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Research paper

# In vivo–in vitro study of biodegradable and osteointegrable gentamicin bone implants

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**Abstract**

Three implants composed of phosphate (25% hydroxyapatite, 75% tricalcium phosphate), 20% poly(DL-lactide) (DL-PLA; weight-average molecular weight (Mw), 30 kD) and 3% gentamicin sulphate (GS) were assayed in vitro and in vivo to study their release profiles as potential drug delivery systems to prevent or treat osteomyelitis. To prolong GS release, some implants were coated with poly(lactide-co-glycolide) (PLGA; Mw, 100 kD; I-PLGA) or DL-PLA (Mw, 200 kD; I-PLA). GS levels were measured in bone, kidney and blood after implantation into the femur of rats. The release profiles show a burst in the first few days, followed by a slower release rate. After I-PLA implantation, bone antibiotic concentrations higher than the minimum bactericidal concentration were maintained for 4 weeks. A linear correlation between in vitro and in vivo GS release was found to continue until complete drug release. Histological and radiological analysis showed that the implants were well tolerated and gradual new bone formation was observed. 

*Keywords*: Poly(lactide-co-glycolide); Poly(lactic acid); Bone implants; Phosphate; Osteomyelitis

## 1. Introduction

Orthopaedic surgery carries the risk of osteomyelitis. In general, three approaches are used to prevent or treat post-operative bone infections: prolonged systemic antibiotic therapy for 4–6 weeks [1]; local implantation of non-degradable antibiotic carriers like gentamicin polymethyl methacrylate (PMMA) beads; and surgical debridement. However, each method has its disadvantages; with systemic therapy, only a small fraction of the dose reaches the surgical site, producing low therapeutic tissue levels and often various adverse systemic side-effects, including the development of bacterial resistance to drugs and deep-seated mycoses [2]. Therefore, local antibiotic administration seems more convenient. PMMA beads have been employed clinically to prevent or treat osteomyelitis [3–5], however, since PMMA is a non-biodegradable material, secondary surgery is required to remove the beads after 2 weeks, once the gentamicin is released.

Many reports have appeared in the literature showing that ceramic materials of beta-tricalcium phosphate (β-TCP) and hydroxyapatite (HAP) can be used in implantable drug delivery systems for local antibiotic treatment of bone infections [6–12], since these implants provide bone antibiotic levels with reduction of bacteria levels for a long period of time. Having a chemical composition very similar to the mineral phase of bone, calcium phosphates have been applied clinically as a bone substitute due to its osteointegrative properties and also appears to be well tolerated by the bone tissue, being not only biocompatible but also osteointegrative material [13].

Great effort has been made in recent years to obtain biodegradable synthetic polymer implants for treating osteomyelitis. Poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and poly(lactide-co-glycolide) (PLGA) have been extensively tested in recent years for suitability as potential bone material for use in antibiotic bone implants. PLA [14] and PGA [15] have shown sufficient absorption, biocompatibility, mechanical properties and positive effects on new bone formation for fixation of osteotomies. PLA [16], PLGA [17], PGA [18] and other biocompatible and biodegradable polymers, such as polyanhydrides [19] and poly-hydroxybutyrate-co-hydroxyvalerate (PHBV) [20], have

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been used to prepare bone implants with different antibiotics. The results in different animal species show the effectiveness of these materials as antibiotic carriers for bone implantation in the treatment of local infections.

More recently, Sasaki and Ishii [21] have tested an implant composed of calcium phosphate cement, gentamicin and poly(L-lactic acid), that can prevent the progression of osteomyelitis in rabbits and induce local bone formation.

All of these systems have the advantage over PMMA beads that no second surgical procedure is required for implant removal, along with certain osteoregenerative properties.

The aim of the present work was to test in vivo three types of biodegradable/bioresorbable implants, composed of blends of β-TCP, HAP, PLA and gentamicin, as drug delivery systems to provide local bactericidal concentrations of gentamicin sulphate (GS) for at least 4–6 weeks. After bone implantation, the tissue tolerance and osteointegrity of the implants were monitored for several weeks. The in vivo amount of gentamicin delivered from these systems was also determined to obtain an in vitro–in vivo correlation. This allowed them to be modified in order to achieve adequate bone levels of gentamicin to treat local infection.

The use of such materials as the matrix of an implantable drug delivery system for potential prevention and treatment of osteomyelitis could be of great clinical interest.

## 2. Materials and methods

### 2.1. Materials

HAP powder ($Ca_5(PO_4)_3OH$) and tricalcium phosphate powder (TCP, $Ca_3(PO_4)_2$) were a gift from Traiber S.A. (Spain). Poly(DL-lactic acid) (DL-PLA) with a weight-average molecular weight (Mw) of 30 kD, polydispersity (pd) of 1.74 (DL-PLA-30, Resomer® R203), DL-PLA with Mw of 200 kD and pd of 1.50 (DL-PLA-200, Resomer® R207) and poly(DL-lactic-co-glycolic acid) with a molar ratio of 50:50, Mw of 100 kD and pd of 1.56 (PLGA-50:50–100, Resomer® RG506) were purchased from Boehringer Ingelheim KG, Germany. GS powder was a gift from Normon Laboratory, S.A. (Spain) and *o*-phthaldialdehyde reagent solution was supplied by Sigma Chemical Co., St. Louis, MO.

