (Y.S.B. and G.M). The first evaluation was done 21 days following inoculation, before implanting the polymer, and the 2nd evaluation was done 21 days later, after sacrificing the rats (Fig. 2).

The radiographic changes were assigned severity grades of 0–3 representing unremarkable, mild, moderate and severe changes, respectively. The evaluated parameters included lucentic lesions, periosteal reaction, cortex irregularity, widening of the metaphysis, and growth plate irregularity. The radiographic evaluation is shown in Table 1.

Statistical methods

Since the sample size was small and paired, we used nonparametric bootstrap methodology where we resampled the entire record on each animal and then, within each selected animal, randomly permuted the left and right leg to generate the null distribution for computing p-values. Since the data are ordinal, we tested for the null hypothesis that the treatment group and control group developed equally intramedullary abscesses against the alternative that the treated group has stochastically fewer abscesses compared to the control group.

Results

The 13 rats that were treated (with and without gentamicin) survived the entire study period.

Macropathologic evaluation during study

The infected legs were swollen and hyperemic as a result of the inflammatory process by 21 days after inoculation. The infected tibiae were much softer for drilling treatment. There were no macropathologic differences between treated and untreated tibiae on day of sacrifice. Although the rats suffered from osteomyelitis, they could walk.

Radiographic evaluation

The first X ray evaluation, 21 days post-inoculation, revealed area changes attributed to the osteomyelitic process including bone lucency mostly, cortex irregularity, periosteal reaction, metaphysis widening, and growth plate irregularity in the affected area. The second X ray evaluation, 21 days following treatment, revealed no significant differences from the former. No clear radiographic differences occurred between treated and untreated tibiae. The radiographic results are summarized in Table 1.

Histopathologic evaluation

In several rats, a significant difference between the right and left tibiae was noted. Typical changes in the left tibiae treated by injection of polymer without gentamicin included the presence of mild (Grade 2) to moderate (Grade 3) intramedullary abscess formation. Typical changes in the right tibia of some rats treated with intramedullary injection of degradable polymer with gentamicin included the absence (or minimal grade only) of abscesses, associated with the presence of stromal tissue having multiple cavities from where the test compound was washed out during the histological processing.

A photographic representation of the tissues treated by injection of polymer with or without gentamicin is presented in Fig. 3 (A, B). All the histological results are summarized in Table 2.

The results of the Gram staining evaluation are presented in Table 2, and representative photos are presented in Fig. 3 (C - F). The data indicate that, in some of the examined left tibiae (treated with the plain polymer), bacterial colonies were present, while in contralateral right tibiae, bacterial colonies were not identified. In some of the left tibial samples, bacterial colonies were not identified.

Discussion

Local antibiotic therapy contributes a critical part of treatment protocol in cases of chronic osteomyelitis and postarthroplasty osteomyelitis [27,28]. It is also used as a preventive device in cases of open fractures [29,30,31].

We induced osteomyelitis in rat tibiae by *S. aureus* injection and treated the infected site with p(SA:RA)s used as a vehicle for local delivery of gentamicin. Many authors have described the usage of PMMA as a standard delivery vehicle for depot antibiotics in orthopedic surgery [32–36]. Recently, a search for alternative vehicles has been made because PMMA has some important limitations [37].

Various biodegradable systems have been evaluated for local delivery of antibiotics [38]. Polyanhydrides [14,39], calcium sulfate [40], hydroxyapatite [41] morselized bone [42], and fibrin sealant materials [43] have all shown some promise, but none of them is being used routinely as a vehicle for local antibiotic delivery.

Biodegradable implants releasing aminoglycosides have been found effective in treating osteomyelitis [13,14,22]. Recently, we have explored the use of injectable biodegradable

(SA:RA)s as carrier of paclitaxel for the treatment of solid tumors [20,22] and as carrier of gentamicin for the treatment of osteomyelitis [44]. Its main advantages include release of gentamicin for longer periods, no need for a removal operation, injectability of the polymer, and no exothermic reaction when injected into bone.

