Local Antibiotic Delivery with Bovine Cancellous Chips

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ABSTRACT: Infected bone defects and osteomyelitis are encountered frequently in trauma cases. Currently, the standard of care for osteomyelitis cases is prolonged systemic antibiotic therapy and implantation of antibiotic carrier beads. However, this method requires a secondary surgery to remove the beads after the infection has cleared. In the present study a common bone void filler was investigated for its ability to be infused with an antibiotic. This study demonstrates that the xenograft material tested can be loaded with gentamicin and release clinically relevant levels of the drug for at least 14 days *in vitro* allowing for the inhibition of bacterial growth on the graft. This study also demonstrates that the levels of gentamicin released did not have an adverse effect on primary osteoblast cell proliferation or ability to generate alkaline phosphatase. This bone void filler may represent a viable alternative to current methods of local antibiotic delivery in orthopedic applications.

KEY WORDS: antibiotic, xenograft, osteomyelitis, cancellous bone, *Staphylococcus aureus*.

INTRODUCTION

Open fractures are often contaminated and the development of infection is a major complication. Osteomyelitis, a deep bone infection, most frequently caused by *staphylococcal* species, can result

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from the initial trauma or as a result of a nosocomial infection [1,2]. Infection leads to the devitalization of bone and soft tissue as well as loss of skeletal stability. Bone infections associated with foreign bodies such as prostheses or other orthopedic devices such as cancellous chips are especially difficult to treat. Once established, these infections are routinely treated by debridement of the surrounding tissue and bone as well as an aggressive antibiotic treatment [3–5]. The current standard of care uses systemic antibiotic delivery for prophylactic treatment. Treatment with systemic antibiotics alone suffers from several drawbacks including poor penetration into ischemic and necrotic tissue at the wound site as well as a potential for systemic toxicity.

To circumvent the accessibility and toxicity issues associated with systemic antibiotic treatments, various local antibiotic systems have been developed including antibiotic-loaded bone cements, organic polymers, and beads [6–10]. The use of these materials has offered an important adjunctive route to administer antibiotics for the treatment or prevention of osteomyelitis [11–13]. Although the use of antibiotic-loaded methylmethacrylate cement improves the outcome of periprosthetic infections [5–9], this delivery system has substantial limitations [12]. Elution rates of common antibiotics mixed with calcium sulfate or polymethylmethacrylate (PMMA) varied considerably depending on the physical environment [14–17]. In addition to high variability in elution rates, the burst release phase leads to concerns for local and systemic toxicity.

PMMA beads are commonly used in clinical orthopedic settings for local antibiotic delivery [12]. This delivery system has the advantage of providing locally high levels of the antibiotic with relatively low serum levels. However, this treatment regimen requires two surgical procedures and a large percentage of the incorporated antibiotic is not released [18]. There have been several reports demonstrating the benefits of local delivery of antibiotics in combination with systemic administration in patients with osteomyelitis [12,19–21]. Gentamicin, an aminoglycoside, is an antimicrobial that is effective against Gram-negative bacteria. This is one of several antibiotics that are currently being used for effective treatment of osteomyelitis due to its ability to combat Gram-negative bacteria as well as *Staphylococcus aureus*, which has been found to be a leading cause of infection [22,23].

Ideal antibiotic delivery devices for orthopedic applications should be biocompatible, degrade in concert with bony replacement, and possess osteoinductive and osteoconductive properties. The use of allograft bone is well established in many orthopedic settings. For some surgeons,

however, autograft material remains the gold standard for use in many orthopedic procedures due to its osteogenic potential. However, there can be significant levels of pain and discomfort associated with harvesting bone from the iliac crest [24–26]. The use of cancellous bone as an osteoconductive matrix is well established [27,28] and may serve as an ideal drug delivery device in the treatment of osteomyelitis due to its enhanced surface area for drug binding as compared to cortical bone and ability to allow bone in growth as a result of the porosity of the structure. In this context, osteoconductivity is defined as the ability to provide a three-dimensional configuration for in-growth of host capillaries, peri-vascular tissue, and osteoprogenitor cells into the graft. Here we present a gentamicin-impregnated bovine cancellous bone material that could be used as a prophylactic measure in orthopedic applications. Data is presented demonstrating the release of gentamicin at levels that are above its minimal inhibitory concentration for *S. aureus* and that are nontoxic to mammalian cells.