### 2.2. Implant preparation

The implants were prepared following the method previously described [22]. Briefly, to prepare 10 g of granulate, an emulsion was formed by homogenization of 0.5 ml aqueous GS solution (700 mg/ml) with a solution 1.9 g of DL-PLA-30 in 1.9 ml of methylene chloride (50% w/v). Homogenization was performed by sonication at output 4 (Sonicator® ultrasonic processor XL 2020) for 15 s in an ice bath. This emulsion was added to the HAP (25%) and TCP (75%) paste (1.9 g of HAP, 5.8 g of TCP and 7.7 ml of water), then mixed, dried at 60°C for 2 h and further granulated and dried at 40°C overnight.

In order to check their uniformity, three batches of granulates were prepared.

Implants ($2.5 \times 12$ mm) were prepared by compressing 500 mg of the resulting granulate at a force of 8 metric tons for 5 min, using a Carver hydraulic press at room temperature; then cut to smaller cylinders of $6 \times 2.5 \times 1$ mm for implantation in rats. Some implants were coated with PLGA-50:50–100 or DL-PLA-200 (concentration, 10% w/v) solution in methylene chloride and then completely dried. Thus, we have three types of formulations, non-coated implants (I-NC), implants coated with PLGA-50:50–100 (I-PLGA) and implants coated with DL-PLA-200 (I-PLA).

### 2.3. Analysis of GS

The drug content of each granulate lot and their released GS concentrations were determined spectrophotometrically after derivatization with *o*-phthaldialdehyde (C.V., 2.3%) by Zhang's method [23].

### 2.4. Assay of drug content

Triplicate samples (25 mg) of each GS granulate lot were placed in methylene chloride to dissolve the DL-PLA-30, and the gentamicin was then extracted four times with 5 ml of distilled water. Aliquots of the aqueous extracts were assayed as in Section 2.3 above. Percentage yields were calculated from the theoretical yields.

### 2.5. In vitro release assay

Release of GS from the implants was assayed in triplicate under sink conditions. The implants were placed in a flask with 25 ml isotonic phosphate buffer solution of pH 7.4 and 0.02% w/v sodium azide, agitated at 80 revs./min in a horizontally shaking water bath maintained at 37°C. At suitable time intervals, 1 ml aliquots of the aqueous solution were withdrawn and replaced immediately with fresh buffer.

### 2.6. In vivo release assay

The local committee for animal studies of the University of La Laguna had previously approved animal experiments.

Male Wistar rats, weighing 250–280 g, were anaesthetized intramuscularly with ketamine (75 mg/kg) and xylazine (10 mg/kg), and their right hind legs were shaven and disinfected. A vertical external parapatellar incision was made in the knee, a dislocation to the medial side of the patellar tendon and quadriceps allowed access to the femoral condyles. A hole in the intercondylar space was made with a 1.2 mm odontologic burr to reach the medullary cavity, avoiding damage to the ligament insertions. After the implant was gently inserted into the medullar space, the patella and the patellar tendon were reduced, closing the structures with stitches. The surgical wound was disinfected and an IM injection of magnesium metamizol

(60 mg/kg) was administered. After the animals recovered from anaesthesia, they were allowed free movement in their cages. The blood loss with this surgical approach was scarce.

One hundred and eight rats were divided into three groups, the first group had an I-NC inserted in the right femur, with the second and third groups receiving an I-PLA or I-PLGA, respectively. Four rats of each group were sacrificed after 1 and 3 days and at 1, 2, 3, 4, 5, 6 and 7 weeks. The femurs of three rats from each group were extracted and kept frozen (−20°C) for gentamicin determination. One leg from each rat group was kept in formaldehyde (10%) for later radiological and histological studies. At each time interval, blood samples (cardiac punction) were extracted. Serum samples were frozen until gentamicin and creatinine levels were analyzed.

### 2.7. In vivo GS determination

To extract GS from bone or kidney, the sample was milled or cut into small pieces, placed in a glass flask containing 5 ml of NaOH (0.1 N), homogenized for 5 min (Silverson®, L4 RT) and then shaken overnight.

GS levels in serum and homogenized tissue were evaluated by microbiological assays. The samples were assayed in quintuplicate by an agar diffusion paper-disc method using *Bacillus subtilis* (ATCC 6633).

### 2.8. Radiography

Radiography was performed at the same times as the bone sample analysis, over a period of 20 weeks. Radiographic images were taken with Philips Optimus X-ray equipment and the exposure conditions were 42 kv, 2.50 mA/s and 11.4 ms.

### 2.9. Histopathology

In one animal of each group, the femur was dissected free of soft tissues and fixed with 10% buffered formalin. Fixed specimens were decalcified in 10% formic acid, embedded in paraffin wax, cut into 6 μm sections and stained with haematoxylin and eosin for light microscope examination.