In the present study all the animals developed osteomyelitis within 3 weeks following inoculation confirmed by X ray and histology (Table 1, Fig. 2). Clinical evidence for the involvement of the surrounding soft tissue in the inflammation process was noticed as well. Three weeks after site treatment with the gentamicin containing polymer, a reduction of bony abscess formation at the area of infection, confirmed histologically, supported the approach of local antibiotic treatment by a slow-release polymeric device. Local treatments of osteomyelitis, combined with a systemic antibiotic, have proved successful. Reduction in bacterial count occurred in rabbits treated for four weeks with poly(lactic-co-glycolic acid) (PLGA) microspheres containing tobramycin together with parenteral cephazolin or with PMMA containing tobramycin together with parenteral cephalosporin [45]. One group demonstrated a decline in bacterial count in rats treated for 4 weeks with a gentamicin-containing collagen sponge or PMMA containing gentamicin. The effect was even better when combined with systemic cephalosporin treatment. [46].

Others have demonstrated a successful local antibiotic treatment with short-term perioperatively systemic antibiotic administration in a human infected non-union and chronic osteomyelitis [27,28]. The animals in our study were treated locally only. Were systemic antibiotic therapy added, the results might be superior [45,46]; we aim to find a substitute for long-term systemic antibiotic treatment.

Most researchers have evaluated treatment results based on bacterial count [45–49]. Some evaluations of macropathologic changes have been made as well [46,48,50]. One group, in addition to bacterial counting, evaluated radiographic and histopathologic changes, but did not describe those results in detail. They did not find histopathological differences among the various groups, and radiographic changes were observed in rabbits that were treated locally with PMMA-containing tobramycin and systemic cephalosporin therapy [45]. Histopathological changes were assessed in other studies as well [49,50].

We performed histopathological, radiographic, and macropathologic evaluations. Histopathological results revealed improvement in the infection process in tibiae treated with polymer containing gentamicin. On the other hand, in tibiae that were treated with polymer only, formation of intramedullary abscesses indicated no improvement. We used nonparametric bootstrap methodology to resample the entire record on each animal; then, within each selected animal we randomly permuted the left and right leg to generate the null distribution for computing p values. The base for comparison was the number of intramedullary abscesses. Since the data are ordinal, we tested for the null hypothesis that the treatment group and control group equally developed intramedullary abscesses against the alternative that the treated group has stochastically fewer abscesses compared to the control.

The p value of 0.028 shows a significant change between the treated and control groups. Application of Gram staining was not consistently suitable to demonstrate the presence of bacteria. These findings are consistent with published reports that clinical cases of chronic osteomyelitis may not show the presence of bacteria, even though bacterial infection was the cause [51].

The treatment period was differed among various animal studies. Infected rats treated for 6 weeks with vancomycin-loaded PMMA manifested reduction of bacterial count [47]. In all New Zealand rabbits treated 2 and 4 weeks locally with gatifloxacin-containing polycaprolactone a significant decrease in bacterial count was reported [48]. Therapeutic effect

in rats after 2 week treatment with gentamicin-containing collagen sponge or gentamicin-containing PMMA was improved after 4 weeks of treatment [46]. Decline in bacterial count was observed in New Zealand rabbits after 4 weeks of treatment with PLGA microspheres containing tobramycin together with parenteral cephazolin treatment [45]. Rabbits treated locally with tazocin-loaded PMMA exhibited complete physical, radiological, and histological healing after 8 weeks [50]. Rabbits treated with polyanhydride-gentamicin beads for 4 weeks showed 97% elimination of infection [51].

Human osteomyelitis in long bones has been treated traditionally for 4–6 weeks [7]. The rats in our study were treated for a period of 3 weeks with successful results. A longer treatment period might further improve the results.

We opine X rays together with other diagnostic tools contribute an integral part of evaluation and treatment of human osteomyelitis. Two groups of researchers [47,48] evaluated radiographically only the state of infection. One evaluated bone healing radiographically [45]. Bone infection and healing were another focus [49]. Our radiographic results demonstrate changes attributed to the infection process in all tibiae 3 weeks following inoculation. The second radiographic evaluation was performed 3 weeks after initiation of treatment. We did not notice a radiographic significant change between the two periods and between treated and untreated tibiae at the end of the study. Radiographic evidence of improvement may lag behind clinical recovery, even when the patient is receiving appropriate antimicrobial therapy. [7, 52].