MATERIALS AND METHODS

Bovine Bone

Bovine cancellous chips (1–2 mm) were attained from the long bones of 18- to 24-month-old cows from an organically fed and registered closed herd at Prather Ranch, CA. The bovine cancellous bone then underwent RTI Biologics™ patented BioCleanse® tissue sterilization process to remove lipids and cellular debris from xenograft material. The BioCleanse® process uses a low temperature cleaning and sterilization system that consecutively exposes the bone to a variety of chemical solutions including isopropanol and hydrogen peroxide. The process provides for complete penetration of the inner matrices of tissue, removal of any contaminants as well as blood, bone marrow, and other unwanted endogenous materials through an extensive serial dilution process [29,30]. As part of the BioCleanse® process, two detergents, which have been proven to be inactivators of enveloped viruses, are used in combination [31,32]. In accordance with the standardized BioCleanse® protocol, bovine bone samples were submerged in a closed chamber and exposed to the previously mentioned cleansing solutions at varying pressures and alternated with vacuum cycles. The bovine bone was then loaded with 1, 5, or 10 mg/mL gentamicin sulfate solution using a proprietary process and terminally sterilized using 20.5–25 kGy of gamma irradiation.

Release Kinetics

Quantification of gentamicin (Baxter Healthcare, Deerfield, IL) was performed using a C18 reverse phase column and High Performance Liquid Chromatography (HPLC) (1200 Series, Agilent, Santa Clara, CA) with an ultraviolet detector (Agilent, Santa Clara, CA). One gram of bovine cancellous bone loaded with gentamicin was added to 5 mL of phosphate buffered saline (PBS) and incubated for 1 h at 37°C. At the designated time points, 1 mL of the solution was removed, stored at 4°C, and then analyzed via HPLC. The remaining PBS solution was removed, replaced with fresh PBS, vortexed, and incubated for an additional 24 h at 37°C. This process was performed in quadruplicate and repeated for 14 days.

Bacteria Zone of Inhibition Study

A biofilm of *S. aureus* (ATCC 6538, Microbiologics, Saint Cloud, MN) was formed on Mueller-Hinton agar plates (Microbiologics, Saint Cloud, MN). A single lyfodisk (Microbiologics) was placed in the center of the agar plate and 100 μL of various concentrations of gentamicin solution was added. The agar plates were then incubated for 16 h at 37°C at which time the distance between the edge of the lyfodisk and the bacteria was measured. The study was performed in triplicate.

Bacteria Colonization Study

Four grams of antibiotic-loaded bovine cancellous bone were added to 20 mL of 10^7 CFU/mL of *S. aureus* in 37°C Mueller-Hinton broth (Becton Dickinson Difco, Franklin Lakes, NJ) and allowed to incubate at 37°C for 1 h while shaking at 55 rpm. After 1 h, 250 ± 25 mg of bone were removed, added to 1 mL of 37°C sterile PBS, and vortexed for 30 ± 1 s to remove all adherent bacteria. One hundred microliters of the resultant solution was diluted with additional PBS and streaked on Mueller-Hinton agar plates, then incubated at 37°C for 16 h. The colonies present on each agar plate were counted and recorded. The remaining Mueller-Hinton broth was removed, replaced with fresh 37°C broth, vortexed, and incubated for an additional 24 h at 37°C. This was performed in triplicate and repeated on a daily basis for 14 days.

Osteoblast Proliferation

Rat calvarial osteoblasts (OB) were isolated and characterized as previously described [33]. Osteoblasts were cultured in basal media (BM), which consists of high glucose DMEM (Invitrogen, Carlsbad, CA)

supplemented with 10% FBS (HyClone, Logan, UT) and 1% antibiotic/antimycotic (Invitrogen). OB were plated in triplicate at a density of 20,000 cells per well in 1 mL of basal media and incubated under standard cell culture conditions for 24 h. The media was then aspirated and the cells were rinsed with 1 mL of PBS. One milliliter of basal media lacking antibiotic/antimycotic but containing either 100, 250, or 500 μg/mL of the gentamicin was added to the cells which were then maintained in culture for 10 days. Cell proliferation was measured at 3, 5, and 10 days using AlamarBlue reagent following instructions recommended by manufacturer (Serotec, Oxford, UK).

Osteoblast Attachment

Rat calvarial osteoblasts, at a density of 40,000 cells per well, were incubated with 65 ± 10 mg of bovine cancellous bone. After 5 days in standard culture conditions cells were visualized under fluorescent microscopy using the LIVE/DEAD Viability Kit following the manufacturer's instructions (Lonza, Basel, Switzerland).

Alkaline Phosphatase Activity

Rat calvarial osteoblasts were plated in triplicate at a density of 40,000 cells per well in 1 mL of basal media and incubated under standard cell culture conditions for 24 h. The media was then aspirated and the cells were rinsed with 1 mL of PBS. Basal media lacking antibiotic/antimycotic (1.5 mL) was added to cells in each well. Transwell inserts loaded with approximately 65 mg of antibiotic infused cancellous bone and 0.5 mL of antibiotic/antiomycotic free basal media were placed into wells containing osteoblasts. Cells were incubated for 3, 5, and 10 days. At each designated time point cell proliferation was measured using AlamarBlue reagent following instructions recommended by manufacturer (Serotec, Oxford, UK). Upon completion of the AlamarBlue assay, alkaline phosphatase (AP) measurements were obtained from exposing cell lysates to 4-nitrophenyl phosphate (Sigma, St. Louis, MO). Activity was normalized per cell.