## 3. Results and discussion

Chronic osteomyelitis is difficult to treat because of bone characteristics. Localized necrosis of the bone can occur providing an environment suitable for bacteria to develop, forming a biofilm [24]. Therefore, it is necessary to achieve and maintain prolonged high local antibiotic concentrations to eradicate the infection. To achieve this, one approach is the use of osteocompatible and osteointegratable antibiotic delivery systems. In a previous paper [22], we characterized 2.5 × 12 mm implants blended from β-TCP, HAP, DL-poly(lactic acid) and gentamicin in terms of morphology, internal structure, X-ray analysis, preparation reproducibility, polymer degradation and in vitro release. In the present work, three types of implants (I-NC, I-PLA and I-PLGA) are studied in the size suited for rat femur implantation. These implants were characterized in terms of in vitro and in vivo release and tissue reactions (radiographic and histological studies).

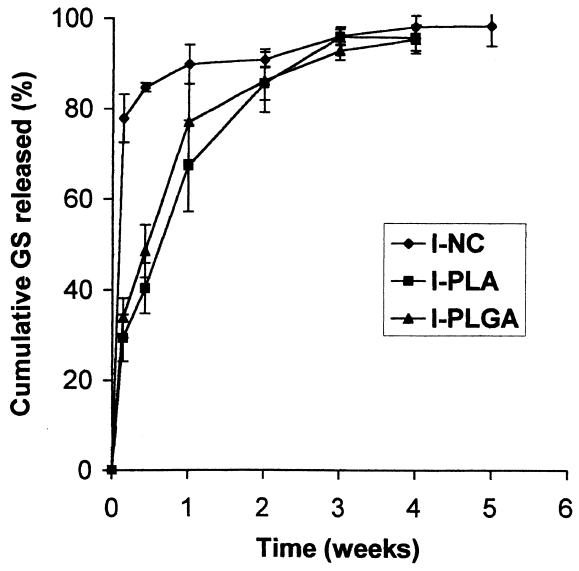


Fig. 1. In vitro release of gentamicin sulfate from the implants into isotonic (pH 7.4) phosphate buffer solution.

### 3.1. GS in vitro release

The accumulated in vitro profile is shown in Fig. 1, about 30, 33 and 78% of GS is released in the first 24 h from I-PLA, I-PLGA and I-NC, respectively. The non-coated formulation (I-NC) released more than 95% of the dose in

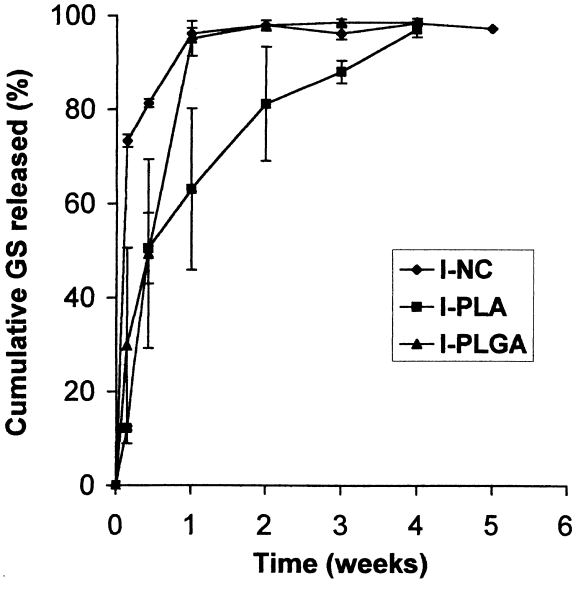


Fig. 2. In vivo GS release profile into bone after implantation of different formulations.

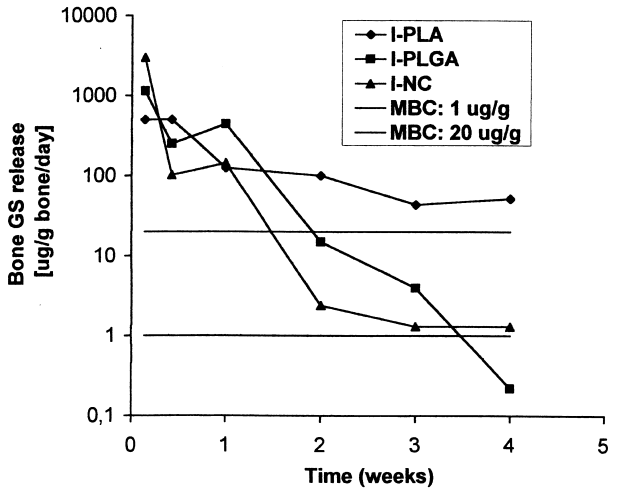


Fig. 3. Available GS in bone from the three different preparations, together with the MBC range for *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

the first week. The GS release rate from coated implants is slower than that from non-coated ones. No great differences were found between the GS percentage released from coated implants during the first 3 days: approximately 40 (I-PLA) and 50% (I-PLGA) of the dose. The release rate was slightly slower from I-PLA than from I-PLGA during the in vitro release assay. As we have already shown, the PLGA coating has a faster degradation rate than PLA film. Both formulations released 95% of the dose in 4 weeks.