While our study did not demonstrate eradication of the infection, histological indicators showed improvement in the infection and in bacterial presence in the infected bone. Subsequent studies are planned to use more animals, perform bone cultures, and incorporate longer periods of treatment to enhance the radiographic, macropathologic, and information gleaned

Conclusions

The local injected p(SA-RA)s polymeric device releasing gentamicin proved to be efficient in treating osteomyelitis. This technique might prove to be an adjunct or alternative to existing treatments and might spare antibiotic and operative treatment. Further research with a larger samples and longer periods of treatment is required.

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References

1. Chambers HF. The changing epidemiology of *S. aureus*. *Emerg Infect Dis* 2001;7:178–182. [PubMed: 11294701]
2. Rubin RJ, Harrington CA, Poon A. The economic impact of *S. aureus* infection in NY City hospitals. *Emerg Infect Dis* 1999;5:9–17. [PubMed: 10081667]
3. Mader JT, Cripps MW, Calhoun JH. Adult posttraumatic osteomyelitis of the tibia. *Clin Orthop Relat Res* 1999;360:14–21. [PubMed: 10101306]
4. Cre'mieux AC, Carbon C. Experimental models of bone and prosthetic joint infections. *Clin Infect Dis* 1997;25:1295–1302. [PubMed: 9431367]
5. Waldvogel FA, Medoff G, Swartz MN. Osteomyelitis: A review of clinical features, therapeutic considerations and unusual aspects. *N Engl J Med* 1970;282:198–206. [PubMed: 4902833]

6. Costerton JW. Biofilm theory can guide the treatment of device-related orthopaedic infections. *Clin Orthop Relat Res* 2005;437:7–11. [PubMed: 16056019]
7. Lazzarini L, Mader JT, Calhoun H. Osteomyelitis in long bones. *J Bone Joint Surg Am* 2004;86-A(10):2305–2318. [PubMed: 15466746]
8. Walenkamp GH, Kleijn LL, de Leeuw M. Osteomyelitis treated with gentamicin-PMMA beads: 100 patients followed for 1–12 years. *Acta Orthop Scand* 1998;69:518–522. [PubMed: 9855236]
9. Sasaki T, Ishibashi Y, Katano H, Nagumo A, Toh S. In vitro elution of vancomycin from calcium phosphate cement. *J Arthroplasty* 2005;20:1055–1059. [PubMed: 16376263]
10. Stern JA, Clemens JQ. Osteomyelitis of the pubis: a complication of a chronic indwelling catheter. *Urology* 2003;61:4621–4622.
11. Wong MW, Hui M. Development of gentamicin resistance after gentamicin-PMMA beads for treatment of foot osteomyelitis: report of two cases. *Foot Ankle Int* 2005;26:1093–1105. [PubMed: 16390644]
12. Schmidt C, Wenz R, Nies B. Antibiotic in vivo/in vitro release, histocompatibility and degradation of gentamicin implants based on lactic acid and copolymers. *J Control Release* 1995;37:83–94.
13. Domb AJ, Rosenberg AE, Hanff P. Bioerodible polyanhydrides for antibiotic drug delivery: in vivo osteomyelitis treatment in a rat model system. *J Orthop Res* 1993;1:256–262. [PubMed: 8483038]