Statistical Analysis

Data were analyzed with the Holm-Sidak test using the Sigma Stat software for Windows Version 3.5. Intergroup comparisons were made using a two-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

Table 1. Determination of minimum effective concentration for S. aureus.

Concentration (μg/mL)	Distance of bacterial inhibition (mm)				
	Trial 1	Trial 2	Trial 3	Average	Standard deviation
1000	11.45	11.05	11.1	11.20	0.22
250	9.55	10.05	10.45	10.02	0.45
62.50	7.55	8.35	7.25	7.72	0.57
15.60	5.20	5.40	5.35	5.32	0.10
7.80	4.25	3.80	3.70	3.92	0.29
3.90	2.5	1.95	1.85	2.10	0.35
3.13	1.85	1.55	1.15	1.52	0.35
2.50	1.50	0.95	0.80	1.08	0.37
1.95	0.90	0.50	0.60	0.67	0.21
1.56	0.75	0.00	0.40	0.38	0.38
0.98	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00

RESULTS

Zone of Inhibition

To determine the minimum concentration of gentamicin to inhibit bacterial growth, *S. aureus* at a concentration of 10^7 CFU/mL were plated on Mueller-Hinton agar plates followed by the addition of a single lyfodisk. Various antibiotic concentrations were added to the lyfodisks and the plates were incubated for 16 h at 37°C. The distance from the edge of the lyfodisk to the edge of the bacteria biofilm was than measured (Table 1). The data shown in Table 1 demonstrate that the minimal inhibitory concentration (MIC) of gentamicin for the *S. aureus* strain tested in this experiment was 1.56 μg/mL.

Release Kinetics

The kinetics of gentamicin release from bovine cancellous bone was analyzed using three different initial loading concentrations. As shown in Figure 1, there is a rapid release of the gentamicin during the first 2 days. The release rate begins to plateaus and all three concentration eluted statistically similar quantities by day 4 out to day 14 ($p > 0.10$). The drug concentration remains above the MIC out to 14 days irrespective of the initial drug loading concentration.

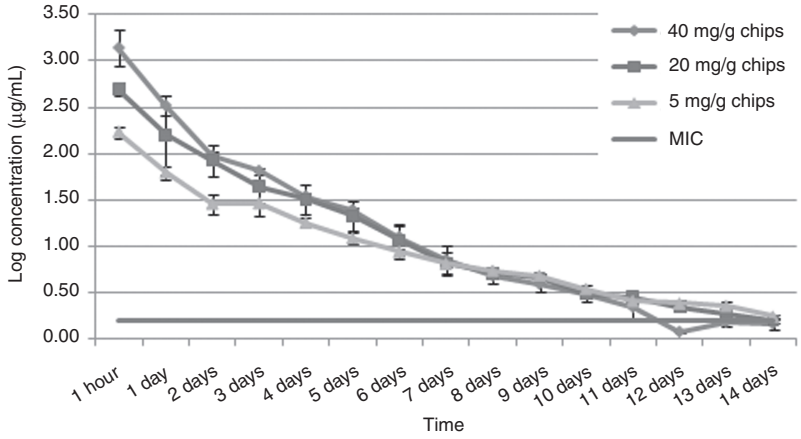

Figure 1. Quantity of drug released within 14 days from 1 g of xenograft when loaded with 5, 20, and 40 mg of gentamicin.

Inhibition of Bacterial Colonization on Bovine Cancellous Bone

The ability of the antibiotic loaded graft to inhibit bacterial biofilm formation was investigated over a consecutive 14-day period. The antibiotic loaded bone was exposed to 10^7 CFU/mL *S. aureus*. At 24 h intervals, an aliquot of 250 ± 25 mg of bone was removed from the broth and vortexed in sterile 37°C PBS. The resultant solution was plated. The number of colonies on each plate was recorded for each time point. The results demonstrated ($p = 0.001$) complete inhibition of bacterial growth on all the antibiotic loaded grafts by the first hour (Figure 2). The nonantibiotic loaded samples did not inhibit bacterial colonization throughout the entire 14 days and maintained a colony count of 10^7 CFU/mL throughout the experiment.

After the 14-day study was complete, 1 g of remaining bovine cancellous bone was placed on a Mueller-Hinton agar plate that contained *S. aureus*. After incubating at 37°C for 16 h the plates were removed and the zone of inhibition from the edge of the bone to the edge of the bacterial lawn was measured. The data in Table 2 shows that even after 14 days of elution, the cancellous chips still retain a sufficient amount of antibiotic to inhibit bacterial growth in and around the bone.

