

Fabrication of cefuroxime-impregnated calcium sulfate: Polycaprolactone composite implant for osteomyelitis

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Osteomyelitis is characterized as an inflammatory bone disease caused by pyrogenic bacteria. As oral bioavailabilities of antibiotics are low, a regimen of 6 weeks of intravenous antibiotic is necessary for adequate therapy. Although the dose of antibiotic administered systemically is high, therapeutically effective drug concentrations are not always achieved at the site of infection. This problem can be overcome by the use of local antibiotics from a biodegradable implant for chronic osteomyelitis that can deliver the drug at least for 6 weeks. The implant delivers high antibiotic concentration at tissue levels, obliterates dead space, aids bone repair and does not need to be removed. The aim of this study was to develop and evaluate a calcium sulfate and polycaprolactone (PCL)-based composite biodegradable implantable delivery system of cefuroxime for the localized treatment of osteomyelitis that can deliver the drug for at least 6 weeks. The PCL and calcium sulfate composite system has not been studied yet. Interaction studies were carried out to check any incompatibility between the ingredients. Implants were prepared by a modified fabrication technique to avoid solvent use. The prepared implants were evaluated for various *in vitro* parameters like dimensions, hardness, tensile strength, drug release profile, sterility test and morphological changes in pellet before and after drug release. The pellets were also tested for microbiological efficacy and compared with a plain drug solution in different concentrations. Developed pellets are regular in shape and size with good tensile strength. The release profile displayed drug levels above the minimum inhibitory concentration continuously for up to 2 months. A wide zone of inhibition by the pellet against *Staphylococcus aureus* as compared with the drug solution proves its efficacy in the treatment of osteomyelitis. Results show that the developed calcium sulfate and PCL-based composite biodegradable implantable delivery system of cefuroxime is a good alternate system and can deliver the drug for more than 6 weeks, maintaining an adequate inhibitory concentration at the site.

Key words: Calcium sulfate, cefuroxime sodium, implants, osteomyelitis, polycaprolactone

INTRODUCTION

Osteomyelitis is an infection of the bones characterized by pain, nausea, pus formation, edema and warmth over the affected bone and rigid overlying muscles. It is often caused by bacteria, usually *Staphylococcus aureus*. The bacteria may reach the bone from outside the body (through open fractures, penetrating wounds or orthopedic surgical procedure), from other sites of infection in the body (abscessed teeth, burn infection, urinary tract infections (UTIs) or upper respiratory tract infections) via the blood or from adjacent soft tissue infections (as occurs in diabetes

mellitus).^[1] The treatment of chronic osteomyelitis includes debridement of the dead infected tissue, obliteration of dead space, osseous repair, adequate soft tissue coverage and systemic antibiotics. The delivery of antibiotics to the bone varies considerably. Oral antibiotics are unpredictable, with relatively low bone levels, and are used infrequently. Intravenous antibiotics are used commonly in the treatment of chronic osteomyelitis.^[2] Six weeks of intravenous antibiotic therapy is necessary for adequate treatment, although prolonged usage of intravenous antibiotics has a significant relapse rate. To supplement systemic antibiotics, local antibiotic delivery has been tried for many years.

The primary benefit achieved with a local antibiotic delivery vehicle is the ability to obtain extremely high levels of antibiotics at the site of infection.^[3] This avoids some of the toxicity associated with systemic

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antibiotics. Several antibiotic implantable drug delivery systems have been developed for the treatment of bone infection. The advantage of this approach is that an effective drug concentration is attained at the site of infection while the systemic drug concentration remains very low.^[2] The main disadvantage is that the implants should be removed at the end of the treatment period. Implantable biodegradable polymer systems have a unique advantage in that the dosage form need not be removed from the body and thus saves cost and risk for the patient.