14. Li LC, Deng J, Stephens D. Polyanhydride implant for antibiotic delivery—from the bench to the clinic. *Adv Drug Deliv Rev* 2002;54:963–986. [PubMed: 12384317]
15. Overbeck JP, Winckler ST, Meffert R. Penetration of ciprofloxacin into bone: a new bioabsorbable implant. *J Invest Surg* 1995;8:155–162. [PubMed: 7547723]
16. Garvin KL, Miyano JA, Robinson D. Polylactide / polyglycolide antibiotic implants in the treatment of osteomyelitis. A canine model. *J Bone Joint Surg. Am* 1994;76:1500–1506. [PubMed: 7929497]
17. Leyh RG, Bartels C, Sievers HH. Adjuvant treatment of deep sternal wound infection with collagenous gentamicin. *Ann Thorac Surg* 1999;68:1648–1651. [PubMed: 10585036]
18. Aimin C, Chunlin H, Juliang B. Antibiotic loaded chitosan bar. An in vitro, in vivo study of a possible treatment for osteomyelitis. *Clin Orthop Relat Res* 1999;366:239–247. [PubMed: 10627741]
19. Gursel I, Korkusuz F, Turesin F. In vivo application of biodegradable controlled antibiotic release systems for the treatment of implant-related osteomyelitis. *Biomaterials* 2001;22:73–80. [PubMed: 11085386]
20. Shikanov B, Vaisman MY, Krasko A, Nyska A, Domb J. Poly(sebacic acid-coricinoleic acid) biodegradable carrier for paclitaxel: in vitro release and in vivo toxicity. *J Biomed Mater Res* 2004;69:47–54.
21. Gracia E, Lacleriga A, Monzon M, Levia J, Oteiza C, Amorena B. Application of a rat osteomyelitis model to compare in vivo and in vitro the antibiotic efficacy against bacteria with high capacity to form biofilms. *J Surg Res* 1998;79(2):146–153. [PubMed: 9758730]
22. Shikanov A, Ezra A, Domb AJ. Poly(sebacic acid-co-ricinoleic acid) biodegradable carrier for paclitaxel—effect of additives. *J Control Release* 2005;105(1–2):52–67. [PubMed: 15955366]
23. Teomim D, Domb AJ. Nonlinear fatty acid terminated polyanhydrides. *Biomacromolecules* 2001;2(1):37–44. [PubMed: 11749153]
24. Sampath SS, Robinson DH. Comparison of New and Existing Spectrophotometric Methods for the Analysis of Tobramycin and Other Aminoglycosides. *J Pharm Sci* 1990;79(5):428–431. [PubMed: 2352163]
25. Zak, OZF.; Rich, R.; Tosch, W.; Kradolfer, F.; Sheld, WM. Experimental staphylococcal osteomyelitis in rats: therapy with rifampin and cloxacillin, alone or in combination. Current chemotherapy and immunotherapy. GG Periti, P., editor. Washington: American Society for Microbiology; 1982. p. 973-974.
26. Lucke M, Schmidmajer G, Sadoni S, Widemann B, Haas NP, Rasche M. Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. *Bone* 32:521–531. [PubMed: 12753868]
27. Patzakis MJ, Mazur K, Wilkins J, Sherman R, Holtom P. Septopal beads and autogenous bone grafting for bone defects in patients with chronic osteomyelitis. *Clin Orthop Relat Res* 1993;295:112–118. [PubMed: 8403634]