### 3.2. In vivo evaluation

#### 3.2.1. in vivo GS release

The differences in the in vivo behaviour of the three types of implants were more noticeable. Their release profiles are shown in Fig. 2. I-NC and I-PLGA released 95% in 1 week. I-PLA presented a more progressive GS release, approximately 60% in the first week and practically the total dose in 4 weeks. Obviously, the PLA film modulates the GS release rate as already shown [22]. Even for I-PLA, the initial GS release percentage could be too high, but according to the GS antimicrobial pharmacodynamics mechanism, i.e. concentration-dependent bactericidal effects coupled with

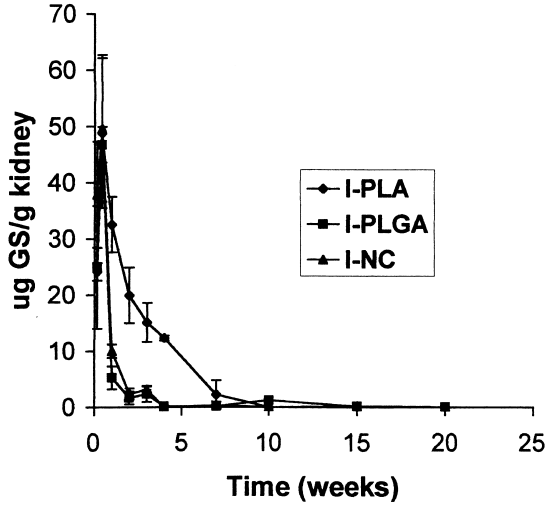


Fig. 4. GS concentrations in kidney after implantation.

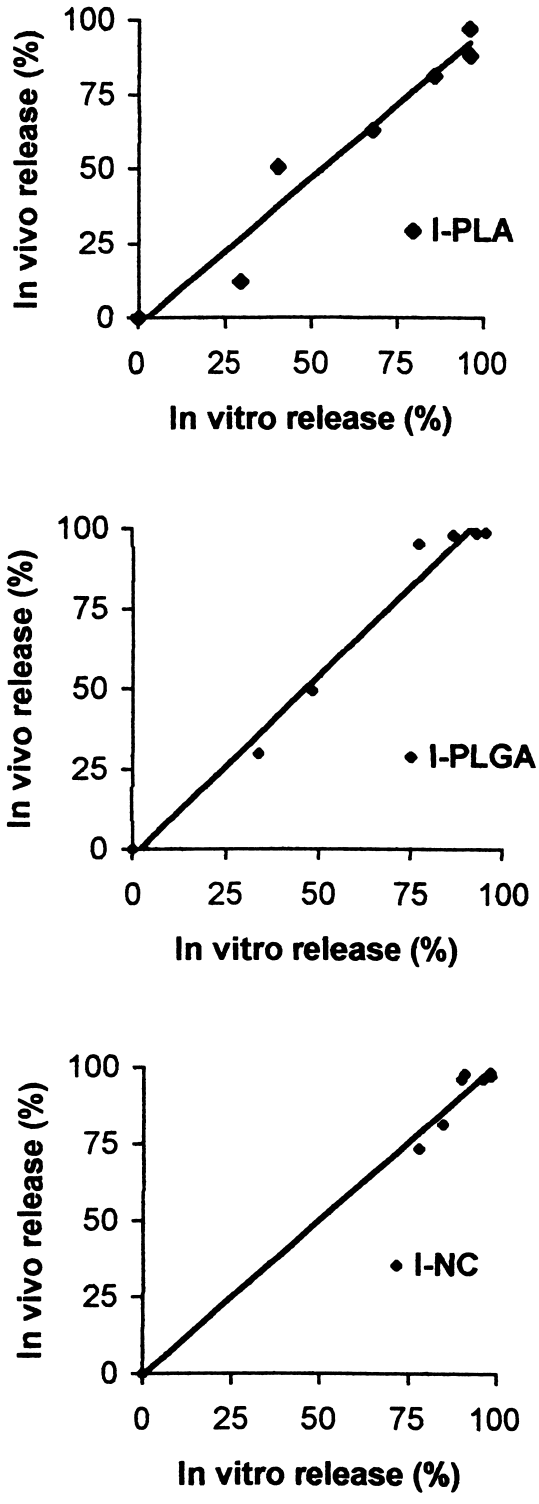


Fig. 5. In vivo–in vitro correlation for the three different implants.

Table 1
Results of in vivo–in vitro linear relationship[a]

| | I-NC | I-PLA | I-PLGA |
|---|---|---|---|
| $b_0$ | − 17.32 | − 6.22 | − 6.98 |
| $b_1$ | 1.19 | 1.04 | 1.18 |
| $R^2$ | 0.905 | 0.956 | 0.977 |
| $f_2$ | 66.62 | 53.02 | 51.36 |

[a] $b_0$, intercept; $b_1$, slope; $R^2$, linear correlation coefficient; $f_2$, similarity factor.

a prolonged post-antibiotic effect [25], the initial high release dose could be more convenient than small doses for a longer period. The best dosage regime with the optimal release rate should be checked in infected animals.