Composite materials are preferred over a single polymer as the implant material has the possibility of variation in physical properties. Various materials other than metal, like organic biomaterials, inorganic biomaterials and their composites, have been used as artificial bone materials to fill bone defects or to replace bony structures.^[4] Composite materials often show an excellent balance between strength and toughness and usually show improved characteristics compared with their separate components.^[5,6] The composite materials such as ceramics/ceramic composites, hydroxyapatite/plaster of paris,^[7,8] calcium phosphate/calcium carbonate,^[9] etc., ceramics/polymer composites, hydroxyl apatite (HA)-poly (lactic acid) (PLA),^[10,11] HA-collagen^[12] and HA-chitin^[13] etc. have been tried.

Calcium sulfate was the first material to be used as bone replacement.^[14] We are using calcium sulfate hemihydrate, which is commonly known as plaster of paris. The plaster has a compressive strength of ~24 MPa.^[15] Calcium sulfate is also characterized by its ease of sterilization, complete resorption, biocompatibility and stimulation of bone when in contact with periosteum. Calcium sulfate may act as binder, facilitating healing and preventing loss of the grafting material.^[16] Moreover, it is tissue compatible and thus does not interfere with the healing process. Calcium sulfate has also been used with other materials, such as autogenous bone,^[17] demineralized freeze-dried bone (DFDBA),^[18] polymers,^[19] etc. Calcium powder acts as a direct source of calcium supply. It completely resorbs after 30-60 days of implantation. Calcium sulfate has been approved by the Food and Drug Administration for use in the fixation of distal radial fracture. Calcium sulfate had been reported as repairing fillers for the treatment of bone deflection.^[20]

Polycaprolactones (PCLs) are polyesters related to poly lactic acid and poly glycolic acid with similar biocompatibility.^[21] PCL has been used in different biomedical applications, such as in scaffolds for tissue engineering of the bone and cartilage.^[22]

Cefuroxime is a second-generation cephalosporin antibiotic. Cefuroxime is active against a wide range of Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumonia* and *Neisseria* spp. Cefuroxime interferes with the cell wall synthesis in the bacteria. Cefuroxime sodium (CFS) is used in treating infections

of the upper and lower respiratory tract, skin and soft tissue, UTI, bone and joint infections and gonococcal infections.

Although an implantable system for ciprofloxacin^[23-25] and gatifloxacin^[26] has been extensively studied for the treatment of bone infection, the polycaprolactone and calcium sulfate composite systems with cefuroxime have not yet been studied.

Our present work describes the formulation and evaluation of a composite-based biodegradable CFS-impregnated implant for the treatment of osteomyelitis. Both calcium sulfate and PCL were used in its preparation to impart biodegradability and strength.

MATERIALS AND METHODS

CFS (purity \geq 99%; MW 446.37) was received as a gift sample from Torque Pharmaceuticals Pvt. Ltd., Chandigarh, India. PCL (MW 14,000; Mn 10,000) and calcium sulfate hemihydrate (assay 98%) was purchased from Sigma Aldrich, Mo, USA. All other chemicals and solvents used were purchased from local suppliers and were of analytical grade, unless mentioned otherwise.

Drug-polymer interaction studies

The samples of calcium sulfate hemihydrate, PCL and cefuroxime were prepared individually and in combinations and were sterilized using ethylene oxide. The infrared spectra were taken before and after sterilization at 0 and 24 h using Fourier Transform Infrared spectroscopy (FTIR 8400 S, Shimadzu, Japan). Both spectra were compared for any possible change in content due to interactions between different ingredients or by sterilization.