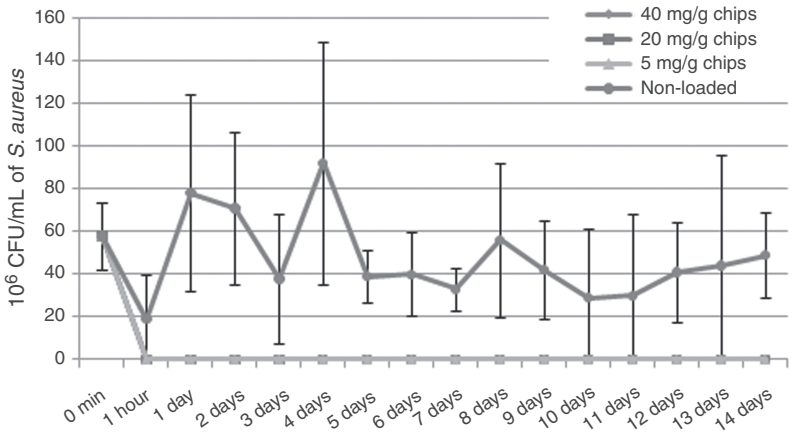

Figure 2. The Effectiveness of various initial loading concentrations on bacterial inhibition for 14 days using *S. aureus* at a concentration of 10^7 CFU/mL.

Table 2. Distance of bacterial zone of inhibition.

Gentamicin (mg)/(g) DBM	Distance (mm)
40	4.8
20	4.6
5	3
0	0

Effect of Gentamicin on Cell Proliferation

The effect of gentamicin on osteoblast cell proliferation was also investigated. Rat calvarial osteoblasts were exposed to various concentrations of gentamicin and the total cell numbers were evaluated. At both the 3- and 5-day time points the 100 and 250 μg/mL show statistically similar cell quantities when compared to the 0 μg/mL media (p-values were 0.660 and 0.432, respectively). By day 10 the cell proliferation of the 100 μg/mL solution was the only concentration that was statistically similar to the 0 μg/mL media ($p = 0.910$) (Figure 3). However, after 3, 5, and 10 days in culture, the number of cells present in each well was greater than that of the original 20,000 cells that were seeded. To further investigate the effects of gentamicin on cell proliferation, osteoblasts were seeded directly onto bovine cancellous bone that was loaded with 5, 20, or 40 mg of

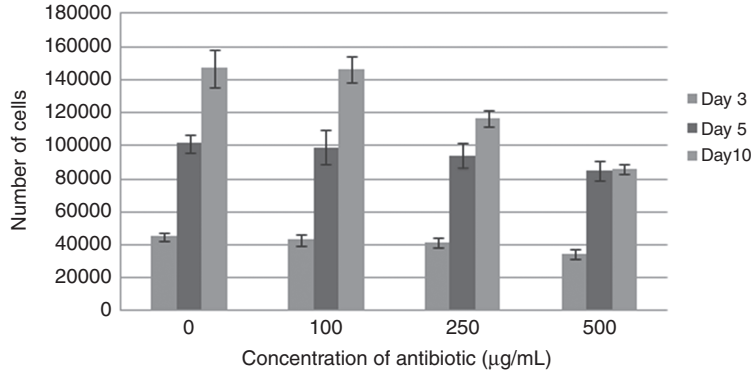

Figure 3. Effect of various gentamicin concentrations on osteoblast cell proliferation over the course of 3, 5, and 10 days using an AlamarBlue assay. This experiment was performed three times and a representative experiment is shown.

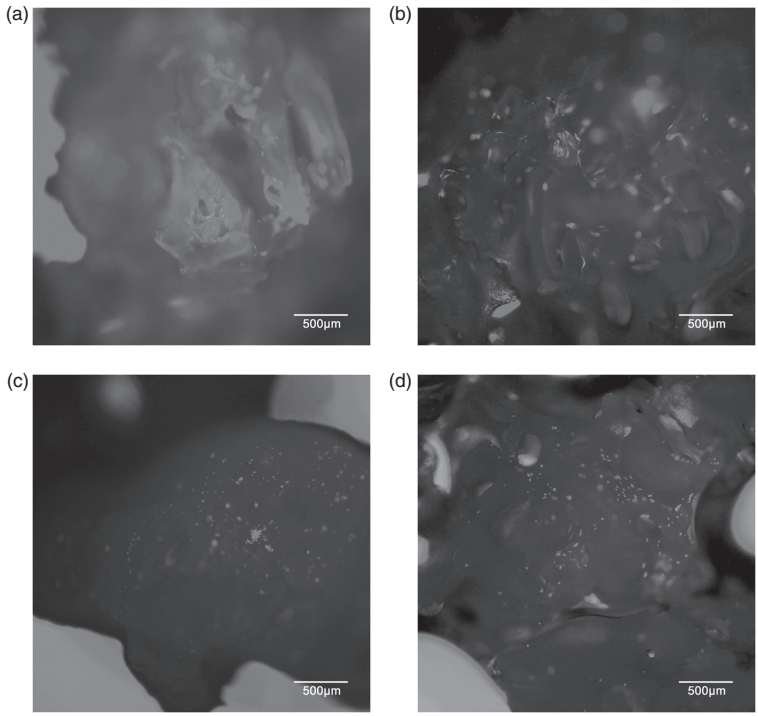

Figure 4. Effect of osteoblast cell adhesion directly on graft loaded with 5 mg/g, 20 mg/g, and 40 mg/g of gentamicin on 1 g of chips. After 5 days the cells were stained with calcein and photographed. (a) Nonloaded, (b) 5 mg/g chips, (c) 20 mg/g chips, (d) 40 mg/g chips. A 40 × magnification was used.