The minimum inhibitory concentrations (MIC) of GS against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, which are the main causative bacteria of osteomyelitis, are 0.03–0.12, 0.25–1 and 0.25–2 μg/g, respectively [26], but the antibiotic concentration at the infected site should be close to the minimum bactericidal concentration (MBC), which is 10 times the MIC, i.e. 1.2, 10 and 20 μg/g. Fig. 3 shows the available amount of GS in bone versus time, obtained after implantation of the three different formulations together with the MBC range for the major causative bacteria. The I-PLA gave bone concentrations higher than the MBC for the three organisms during 4 weeks, while I-NC and I-PLGA produced bone concentrations higher than 10 μg/g during 1 and 2 weeks, respectively.

### 3.2.2. Serum and kidney monitoring

Serum samples were evaluated at the same times as the bones, but the concentrations were at non-detectable levels. In contrast, GS reached a peak level, around 40–50 μg/g in the kidney, 3 days after all implant formulations. Kidney concentrations were detectable until 7 weeks later, at 0.5, 0.07 and 0.03 μg/g for I-PLA, I-PLGA and I-NC, respec-

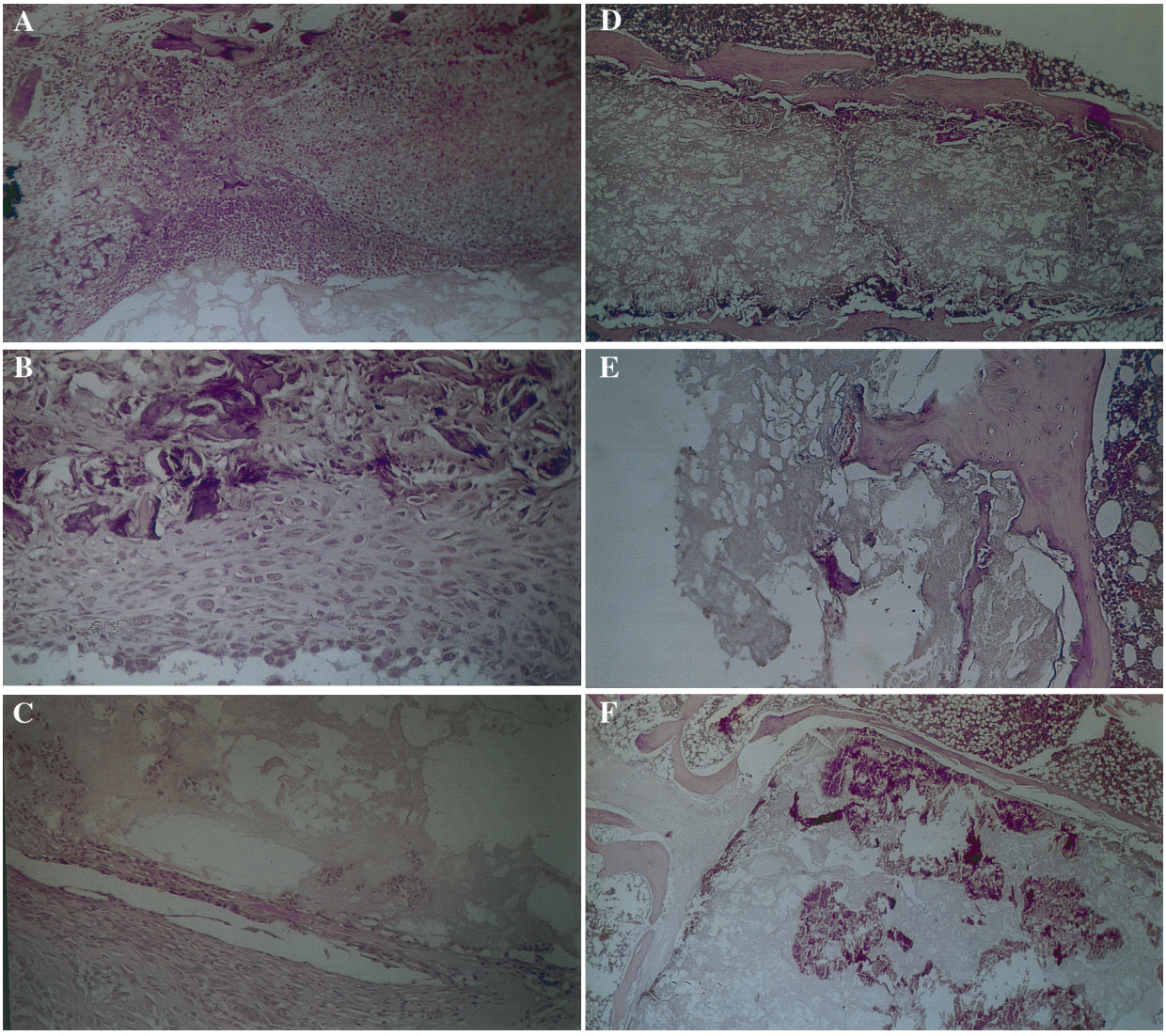


Fig. 6. Histopathological findings after implantation. (A)–(F) correspond to 1 day and 1, 3, 6, 10 and 20 weeks after implantation, respectively.

tively, as illustrated in Fig. 4. As gentamicin is excreted by the renal route and is accumulated in the kidney, it is important to monitor renal function. The serum creatinine level was used for this, no increase in this parameter was observed in any of the animals. The mean concentration in the control group was $0.82 \pm 0.05$ mg/dl ($n = 6$) and the treated rats were in the range of 0.5–0.9 mg/dl.