Preparation of implants

Pellets were prepared by a modified fabrication technique. The pellets were prepared by a modified fabrication technique. Different placebo combinations [Table 1] were tested before final selection of the formulation. Calcium sulfate is responsible for the hardness, but a high concentration of calcium sulfate also gives an eroded surface after keeping in the simulated body fluid (SBF) for 1-2 weeks, which leads to a fast release of the medicament. The F3 formulation with an equal concentration of both polymers was found to

Table 1: Different placebo combinations tested

Codes	PCL (mg)	Calcium sulfate hemihydrate (mg)	Hardness	Surface erosion after 2 weeks
F1	150	50	8.08 \pm 0.78	Eroded
F2	100	100	11.08 \pm 0.59	Not eroded
F3	50	150	13.08 \pm 0.24	Rough surface with some erosion

Values are expressed as mean \pm SD (n = 6), PCL - Polycaprolactone



Figure 1: Cefuroxime-impregnated calcium sulfate–polycaprolactone composite pellets

be most suitable in terms of hardness and surface erosion even after 2-3 weeks. Hence, F3 with equal quantities of PCL and calcium sulfate, i.e. 100 mg, is selected for further studies. PCL polymer, calcium sulfate hemihydrate and cefuroxime were taken in a ratio of 1:1:0.75 w/w. PCL was first melted at 60°C and a blend of drug and calcium sulfate hemihydrate were mixed in the melted polymer because PCL gets solidified almost immediately. The mixture was stirred by a mechanical stirrer for 10 min to ensure proper mixing of the drug with the polymer matrix. The composite mixture gets solidified immediately. The compact mass was then passed through a sieve no. 16 to form granules. These granules were compressed as pellets in a tablet punching machine (Mini press; Karnavati, India) [Figure 1]. A batch of 50 pellets was prepared for further studies. The compressed pellets were collected, packed in aluminum foil and stored in a refrigerator under dry conditions. All pellets were sterilized by gas (ethylene oxide) sterilization.

Physicochemical characterization

The prepared pellets were subjected to physicochemical characterization and data are shown in Table 2.

Dimensional analysis

Twenty pellets were randomly selected and subjected to dimensional analysis. The diameter and thickness of the pellets were determined by a digital vernier calliper (Mitutiyo digimatic calliper; Mitutiyo Cooperation, Japan).

Hardness

Six pellets were subjected to hardness analysis. Hardness of the pellets was calculated by a Monsanto hardness tester (model HT01; Orchid Scientifics, Nashik, India). The force was applied diametrically for determining the hardness.

Tensile strength

Rectangular strips of 20 × 15 mm size were cut and

Table 2: Physicochemical properties of the developed pellets

Parameters	Inference
Diameter (mm)	6.06 ± 0.015
Thickness (mm)	7.01 ± 0.015
Hardness (kg/cm ²)	11.08 ± 0.59
Tensile strength (N)	1N ± 0.002

Values are expressed as mean ± SD (n = 6)

subjected to tensile strength analysis using an Instron 5842 (Instron, Canton, MA, USA) using the associated software “Merlin.” Tensile strength at the breakpoint was measured. Five measurements were taken and the data were averaged to obtain a mean value.

Test for sterility

Sterility tests were performed for the sterilized pellet to ensure proper sterilization. Three sterilized pellets were taken and each was placed in a Petri dish containing 10 ml of the culture medium (peptic digest of animal tissue 5 g, sodium chloride 5 g, beef extract 1.5 g, yeast extract 1.5 g, agar 15 g, water q.s. 100 ml) and incubated at 37°C for 3 weeks. Petri dishes were taken out after 3 weeks and evaluated for any growth.

In vitro release profile^[27,28]

The pellet was taken in the dialysis tube (Sigma Chemicals, Mo, USA), which was suspended in a beaker at 37 ± 0.5°C containing 5 ml of artificial SBF, pH 7.4. The composition of artificial SBF used was tris base 6.051 g, NaHCO₃ 0.35 g, K₂HPO₄ 0.171 g, Na₂SO₄·10H₂O 0.16 g, KCl 0.224 g, CaCl₂·2H₂O 0.368 g, MgCl₂·6H₂O 0.305 g, NaCl 7.996 g, 1N HCl 40 ml and water q.s. 1000 ml. Aliquots of the medium were withdrawn at different time intervals (24 h duration for the first 10 days followed by 72 h duration for the next 45 days) and equal volumes of fresh media were added to replace the withdrawn samples. The withdrawn samples were filtered, diluted appropriately and estimated for the drug content by ultraviolet spectrophotometry at 215 nm (calibration curve of the drug in SBF was plotted with R² = 0.999). The cumulative percent drug released was calculated.