28. Calhoun JH, Henry SL, Anger DM, Cobos JA, Mader JT. The treatment of infected nonunions with gentamicin-polymethylmethacrylate antibiotic beads. *Clin Orthop* 1993;295:23–27. [PubMed: 8403654]
29. DeSilva GL, Fritzler A, DeSilva SP. Antibiotic-impregnated cement spacer for bone defects of the forearm and hand. *Tech Hand Up Extrem Surg* 2007;11(2):163–167. [PubMed: 17549024]
30. Holtom PD. *J Am Acad Orthop Surg* 2006 Oct;14(10 Suppl):S98–S100. [PubMed: 17003220]
31. Buchholz HW, Engelbrecht H. Depot effects of various antibiotics mixed with Palacos resins. *Chirurg* 1970;41:511–515. [PubMed: 5487941]
32. Evans RP, Nelson CL. Gentamicin-impregnated polymethylmethacrylate beads compared with systemic antibiotic therapy in the treatment of chronic osteomyelitis. *Clin Orthop Relat Res* 1993; (295):37–42. [PubMed: 8403668]
33. Jenny G. Local antibiotic therapy using gentamicin-PMMA chains in post-traumatic bone infections. Short and long-term results. *Reconstr Surg Traumatol* 1988;20:36–46. [PubMed: 3399738]
34. Klemm KW. Antibiotic bead chains. *Clin Orthop Relat Res* 1993;295:63–76. [PubMed: 8403672]
35. Klemm KW. Gentamicin-PMMA chains (Septopal chains) for the local antibiotic treatment of chronic osteomyelitis. *Reconstr Surg Traumatol* 1988;20:11–35. [PubMed: 3399737]
36. Ambrose CG, Clyburn TA, Loudon K, Joseph J, Wright J, Gulati P, Gogola GR, Mikos AG. Effective treatment of osteomyelitis with biodegradable microspheres in a rabbit model. *Clin Orthop Relat Res* 2004 Apr;421:293–299. [PubMed: 15123963]
37. van de Belt, Neut, D.; Schenk, W.; van Horn, JR.; van der Mei, HC.; Busscher, HJ. Gentamicin release from polymethylmethacrylate bone cements and *Staphylococcus aureus* biofilm formation. *Acta Orthop Scand* 2000;71:625–629. [PubMed: 11145392]
38. Schmidt C, Wenz R, Nies B. Antibiotic in vivo/in vitro release, histocompatibility and degradation of gentamicin implants based on lactic acid and copolymers. *J. Control. Release* 1995;37:83–94.
39. Quick DJ, Macdonald KK, Anseth KS. Delivering DNA from photocrosslinked, surface eroding polyanhydrides. *J. Control. Release* 2004;97:333–343. [PubMed: 15196760]
40. McLaren AC, McLaren SG, Nelson CL, Wassell DL, Olsen KM. The effect of sampling method on the elution of tobramycin from calcium sulfate. *Clin Orthop Relat Res* 2002;403:54–57. [PubMed: 12360008]
41. Jansen JA, van de Waerden JP, Wolke JG, Groot K. Histologic evaluation of the osseous adaptation to titanium and hydroxyapatite-coated titanium implants. *J Biomed Mater Res* 1991;25:973–989. [PubMed: 1655798]
42. McLaren AC, Miniaci A. Pharmacokinetics of depot administration of tobramycin mixed with morselized cancellous bone. *Trans Orthop Res Soc* 1987:12.
43. Readl H, Schlag G, Stanek G, Hirschl A, Seelich T. In vitro properties of mixtures of fibrin seal and antibiotics. *Biomaterials* 1983;4:29–32. [PubMed: 6601497]
44. Krasko MY, Golenser J, Nyska A, Nyska M, Brin YS, Domb AJ. Gentamicin extended release from an injectable polymeric implant. *J Control Release* 2007;117(1):90–96. [PubMed: 17150275]
45. Ambrose CG, Clyburn TA, Loudon K, Josef J, Wright J, Gulati P, Gogola GR, Mikos AG. Effective treatment of osteomyelitis with biodegradable microspheres in a rabbit model. *Clin Orthop Relat Res* 2004;421:293–299. [PubMed: 15123963]
46. Mendel V, Simanowski HJ, Scholz HC, Heymann H. Therapy with gentamicin-PMMA beads, gentamicin-collagen sponge, and cefazolin for experimental osteomyelitis due to *Staphylococcus aureus* in rats. *Arch Orthop Trauma Surg* 2005;125(6):363–368. [PubMed: 15864679]
47. Zelken J, Wanich T, Gardner M, Griffith M, Bostrom M. PMMA is superior to hydroxyapatite for colony reduction in induced osteomyelitis. *Clin Orthop Relat Res* 2007;462:190–194. [PubMed: 17514008]
48. Kamel AH, Baddour MM. Gatifloxacin biodegradable implant for treatment of experimental osteomyelitis: in vitro and in vivo evaluation. *Drug Deliv* 2007;14(6):349–356. [PubMed: 17701524]
49. Nelson CL, Hickmon SG, Skinner RA. Treatment of experimental osteomyelitis by surgical debridement and the implantation of biodegradable, polyanhydridegentamicin beads. *J Orthop Res* 1997;15:249–255. [PubMed: 9167628]