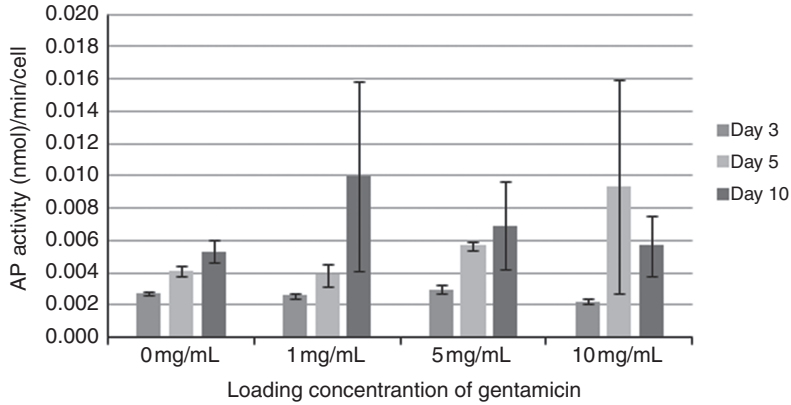

Figure 5. The effects of bovine bone loaded with various gentamicin concentrations on osteoblast AP activity over the course of 3, 5, and 10 days. AP measurements were obtained with cell lysates using 4-nitrophenyl phosphate (Sigma, St. Louis, MO) as a substrate; activity was normalized per gram cell.

gentamicin per gram of DBM. As shown in Figure 4, cell attachment and proliferation was seen at all antibiotic concentrations after the 5-day incubation period.

Effect of Gentamicin AP Production

The effect of gentamicin on the osteoblast produced AP was investigated. AP is an early marker for osteoblastic development. Rat calvarial osteoblasts were seeded on the bottom of a transwell dish and the inserts were loaded with approximately 65 mg of antibiotic infused cancellous bone. After 3, 5, or 10 days of incubation the AP activity was measured. There were no statistically significant differences in the levels of AP at the 5, 20, or 40 mg/g concentrations of gentamicin that were investigated over the 10-day period (p-values were 0.010, 0.013, and 0.009, respectively) (Figure 5).

DISCUSSION

Structural bone allografts have provided a solution to many reconstructive problems in musculoskeletal and maxillofacial surgeries, but infection of the surgical site remains a significant issue. Infection rates of 4–12% have been reported in various orthopedic surgeries involving allograft [34–39]. These infections are commonly caused by *staphylococcal* species and can result from the initial trauma or as a

result of the treatment regimen. These high infection rates are not surprising because the surgeries often involve extensive soft tissue excision, large wounds, and long operating times. Treatment of musculoskeletal infections generally involves surgical debridement of necrotic tissue, followed by 4–6 weeks of systemic antibiotics. To obtain adequate antibiotic concentrations at the site of infection, high serum concentrations have to be obtained, which may carry the risk of systemic toxicity. Local antibiotic delivery systems have become increasingly popular for treatment and prophylaxis of orthopedic infections. Such systems yield higher local concentrations of antibiotic than possible with parenteral administration while reducing the risk of systemic side-effects. High local antibiotic concentrations are especially beneficial in the treatment of relatively avascular areas and organisms resistant to antibiotic levels obtained via parenteral administration, including organisms in biofilms [40,41].

There have been several attempts to supplement allografts with antibiotics [42–46]. In an effort to achieve a more sustained release of antibiotics, demineralized allograft mixed with antibiotic powders and gelatin was used to reduce infection in a dog model [47]. The results of the treatment with antibiotic-supplemented bone were compared to the standard of care. All fractures treated by the conventional method developed acute osteomyelitis and nonunion, whereas the antibiotic treatment group resulted in bone union without infection. This study demonstrated the potential for an antibiotic impregnated bone construct to assist in reducing the rate of infection. In a study by Witso et al. [48], cortical allograft loaded with four different antibiotics were implanted into the meduallar canal of outbred Wistar rats that had been infected with 10^7 CFU/mL of *S. aureus* to determine the efficacy of this type of delivery device. Their results indicated that three of the four antibiotics tested eradicated the *S. aureus* infection. These results showed significant promise in utilizing bone grafts as a local delivery device.

In the present study, gentamicin was loaded onto cancellous bone in a consistent and dose-dependent manner. The data presented in this study showed a bulk release of gentamicin within the first 48 h as well as clinically relevant concentrations of drug continued to elute up to 14 days *in vitro*.