Scanning electron microscopy studies

Cefuroxime-loaded pellets, before and after *in vitro* release, were coated with gold under vacuum and scanning electron micrographs were obtained using a Jeol, Peabody, MA (JSM-400, Japan) scanning electron microscope. Morphological and topographical changes due to antibiotic release were assessed from the micrographs.

Microbiological assay

The cup plate assay was performed on developed pellets as well as on different concentrations of plain cefuroxime solution in different Petri plates. Nutrient agar was selected as a nutrient media while *Staphylococcus aureus* MTCC 1430 was procured from Institute of Microbial Technology, Chandigarh, India, was

used as an indicator organism. A previously prepared dilute sample of inoculum was plated over melted agar medium. The agar medium was maintained in a liquid state by maintaining the temperature at 45°C. The medium was mixed with inoculum and then poured into sterile Petri plates. A cup was made in the plates with the help of a cork borer. Three cups numbered 1, 2 and 3 were prepared in each plate carrying the antibiotic solution of 30, 15 and 0 µg/ml (blank) of cefuroxime, respectively. These cups were then filled with the drug solution and the plates were incubated at 37°C for 48 h. The same procedure was followed for the pellets by replacing the drug solution with the pellets.

RESULT AND DISCUSSION

The interaction studies were carried out to check any possible incompatibility among the formulation ingredients. Infrared spectra of the ingredients before and after sterilization at 0 and 24 h were identical. No additional peak was observed that confirmed that the formulation ingredients were compatible with each other and no physicochemical reactions took place due to sterilization by ethylene oxide and during the manufacturing process.

Pellets formed are shown in Figure 1. All pellets formed were of uniform shape and size. Pellets formed were found to be of uniform thickness. The measured tensile strength of all the pellets was 1 N, indicating reasonable mechanical properties of the prepared implant [Table 2].

Sterilization of the packaged product was performed by gaseous sterilization using ethylene oxide and the test for sterility was performed on a sterilized packaging according to the IP 1996 standards. No microbial growth/microbial contamination were observed up to 14 days of incubation. Hence, the formulation passed the sterility test.

The *in vitro* release behavior of the formulations was determined in artificial SBF. The release profile of the developed cefuroxime composite implant revealed that 53.3% of the drug content of the implant was released in the first 5 days. Then, there is a continuous release of 73.2% of the drug in 10 days followed by a slow release till 55 days. The release profile was characterized by an initial burst followed by a second stage of gradual delivery of 91.1% over 55 days [Figure 2]. Burst release is a phenomenon frequently associated with polymeric-controlled drug delivery systems. Maintaining a high antibiotic concentration at the site of infection is highly appropriate for treating multibacterial infections, which consequently increases the effectiveness of the treatment.

Scanning electron microscopy studies

In general, rods displayed a very similar texture and morphology regardless of the polymer and antibiotic type used. Cefuroxime-loaded pellets were examined before and after 15 days in the release medium. It was observed that

before release the antibiotic crystals on the surface of the rods were partially exposed [Figure 3a] and the surface was smooth as compared with the pellet after drug release. When the drug-loaded pellet was placed in the aqueous environment, pores were created on the exposed part due to removal of drug particles through dissolution. These pores led to water penetration into the pellet and caused further drug dissolution [Figure 3b]. Voids generated reveal uniform drug distribution and release due to diffusion from the pellets.