### 3.3. In vivo–in vitro correlation

Fig. 5 shows the relationship found between the in vivo and in vitro percentage released. The slopes are quite close to one, but the intercept is not so close to the ideal value, zero (Table 1). To evaluate how far the in vivo–in vitro linear relationship is from the ideal, the similarity factor $f_2$, proposed by Moore and Flanner [27], was used to assess the equivalence of the in vitro dissolution profiles of two formulations. The $f_2$ value of between 50 and 100 suggests that the test and reference release profiles are similar. Table 1 shows the results obtained applying this procedure, showing $f_2$ values just slightly higher than 50, indicating a good correlation for all formulations. These results allow the effect of formulation modifications on the in vivo release profile to be checked in vitro.

### 3.4. Histological findings

#### 3.4.1. Sequential changes in the femur

One day after implantation, a local accumulation of polymorphonucleocytes and bone trabeculae fractures with hemorrhagic areas were observed (Fig. 6A) as normal consequences of surgical trauma. After 1 week, a layer of polymorphonucleocytes surrounded the implant. Immature fibrosis and immature bone neoformation with microcalcifications were also observed (Fig. 6B). In 3 weeks, the foreign-body reaction was minimal, and a thin layer of bone trabeculae with vascular tracts was observed (Fig. 6C). At 6 weeks, a thin trabecular bone tissue surrounded

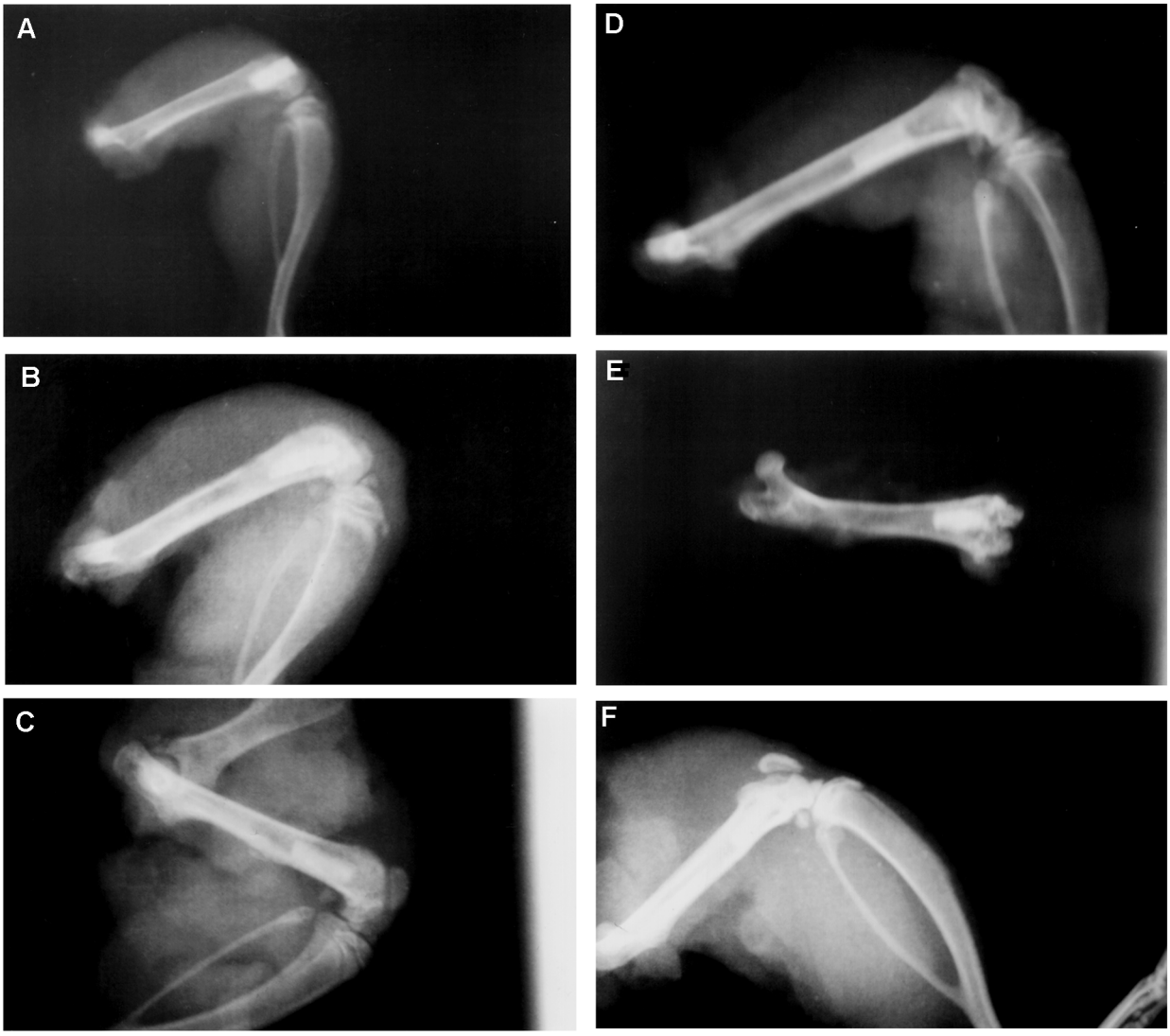


Fig. 7. Radiographic findings. (A)–(F) correspond to 1 day and 1, 3, 6, 10 and 20 weeks after implantation, respectively.