The data presented herein demonstrate that the addition of gentamicin to the graft prevented bacterial adhesion of *S. aureus,* which is a major contributor to osteomyelitis. By supplementing the bone directly; the antibiotic is able to bind to the entire surface including the haversian canals, Volkmann's canals, and the canaliculi. The immediate bulk release of gentamicin coupled with its extended release rate

demonstrates a potential option in choosing an effective local delivery device for infection prophylaxis. An added benefit in utilizing cancellous bone as a vehicle for antibiotic delivery as compared to previously seen cortical scaffolds [48] is the increased surface area that allows for drug binding and bone ingrowth due to the porosity of the structure.

In a study by Ince et al. [49] the effect of gentamicin in media on the cell proliferation and AP activity of C2C12 cells was performed. The results indicated that the expression of AP activity was not inhibited or decreased with a gentamicin concentration less than 800 μg/mL. The potential adverse effects on cell replication and AP production were also studied in the present study. Concentrations similar to amounts determined to elute from the cancellous bone graft were tested. The addition of gentamicin directly into the cell media containing osteoblasts showed that concentrations above 100 μg/mL decreased the rate of proliferation at the later time points. However, the osteoblasts' AP production when exposed to the various gentamicin loaded cancellous bone groups were not statistically different at any of the drug levels as compared to the nonloaded control. These AP results presented herein are in agreement with the data presented by Ince [49].

It is also important to note that the amount of drug being delivered at the site did not adversely affect the osteoconductive properties of the graft *in vitro* via the cell proliferation and AP assays performed. It has also been shown that similar amounts of gentamicin delivered at the site via human DBM does not adversely affect bone growth [50]. The data presented herein clearly demonstrates that the concentration of gentamicin eluted had no adverse effect on the proliferation of osteoblasts. In addition, the concentrations of drug eluting from the graft do not have an adverse effect on one of the accepted phenotypic markers of osteoblastic cells, alkaline phosphatase.

CONCLUSION

The release kinetics, bacterial inhibition, and osteoblast cell data presented herein demonstrate that gentamicin loaded cancellous bone maintains its ability to be an osteoconductive scaffolding while inhibiting colonization of the device. The data also supports the idea of an extended release possibility to continue to prevent bacterial colonization of the graft once it has been implanted. Therefore, this combination device could allow the surgeon to prophylactically treat an orthopedic surgical site with a resorble and osteoconductive biological graft. In conclusion, antibiotic-impregnated cancellous bone may

act as an efficient carrier of antibiotics for a local delivery system in a bony site.

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REFERENCES

1. Cremieux, A.C. and Carbon, C. Experimental Models of Bone and Prosthetic Joint Infections, *Clin. Infect. Dis*., 1997: **25**: 1295–1302.
2. Mader, J.T., Cripps, M.W. and Calhoun, J.H. Adult Posttraumatic Osteomyelitis of the Tibia, *Clin. Orthop. Relat. Res*., 1999: **360**: 14–21.
3. Deirmengian, C., Greenbaum, J., Stern, J. et al. Open Debridement of Acute Gram-Positive Infections after Total Knee Arthroplasty, *Clin. Orthop. Relat. Res*., 2003: **416**: 129–134.
4. Hartman, M.B., Fehring, T.K., Jordan, L. and Norton, H.J. Periprosthetic Knee Sepsis: The role of Irrigation and Debridement, *Clin. Orthop. Relat. Res*., 1991: **273**: 113–118.
5. Waldman, B.J., Hostin, E., Mont, M.A. and Hungerford, D.S. Infected Total Knee Arthroplasty Treated by Arthroscopic Irrigation and Debridement, *J. Arthroplasty*, 2000: **15**: 430–436.
6. Hofmann, A.A., Kane, K.R., Tkach, T.K., Plaster, R.L. and Camargo, M.P. Treatment of Infected Total Knee Arthroplasty Using an Articulating Spacer, *Clin. Orthop. Relat. Res*., 1995: **321**: 45–54.
7. Lynch, M., Esser, M.P., Shelley, P. and Wroblewski, B.M. Deep Infection in Charnley Low-Friction Arthroplasty. Comparison of Plain and Gentamicin-Loaded Cement, *J. Bone Joint Surg. Br*., 1987: **69**: 355–360.
8. Nijhof, M.W., Dhert, W.J., Fleer, A., Vogely, H.C. and Verbout, A.J. Prophylaxis of Implant-Related Staphylococcal Infections Using Tobramycin-Containing Bone Cement, *J. Biomed. Mater. Res*., 2000: **52**: 754–761.
9. Nijhof, M.W., Stallmann, H.P., Vogely, H.C. et al. Prevention of Infection with Tobramycin-Containing Bone Cement or Systemic Cefazolin in an Animal Model, *J. Biomed. Mater. Res*., 2000: **52**: 709–715.
10. Waertel, G. The Role of Antibiotic-Loaded Cement in the Treatment of an Infection after a Hip Replacement, *J. Bone Joint Surg. Am*., 1996: **78**: 472–473.
11. McLaren, A.C. Alternative Materials to Acrylic Bone Cement for Delivery of Depot Antibiotics in Orthopaedic Infections, *Clin. Orthop. Relat. Res*., 2004: **427**: 101–106.
12. Nelson, C.L. The Current Status of Material Used for Depot Delivery of Drugs, *Clin. Orthop. Relat. Res*., 2004: **427**: 72–78.