A cup plate assay of the plain cefuroxime solution showed an inhibitory activity of this drug against *S. aureus*. The zone of inhibition is concentration dependent, as clearly seen [Table 3]. The cup carrying the 30 µg/ml [Figure 4a, no. 1] concentration has a large inhibition zone as compared with the 15 µg/ml concentration [Figure 4a, no. 2]. Diffusion of the drug from the pellets also showed a clear zone of inhibition. A large zone of inhibition (almost covering the entire plate) was observed at 24 h in the plate carrying the intact pellet [Figure 4b]. It indicates efficacy of the developed pellets in comparison with the simple drug solution.

CONCLUSION

Till date, various materials have been tested for the treatment of osteomyelitis either as a single or as a composite material, which can release the drug over a period of 1 month.

Table 3: Zone of inhibition of different concentrations of cefuroxime sodium solution and pellets

Antibiotic medium	Concentration (µg/ml)	Zone of inhibition (mm)
Cefuroxime sodium solution	15	14.33 ± 1.53
Cefuroxime sodium solution	30	26.33 ± 1.53
Cefuroxime sodium pellet	-	38.67 ± 0.577

Values are expressed as mean ± SD (n = 6)

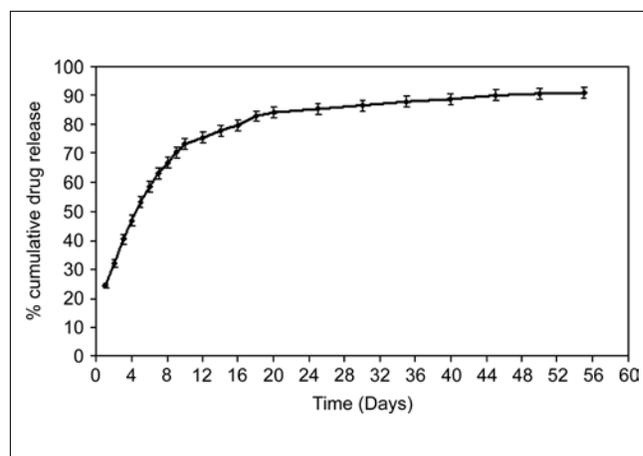


Figure 2: *In vitro* release behavior of cefuroxime sodium formulations in simulated body fluid

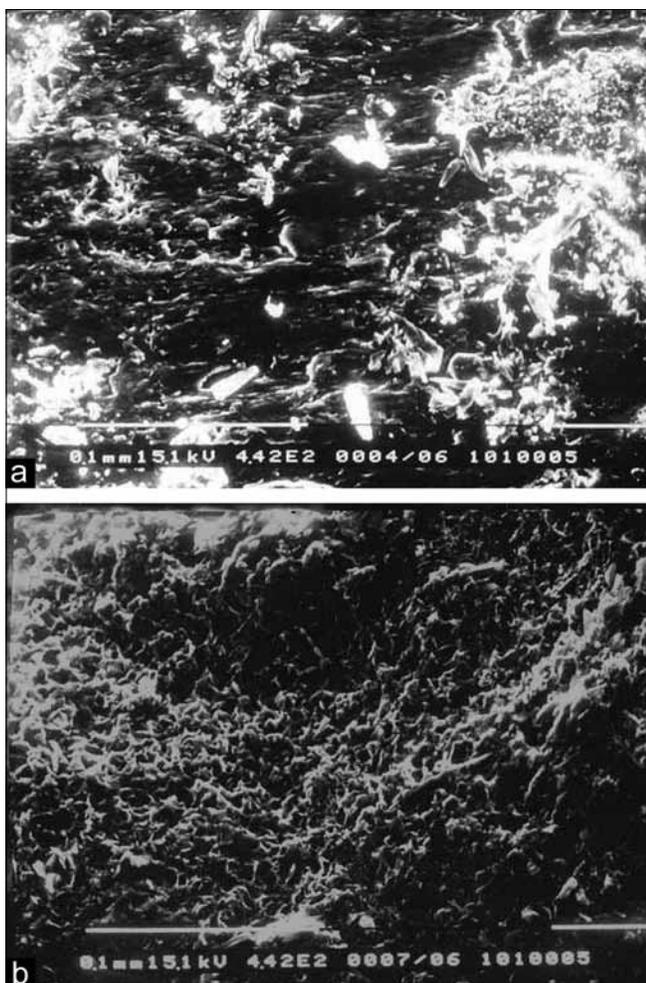


Figure 3: Scanning electron micrographs showing the surface morphology of the pellets before (a) and after (b) *in vitro* release study