the implant, within it a connective tract as well as the formation of nutritial arteries could be identified (Fig. 6D). After 10 weeks, the preparation showed several intraimplant calcifications and a thin vascular network of capillaries inside the implant. A thin mature bone layer (Fig. 6E) also surrounded the implant. The bone formation continued, increasing the intraimplant vascularization and osteoblastic activity. Increase of peri-implant bone tissue, and at week 20, a pure spiculata stellata calcification and new bone formation were observed (Fig. 6F). Similar results were found by other authors using implants composed of polylactic polymers [16], phosphate cements [11] and blends of both. However, although the implants prepared by Sasaki and Ishii [21] were very similar in composition (calcium phosphate cement, gentamicin and poly (L-lactic acid)) to those prepared in this work, the method used for producing the implant was totally different. The differences observed in the sequential bone regeneration lie in process duration, probably due to the different implant compositions and the different animal species.

### *3.5. Radiological findings*

Fig. 7A–F shows the sequential radiological changes observed. On the first day, increases were observed in soft tissue due to the surgical trauma. After 1 week, a sclerotic picture surrounding the implant was seen. After 3 weeks, there was a radiolucent area around the implant and an osteo-tract intraimplant, non-periostic reaction was appreciated. The radiolucent area gradually became indistinct and at the sixth week, a discrete bone neoformation became gradually more clearly defined with a bone wall and a clear osteosclerotic reaction, suggesting bone repair after 10 weeks. Bone formation continued and at week 20, the implant density was very similar to the surrounding bone.

The images in Figs. 6 and 7 correspond to I-PLA, since I-NC and I-PLGA were fragmented throughout the observation period.

The histological and radiological results indicated the good tolerance, bone integration and high bone repair capacity of the implant materials.

## 4. Conclusions

Histological and radiological studies showed, at the very beginning, a normal foreign-body reaction, decreasing early to leave a regenerated bone profile. The implant material was well tolerated and rapidly induced the formation of new bone. Furthermore, the amount of gentamicin released within the first 3 days was around 60% with the coated implant and higher with non-coated implant. The release of gentamicin from the PLA-coated implant in the femur was prolonged to 4 weeks, reaching a concentration above the MBC of the major causative bacteria of osteomyelitis. Gentamicin was accumulated in the kidney according to its long renal half-life, but renal function remained normal. The in vivo–in vitro relationship showed a slope close to one and an $f_2$ within the 50–100 interval. The efficacy of this implant must be checked in infected animals before reformulation according to this correlation.

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## References

[1] S.S. Sampath, K. Garvin, D.H. Robinson, Preparation and characterisation of biodegradable poly(L-lactic acid) gentamicin delivery systems, Int. J. Pharm. 78 (1992) 165–174.
[2] J.D. Hayes, C.R. Wolf, Mechanism of drug resistance, Biochem. J. 272 (1990) 281–295.
[3] A.M. Armynot du Chatelet, S. Fourcade, M.A. Atisso, M. Simeon de Bouchberg, M. Yakoun, A. Varet, C. Piedra, In vitro release of gentamicin from beads, an original galenic form, and in vivo efficacy in abdomino-perineal surgery, Pharmazie 44 (H.2) (1989) 131–132.
[4] R.A. Giuliano, G.A. Verpooten, M.E. de Broe, Renal cortical kinetics of gentamicin after implantation of gentamicin–polymethylmethacrylate beads in rats, Antimicrob. Agents Chemother. 30 (3) (1986) 385–389.
[5] L. Bunetel, A. Segui, M. Cormier, F. Langlais, Comparative study of gentamicin release from normal and low viscosity acrylic bone cement, Clin. Pharmacokinet. 19 (4) (1990) 333–340.
[6] Y. Shinto, A. Uchida, F. Korkusuz, N. Araki, K. Ono, Calcium hydroxyapatite ceramic used as a delivery system for antibiotics, J. Bone Joint Surg. Br. 74 (1992) 600–604.
[7] K. Yamamura, H. Iwata, T. Yotsuyanagi, Synthesis of antibiotic-loaded hydroxyapatite beads and in vitro drug release testing, J. Biomed. Mater. Res. 26 (1992) 1053–1064.
[8] S. Kano, A. Yamazaki, R. Otsuka, M. Ohgaki, M. Akao, H. Aoki, Application of hydroxyapatite–sol as drug carrier, Biomed. Mater. Eng. 4 (4) (1994) 283–290.
[9] F. Korkusuz, A. Uchida, Y. Shinto, N. Araki, K. Inoue, K. Ono, Experimental implant-related osteomyelitis treated by antibiotic–calcium hydroxyapatite ceramic composites, J. Bone Joint Surg. Br. 75 (1993) 111–114.
[10] M. Itokazu, T. Ohno, T. Tanemori, E. Wada, N. Kato, K. Watanabe, Antibiotic-loaded hydroxyapatite blocks in the treatment of experimental osteomyelitis in rats, J. Med. Microbiol. 46 (9) (1997) 779–783.
[11] K. Thoma, R. Alex, J. Randzio, Biodegradable controlled release implants based on β-tricalcium phosphate ceramic. 2. Testing of gentamicin controlled release pellets in vitro and in vivo, Eur. J. Pharm. Biopharm. 38 (3) (1992) 107–112.
[12] J.C. Lambotte, H. Thomazeau, G. Cathelineau, G. Lancien, J. Minet, F. Langlais, Tricalcium phosphate an antibiotic carrier: a study focused on experimental osteomyelitis in rabbits, Chirurgie 123 (6) (1998) 572–579.
[13] Z. Yang, H. Yuan, W. Tong, P. Zou, W. Chen, X. Zhang, Osteogenesis in extraskeletally implanted porous calcium phosphate ceramics: variability among different kinds of animal, Biomaterials 17 (1996) 2131–2137.
[14] A. Majola, S. Vainionpoa, K. Vihtonen, M. Mero, J. Vasenius, P.