13. Zalavras, C.G., Patzakis, M.J. and Holtom, P. Local Antibiotic Therapy in the Treatment of Open Fractures and Osteomyelitis, *Clin. Orthop. Relat. Res*., 2004: **427**: 86–93.
14. Buttaro, M., Valentini, R. and Piccaluga, F. Persistent Infection Associated with Residual Cement after Resection Arthroplasty of the Hip, *Acta Orthop. Scand*., 2004: **75**: 427–429.
15. Kalteis, T., Luring, C., Gugler, G. et al. Acute Tissue Toxicity of PMMA Bone Cements, *Z. Orthop. Ihre. Grenzgeb*., 2004: **142**: 666–672.
16. McLaren, A.C., Nelson, C.L., McLaren, S.G. and De, C.G.R. The Effect of Glycine Filler on the Elution Rate of Gentamicin from Acrylic Bone Cement: A Pilot Study, *Clin. Orthop. Relat. Res*., 2004: **427**: 25–27.
17. Stevens, C.M., Tetsworth, K.D., Calhoun, J.H. and Mader, J.T. An Articulated Antibiotic Spacer Used for Infected Total Knee Arthroplasty: A Comparative *In Vitro* Elution Study of Simplex and Palacos Bone Cements, *J. Orthop. Res*., 2005: **23**: 27–33.
18. Perry, A.C., Rouse, M.S., Khaliq, Y. et al. Antimicrobial Release Kinetics from Polymethylmethacrylate in a Novel Continuous Flow Chamber, *Clin. Orthop. Relat. Res*., 2002: **403**: 49–53.
19. Ostermann, P.A., Seligson, D. and Henry, S.L. Local Antibiotic Therapy for Severe Open Fractures. A Review of 1085 Consecutive Cases, *J. Bone Joint Surg. Br*., 1995: **77**: 93–97.
20. Tice, A.D., Hoaglund, P.A. and Shoultz, D.A. Risk Factors and Treatment Outcomes in Osteomyelitis, *J. Antimicrob. Chemother*., 2003: **51**: 1261–1268.
21. Yarboro, S.R., Baum, E.J. and Dahners, L.E. Locally Administered Antibiotics for Prophylaxis against Surgical Wound Infection. An *In Vivo* Study, *J. Bone Joint Surg. Am*., 2007: **89**: 929–933.
22. Price, J.S., Tencer, A.F., Arm, D.B.M. and Bohach, G.A. Controlled Release of Antibiotics from Coated Orthopedic Implants, *J. Biomed. Mater. Res*., 1996: **30**: 281–286.
23. Stigter, M., Bezemer, J., de Groot, K. and Layrolle, P. Incorporation of Different Antibiotics into Carbonated Hydroxyapatite Coatings on Titanium Implants, Release and Antibiotic Efficacy, *J. Control. Release*, 2004: **99**: 127–137.
24. Coventry, M.B. and Tapper, E.M. Pelvic Instability: A Consequence of Removing Iliac Bone for Grafting, *J. Bone Joint Surg. Am*., 1972: **54**: 83–101.
25. Goulet, J.A., Senunas, L.E., DeSilva, G.L. and Greenfield, M.L. Autogenous Iliac Crest Bone Graft: Complications and Functional Assessment, *Clin. Orthop. Relat. Res*., 1997: **339**: 76–81.
26. Guha, S.C. and Poole, M.D. Stress Fracture of the Iliac Bone with Subfascial Femoral Neuropathy: Unusual Complications at a Bone Graft Donor Site: Case Report, *Br. J. Plast. Surg*., 1983: **36**: 305–306.
27. Kalfas, I.H. Principles of Bone Healing, *Neurosurg. Focus*, 2001: **10**: E1.
28. Muschler, G.F., Lane, J.M. and Dawson, E.G. (1990). The Biology of Spinal Fusion, In: Cotler, J.M. and Cotler, H.P. (eds), *Spinal Fusion: Science and Technique*, Berlin, Springer-Verlag, pp. 9–21.