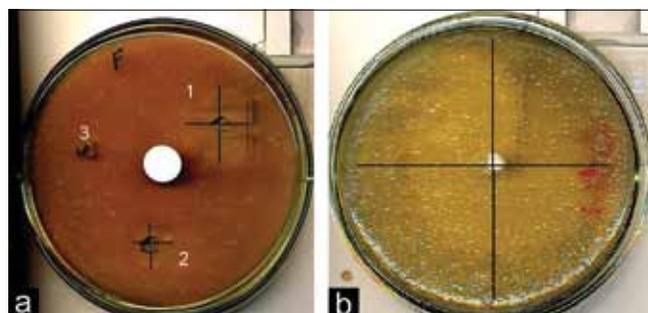


Figure 4: Zone of inhibition in the cup plate assay; (a) Different drug concentration (1) 30 µg/ml concentration, (2) 15 µg/ml concentration and (3) blank solution; (b) Pellet formulation

The developed calcium sulfate and PCL composite prolonged the drug release up to 2 months while maintaining therapeutic levels above the minimum inhibitory concentration of cefuroxime against *S. aureus*. After complete release of the drug and calcium sulfate resorption, the porous PCL scaffold supports the growth of bone cells. Moreover, this composite material has a significant mechanical strength and is suitable for load bearing applications. These pellets are found suitable for

prolonged treatment of osteomyelitis up to 2 months. The implant system can be further evaluated for *in vivo* parameters.