50. Brozsei L, Mintal T, Koos Z, Kocsis B, Helyes Z, Kereskai L, Nyarady J. Examination of a novel, specified local antibiotic therapy through polymethylmethacrylate capsules in a rabbit osteomyelitis model. *Chemotherapy* 2006;52(2):73–79. [PubMed: 16567940]
51. Perry CR, Pearson RL, Miller GA. Accuracy of cultures of material from swabbing of the superficial aspect of the wound and needle biopsy in the preoperative assessment of osteomyelitis. *J Bone Joint Surg Am* 1991;73(5):745–749. [PubMed: 2045400]
52. Butt WP. The radiology of infection. *Clin Orthop* 1973;96:20–30. [PubMed: 4584241]

Figure 1.
the structure of the polymer:

Figure 2.

Rats number 3 and 4. The x-ray was taken 42 days after inoculation.

Drilling was done in the tibia between the two red arrows.

Entry site between tibial plateau and tuberosity.

A KW of 0.8 mm was used to drill down the tibial canal for 1cm.

The rt. tibiae were treated with polymer and gentamicin and the Lt. tibiae were treated with polymer only.

Note the periosteal reaction in the Lt. tibia of rat number 4, and the bone lucency of the Lt. tibia of rat number 3 (arrows).

Figure 3.

- Note: all photos were taken from animal # 2, left and right legs
- B. Right tibia - diaphyseal region – The leg was injected with a polymer loaded with gentamicin. Note: The absence of intramedullary abscesses, which were replaced by stromal tissue having multiple cavities from where the test compound was washed out during the histological processing (arrows). Hematoxylin and Eosin, X 10.

- C. Left tibia - diaphyseal region – The leg was injected with plain polymer. Note: presence of multiple intramedullary abscesses with bluish granules, representing the Gram positive stained, *Staphylococcus aureus* (arrows). Gram staining, X 10.
 - D. Right tibia - intramedullary stromal tissue within the diaphyseal region – The leg was injected with a polymer loaded with gentamicin. Note: There is no Gram positive stained, *Staphylococcus aureus* bacteria (arrows). Gram staining, X 10.
 - E. Left tibia - diaphyseal region – The leg was injected with plain polymer. Note: presence of multiple intramedullary abscesses with bluish granules, representing the Gram positive stained, *Staphylococcus aureus* bacteria (arrows). Gram staining, X 40.
 - F. Right tibia - intramedullary stromal tissue within the diaphyseal region – The leg was injected with a polymer loaded with gentamicin. Note: There is no Gram positive stained, *Staphylococcus aureus* bacteria (arrows). Gram staining, X 40.
1. level of evidence
 2. Shyamal

Table 1
radiographic evaluation

Animal #	1		2		3		4		5		6		7		8		9		10		11		12		13	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
t=21																										
LUCENTIC LEASIONS	2	1	1	1	2	3	1	3	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1
PERIOSTAL REACTION	0	0	0	1	0	1	1	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
CORTEX IRREGULARITY	0	0	1	0	1	3	1	3	1	0	1	1	1	2	1	1	2	0	0	0	0	0	0	0	1	1
WIDENING OF METAPHYSIS	1	1	1	1	0	3	0	1	1	0	1	0	0	1	1	1	2	0	1	1	1	0	0	0	1	1
GROWTHPLATE IRREGULARITY	1	1	1	3	1	2	1	1	1	0	2	1	1	1	2	2	2	2	3	1	1	2	0	1	1	3
t=42																										
LUCENTIC LEASIONS	1	2	1	3	2	3	1	2	1	1	1	1	0	1	2	2	2	1	2	2	2	1	1	2	1	1
PERIOSTAL REACTION	0	0	0	2	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORTEX IRREGULARITY	1	2	0	2	1	2	1	1	1	0	1	1	0	1	1	1	2	1	1	1	0	0	0	1	0	0
WIDENING OF METAPHYSIS	1	1	1	1	0	1	0	0	1	0	1	0	0	1	1	1	2	0	1	0	3	0	0	1	1	1
GROWTHPLATE IRREGULARITY	2	0	1	3	2	2	1	1	2	2	2	1	1	2	3	2	2	2	3	2	3	2	3	2	1	1

0-non, 1- mild, 2- moderate, 3- severe

Table 2
histopathologic evaluation

Animal #	1		2		3		4		5		6		7		8		9		10		11		12		13	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Bone pathological change																										
Treatment:	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP	W+ PG	W+ PP
Medullary cavity, abscesses formation, multifocal	0	0	0	3	1	3	0	3	0	0	0	1	1	3	0	2	0	1	0	1	2	1	0	1	1	1
Results of Gram staining	G- G-		G- G+		G- G+		G- G+		G- G-		G- G-		G- G-		G- G-		G- G-		G- G-		G- G-		G- G-		G- G-	

W=wash with gentamicin sol., S= wash with saline, PP=plain polymer (blank),

PG= polymer loaded with gentamicin There is no mention in the table to wash with saline!

G+ = Presence of Gram + bacteria within the medullary cavity

G- = No presence of Gram + bacteria within the medullary cavity

R= right tibia washed with gentamicin and treated by injection of a polymer loaded with gentamicin

L= left tibia washed with gentamicin and treated by injection of a plain polymer (blank)