29. Mroz, T.E., Lin, E.L., Summit, M.C. et al. Biomechanical Analysis of Allograft Bone Treated with a Novel Tissue Sterilization Process, *Spine J.*, 2006: **6**: 34–39.
30. Schimizzi, A., Wedemeyer, M., Odell, T., Thomas, W., Mahar, A.T. and Pedowitz, R. Effects of a Novel Sterilization Process on Soft Tissue Mechanical Properties for Anterior Cruciate Ligament Allografts, *Am. J. Sports Med.*, 2007: **35**: 612–616.
31. Horowitz, B., Bonomo, R., Prince, A.M., Chin, S.N., Brotman, B. and Shulman, R.W. Solvent/Detergent-Treated Plasma: A Virus-Inactivated Substitute for Fresh Frozen Plasma, *Blood*, 1992: **79**: 826–831.
32. Howett, M.K., Neely, E.B., Christensen, N.D. et al. A Broad-Spectrum Microbicide with Virucidal Activity against Sexually Transmitted Viruses, *Antimicrob. Agents Chemother.*, 1999: **43**: 314–321.
33. Puleo, D.A., Holleran, L.A., Doremus, R.H. and Bizios, R. Osteoblast Responses to Orthopedic Implant Materials *In Vitro, J. Biomed. Mater. Res.*, 1991: **25**: 711–723.
34. Aro, H.T. and Aho, A.J. Clinical Use of Bone Allografts, *Ann. Med.*, 1993: **25**: 403–412.
35. Gross, A.E., Hutchison, C.R., Alexeeff, M., Mahomed, N., Leitch, K. and Morsi, E. Proximal Femoral Allografts for Reconstruction of Bone Stock in Revision Arthroplasty of the Hip, *Clin. Orthop.*, 1995: **319**: 151–158.
36. Haddad, F.S., Spangehl, M.J., Masri, B.A., Garbutz, D.S. and Duncan, C.P. Circumferential Allograft Replacement of the Proximal Femur, *Clin. Orthop.*, 2000: **371**: 98–107.
37. Lord, C.F., Gebhardt, M.C., Tomford, W.W. and Mankin, H.J. Infection in Bone Allografts, *J. Bone Joint Surg. Am.*, 1988: **70**: 369–376.
38. Tomford, W.W., Thongphasuk, J., Mankin, H.J. and Ferraro, M.J. Frozen Muscoskeletal Allografts. A study of the Clinical Incidence and Causes of Infection Associated with their Use, *J. Bone Joint Surg. Am.*, 1990: **72**: 1137–1143.
39. Webb, L.X., Holman, J., de Araujo, B., Zaccaro, D.J. and Gordon, E.S. Antibiotic Resistance in Staphylococci Adherent to Cortical Bone, *J. Orthop. Trauma*, 1994: **8**: 28–33.
40. Hanssen, A.D., Osmon, D.R. and Patel, R. Local Antibiotic Delivery Systems: Where are We and Where are We Going? *Clin. Orthop. Relat. Res.*, 2005:**437**: 111–114.
41. Heijink, A., Yaszemski, M.J., Patel, R., Rouse, M.S., Lewallen, D.G. and Hanssen, A.D. Local Antibiotic Delivery with OsteoSet, DBX, and Collagraft, *Clin. Orthop. Relat. Res.*, 2006: **451**: 29–33.
42. Henry, S.L. and Galloway, K.P. Local Antibacterial Therapy for the Management of Orthopaedic Infections: Pharmacokinetic Considerations, *Clin. Pharmacokinet.*, 1995: **29**: 36–45.
43. Winkler, H., Janata, O., Berger, C., Wein, W. and Georgopoulos, A. *In Vitro* Release of Vancomycin and Tobramycin from Impregnated Human and Bovine Bone Grafts, *J. Antimicrob. Chemother.*, 2000: **46**: 423–428.
44. Witso, E., Persen, L., Benum, P. and Bergh, K. Release of Netilmicin and Vancomycin from Cancellous Bone, *Acta Orthop. Scand.*, 2002: **73**: 199–205.

45. Witso, E., Persen, L., Loseth, K., Benum, P. and Bergh, K. Cancellous Bone as an Antibiotic Carrier, *Acta Orthop. Scand.*, 2000: **71**: 80–84.
46. Witso, E., Persen, L., Loseth, K. and Bergh, K. Adsorption and Release of Antibiotics from Morselized Cancellous Bone. In Vitro Studies of 8 Antibiotics, *Acta Orthop. Scand.*, 1999: **70**: 298–304.
47. Petri 3rd, W.H. and Schaberg, S.J. The Effects of Antibiotic-Supplemented Bone Allografts on Contaminated, Partially Avulsive Fractures of the Canine Ulna, *J. Oral Maxillofac. Surg*., 1984: **42**: 699–704.
48. Witso, E., Persen, L., Benum, P. and Bergh, K. Cortical Allograft as a Vehicle for Antibiotic Delivery, *Acta Orthop.*, 2005: **76**: 481–486.
49. Ince, A., Schütze, N., Karl, N., Löhr, J.F. and Eulert, J. Gentamicin Negatively Influenced Osteogenic Function *In Vitro*, *Int Orthop*., 2007: **31**: 223–228.
50. Lewis, C.S., Cobb, R.R., Supronowicz, P.R. Zhukauskas, R.M. and Gill, E. Local Antibiotic Delivery with Demineralized Bone Matrix (Not yet Published), 2009.