REFERENCES

1. Tortoara GJ, Derrickson B. Ch 6. The Skeletal System: Bone Tissue. In Principles of Anatomy and Physiology. 11th ed. Wiley International; p. 190.
2. Gitelis S, Brebach GT. The treatment of chronic osteomyelitis with a biodegradable antibiotic impregnated implant. J Orthop Surg (Hong Kong) 2002;10:53-60.
3. Hanssen AD. Local antibiotic delivery vehicle in the treatment of musculoskeletal infection. Session IV. Clin Orthop Relat Res 2005;437:91-6.
4. Gao C, Gao J, You X, Huo S, Li X, Zhang Y, et al. Fabrication of calcium sulfate/PLLA composite for bone Repair. J Biomed Mater Res A 2005;73A:244-53.
5. Walsh D, Furuzono T, Tanaka J. Preparation of porous composite implant materials by *in situ* polymerization of porous apatite containing ε-caprolactone or methyl methacrylate. Biomaterial 2001;21:1205-12.
6. Sato S, Koshino T, Saito T. Osteogenic response of rabbit tibia to hydroxyapatite particle: Plaster of Paris mixture. Biomaterial 1998;19:1895-900.
7. Georgiade NG, Hanker J, Levin S, Ruff G. The use of particular hydroxyapatite and Plaster of Paris in aesthetic and reconstructive surgery. Aesthetic Plast Surg 1993;17:5-92.
8. Khairoun I, Boltong MG, Driessens FC, Planell JA. Effect of calcium carbonate on the compliance of apatitic calcium phosphate bone cement. Biomaterial 1997;18:1535-9.
9. Higashi S, Yamamuro T, Nakamura T, Ikada Y, Hyon SH, Jamshidi K. Polymer-hydroxyapatite composites for biodegradable bone fillers. Biomaterial 1986;7:183-7.
10. Chen G, Ushida T, Tateishi T. Poly (DL-lactic-co-glycolic acid) sponge hybridized with collagen microsponges and deposited apatite particulates. J Biomed Mater Res 2001;57:8-14.
11. Bakos D, Soldán M, Hernández-Fuentes I. Hydroxyapatite-collagen-hyaluronic acid composite. Biomaterial 1999;20:191-5.
12. Tamura H, Tokma S. Carboxymethyl-chitin and hydroxyapatite composite for bone repairing. Polymer Preprints 2000;41:1032-3.
13. Coetzee AS. Regeneration of bone in the presence of calcium sulfate. Arch Otolaryngol 1980;106:405-9.
14. Laurencin CT, Ambrosio AM, Borden MD, Cooper JA Jr. Tissue engineering: Orthopaedic applications. Annu Rev Biomed Eng 1999;1:19-46.
15. Orsini G, Ricci J, Scarano A, Pecora G, Petrone G, Piattelli A. Bone- defect healing with calcium-sulfate particles and cement: An experimental study in rabbit. J Biomed Mater Res B Appl Biomater 2004;68:199-208.
16. Ziran BH, Smith WR, Morgan SJ. Use of calcium-based demineralized bone matrix/allograft for nonunions and posttraumatic reconstruction of the appendicular skeleton: Preliminary results and complications. J Trauma 2007;63:1324-8.
17. Harris RJ. Clinical evaluation of a composite bone graft with a calcium sulfate barrier. J Periodontol 2004;75:685-92.
18. La Gatta A, De Rosa A, Laurienzo P, Malinconico M, De Rosa M, Schiraldi C. A Novel Injectable Poly(epsilon-caprolactone)/Calcium Sulfate System for Bone Regeneration: Synthesis and Characterization. Macromol Biosci 2005;5:1108-17.
19. Gao C, Huo S, Li X, You X, Zhang Y, Gao J. Characteristics of calcium sulfate/gelatin composite biomaterials for bone repair. J Biomater Sci Polym Ed 2007;18:799-824.
20. Agrawal CM, Ray RB. Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. J Biomed Mater Res 2001;55:141-50.
21. Mano JF, Sousa RA, Boesel LF, Neves NM, Reis RL. Bioinert, biodegradable and injectable polymeric matrix composites for hard tissue replacement: State of the art and recent developments. Composites Science and

- Technology 2004; 64: 789-817.
22. Overbeck JP, Winckler ST, Meffert R, Törmälä P, Spiegel HU, Brug E. Penetration of ciprofloxacin into bone: A new bioabsorbable implant. *J Invest* 1995;8:155-62.
 23. Ramchandani M, Robinson D. *In vitro in vivo* release of ciprofloxacin from PLGA 50:50 implants. *J Control Rel* 1998;54:167-75.
 24. Désévaux C, Lenaerts V, Girard C, Dubreuil P. Characterization of crosslinked high amylose starch matrix implants: *In vivo* release of ciprofloxacin. *J Control Rel* 2002;82:95-103.
 25. Désévaux C, Dubreuil P, Lenaerts V. Characterization of crosslinked high amylose starch matrix implants: *In vitro* release of ciprofloxacin. *J Control Rel* 2002;82:83-93.
 26. El-Kamel AH, Baddour MM. Gatifloxacin Biodegradable Implant for Treatment of Experimental Osteomyelitis: *In vitro* and *in vivo* Evaluation. *Drug Deliv* 2007;14:349-56.
 27. Shinto Y, Uchida A, Korkusuz F, Araki N, Ono K. Calcium hydroxyapatite ceramic used as a delivery system for antibiotics. *J Bone Joint Surg Br* 1992;74B:600-4.
 28. Bálint L, Kocsis B, Szántó Z, Szabó G. *In vitro* measurement of the time related efficacy of gentamicin sulfate release from bone cements. *Chemotherapy* 2004;50:302-7.

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