Tormala, P. Rokkanen, Absorption, biocompatibility, and fixation properties of polylactic acid in bone tissue: an experimental study in rats, Clin. Orthop. 268 (1991) 260–269.
[15] N. Ashammakhi, E.A. Makela, K. Vihtonen, P. Rokkanen, P. Tormala, Effect of self-reinforced polyglycolide membranes on cortical bone: an experimental study on rats, J. Biomed. Mater. Res. 29 (6) (1995) 687–694.
[16] G. Wei, Y. Kotoura, M. Oka, T. Yamamuro, R. Wada, S. Hyon, Y. Ikada, A bioabsorbable delivery system for antibiotic treatment of osteomyelitis, J. Bone Joint Surg. Br. 73 (1991) 246–252.
[17] K.L. Garvin, J.A. Miyano, D. Robinson, D. Giger, J. Novak, S. Radio, Polylactide/polyglycolide antibiotic implants in the treatment of osteomyelitis. A canine model, J. Bone Joint Surg. Am. 76 (10) (1994) 1500–1506.
[18] J.P. Overbeck, S.T. Winckler, R. Meffert, P. Tormala, H.V. Spiegel, E. Brug, Penetration of ciprofloxacin into bone: a new bioabsorbable implant, J. Invest. Surg. 8 (3) (1995) 155–162.
[19] C.T. Laurencin, T. Gerhart, P. Witschger, A. Domb, A.E. Rosenberg, P. Hanff, L. Edsberg, W. Hayes, R. Langer, Bioerodible polyanhydrides for antibiotic drug delivery: in vivo osteomyelitis, J. Orthop. Res. 11 (2) (1993) 256–262.
[20] M.F. Yagmurlu, F. Korkusuz, I. Gursel, P. Korkusuz, U. Ors, V. Hasirci, Sulbactam–cefoperazone polyhydroxybutyrate-co-hydroxyvalerate (PHBV) local antibiotic delivery system: in vivo effectiveness and biocompatibility in the treatment of implant-related experimental osteomyelitis, J. Biomed. Mater. Res. 46 (4) (1999) 494–503.
[21] S. Sasaki, Y. Ishii, Apatite cement containing antibiotics: efficacy in treating experimental osteomyelitis, J. Orthop. Sci. 4 (1999) 361–369.
[22] I. Soriano, C. Évora, Formulation of calcium phosphates/poly(DL-lactide) blends containing gentamicin for bone implantation, J. Controlled Release 68 (2000) 121–134.
[23] X. Zhang, U.P. Wyss, D. Pichora, M.F.A. Goosen, Biodegradable controlled antibiotic release devices for osteomyelitis: optimization of release properties, J. Pharm. Pharmacol. 46 (1994) 718–724.
[24] T.J. Marrie, J.W. Costerton, Mode of growth of bacterial pathogens in chronic polymicrobial human osteomyelitis, J. Clin. Microbiol. 22 (1985) 924–933.
[25] S.L. Barrieri, Selection of antimicrobial regimens, in: J.T. Dipiro, R.L. Talbert, G.C. Yee, G.R. Matzke, B.G. Wells, L.M. Posey (Eds.), Pharmacotherapy. A Pathophysiologic Approach, 3rd Edition, Appleton & Lange, Stamford, CT, 1997, p. 1961.
[26] H.P. Lambert, F.W. O'Grady, Antibiotics and Chemotherapy, 6th Edition, Churchill Livingstone, Edinburgh, 1992, p. 11.
[27] J.W. Moore, H.H. Flanner, Mathematical comparison of dissolution profiles, Pharm. Technol. 20 (1996) 64–74.